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Detection of Allergens in a Multiple Allergen Matrix and Study of the Impact of Thermal Processing

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Abstract

The objectives of this work were to (a) determine the percentage recovery of three allergens (casein, egg and soy) simultaneously incurred in a flour matrix, (b) investigate the effect of different baking periods on allergen recovery using two different methods of allergen detection, namely enzyme linked immunosorbent assay (ELISA) and flow cytometry, and c) determine the solubility of proteins in the prepared blank dry cookie mix flour, non-cooked dough and baked cookies after baking at different temperatures using different extraction buffers. Sodium carbonate buffer was least effective in extracting protein. For the blank flour and non-cooked samples, PBS buffer was the most effective at extracting protein, whereas the Tris buffer gave the highest protein recoveries among the three buffers for the cooked samples. For all three allergens, thermal processing greatly reduced allergen recovery in the processed food matrix as detected using both the commercial ELISA kits and flow cytometry. Moreover, allergen recovery in the cooked samples decreased with increasing processing temperatures and ranged from 96% to 20% for casein, 26.5% to 3.7% for soy and 12.8% to 0% for egg in an instance where a false negative was detected when a high processing temperature of 450°F was employed.

Keywords: ELISA; Flow cytometry; Allergen detection; Protein extraction; Solubility

Introduction

The immune system of people with food allergic disease, responds in a way that is harmful to the self following exposure to some specific foods [1]. As these immunological reactions can be very severe, such individuals are often advised to completely avoid culprit foods as even minimal concentrations of allergenic proteins can trigger adverse outcomes [2]. The number of people with food allergies has increased significantly during the past decades, and has resulted in measures by legislators and the food industry to ensure that hidden allergens in final products are clearly stated on labels [3,4].

Heat treatment, a commonly used method in food processing, is usually carried out to enhance texture and flavours or to ensure microbiological safety. One of the major concerns for legislators and food manufacturers is that processing may alter allergen detection in processed foods. In fact, the effect of thermal processing on allergen quantization is of growing interest especially in regards to the detection of allergenic food residues at low levels [5-7]. Food processing treatments such as thermal treatment, pressurization and sterilization cause denaturation of proteins. As such, an antibody generated against a native protein may have lower reactivity to its denatured form because of the structural changes to the protein following its denaturation [8,9].

Detection of allergenic substances in food can be done using DNA-based methods, which are indirect, and/or protein-based methods, which are mostly immunoassays. The DNA-based methods such as polymerase chain reaction (PCR) determine a characteristic sequence of base pairs specific to a food [10,11], whereas the most common protein-based method for allergen detection is the enzyme-linked immunosorbent assay (ELISA) [12], which detects a specific sequence of amino acids (epitope) present in a food that bind to an antibody of interest.

ELISA is accepted as the method of choice for the detection of food allergens by the food industry [10,13]. It is an extremely useful method due to its simplicity, high detection sensitivity, and good quantification of native proteins [14]. One of the major drawbacks of ELISA, however, is that it can only detect one allergen per test. Additionally for processed foods, extraction of denatured or altered proteins from food tends to be difficult due to their reduced solubility as compared to native proteins, which can affect allergen detection when measured by ELISA. In these cases, surfactants and denaturants are sometimes employed in the ELISA kits to solubilise and extract insoluble proteins. However, the use of extraction solutions containing denaturants can affect the accuracy of measurements by ELISA as the antibody can be affected by the denaturant. Diluting the denaturant so as to minimise its effect on the antibody may negatively affect the detection of the extracted protein [14].

Recently, flow cytometry has been introduced as a possible technique that can be used for allergen detection. One of its key advantages is the potential for multiple allergen detection. It is an automated fluorescent microsphere-based multiplex immunoassay that employs Multi-Analyte Profiling (xMAP) technology. The xMAP technology, in theory, enables multiple (up to 100 in some instances) microsphere sets to be distinguished simultaneously, which allows for the binding of multiple allergens on to different beads, enhances ease-of-use and automation and can markedly reduce the time and labour required for allergen detection and quantification [15].

So far flow cytometry has had very limited application in the field of food science. In this field it has been employed to detect: soy, pea

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and soluble wheat protein in milk powder [16], veterinary antibiotic sulphonamides in milk [17], and chemical contaminants such as polycyclic aromatic hydrocarbons which are carcinogenic materials formed from incomplete combustion of organic materials during industrial processing [18] and mycotoxins in feed [19] or in wheat and cereal [20].

The objectives of this work therefore were to (a) determine the percentage recovery of three allergens (casein, egg and soy) simultaneously incurred in a flour matrix, (b) investigate the effect of different baking periods on allergen recovery using ELISA and flow cytometry, and c) determine the solubility of proteins in the blank dry cookie mix flour, non-cooked dough and baked cookies in the different extraction buffers, namely, sodium carbonate buffer, PBS (phosphate buffer saline) and Tris buffer.

Materials and Methods

Materials

Casein (90% protein) and whole egg powder (50% protein) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Soy protein concentrate (81.5% protein) was prepared in-house from press-cake provided from La Ferme Jirah (Ormstown Québec, Canada). Sodium carbonate, sodium bicarbonate, Tris (hydroxymethyl) aminomethane, sodium chloride and skin fish gelatin were obtained from Sigma–Aldrich (St. Louis, MO, USA). PBS (phosphate buffer saline) was provided with the Veratox ELISA kits. Bio-Plex pro magnetic COOH beads were obtained from Bio-Rad (USA). Capture antibodies: rabbit polyclonal anti-casein, rabbit polyclonal anti-ovalbumin and rabbit polyclonal anti-soy protein were obtained from Abcam (Cambridge, MA, USA). Detection (reporter) antibodies were obtained by conjugating the pre-mentioned capture antibodies to phycoerythrin (PE) using a Phycolink R-Phycoerythrin (RPE) conjugation kit and following the manufacturer's recommendations (Promega, USA). N-hydroxysulfosuccinimide (Sulfo-NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), dithiothreitol (DTT), iodoacetamide and sequencing grade trypsin were obtained from Sigma–Aldrich (St. Louis, MO, USA). ELISA kits (Veratox and Biokits) were purchased from Neogen as described in Table 1. All other chemicals used were of analytical grade.

Preparation of incurred flour and cookie matrix (dough and baked cookie)

In this study "Allergen-free samples" is defined as samples free of tested allergen (casein, soy, egg). Allergen-free dry mix flour samples ("dry mix sample") consisting of flour (450 g), sugar (180 g), salt (1.3 g), sodium bicarbonate (2.23 g), baking soda (1.8 g), were prepared by mixing all the ingredients with an electric mixer. The dry mix sample was incurred with casein, whole egg powder, and soy protein concentrate to obtain an incurred flour mix containing 1000 µg of each allergenic protein/g (i.e., 1000 ppm incurred samples). To ensure uniform distribution of the allergens in the dry mix, the incurred samples were mixed in a 'V' shaped blender (Arnold Equipment Company, Twinsburg, OH, USA). The mixing time required to achieve a homogenous distribution, which was about 30 min, was visually estimated using casein at a concentration of 1000 ppm that had been dyed with a coloring agent prior to mixing. To ensure homogenous distribution of the allergen, all samples were mixed for an additional 90 min to a total of 2h and the samples were packaged in sterile sample bags (VMR, Mississauga, ON, CA) and stored at 4°C until use. Tests for homogeneity yielded coefficient of variation values (CV) below 15%.

Allergen-free and incurred cookies (dough and cooked samples) were prepared from the allergen-free or incurred dry mix sample by adding 50 g of the mixture to 90 g of sunflower oil, followed by the addition of 150 g of water to form dough. A sample of the dough was taken for testing and the rest was used to make cookies. The cookies had a uniform weight of 10±0.3 g and dimensions of 38 mm x 58 mm x 76 mm. Cookies were baked in the center of a pre-heated oven at 350°F, 400°F and 450°F for 12 min. The baked cookies were cooled at room temperature and subsequently ground into fine powders. Taking into consideration moisture loss during baking, the final concentration of each allergen in the baked cookie was 600 ppm, 616 ppm, 619 ppm for cookies baked at 350°F, 400°F and 450°F, respectively. The final concentration of each allergen in the non cooked dough was 514 ppm. The employed samples and their respective final allergen concentrations are summarized in Table 1.

ELISA method

All tested allergens were detected using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer instructions. Descriptions of the test kits used are presented in Table 2. In general, a protein extract was prepared by mixing 2 g of sample with 20 or 50 mL of extraction buffer (according to the kit and tested allergen). The mixture was shaken for 15 min in a stirring water bath at 60°C. Subsequently, the mixture was centrifuged at 1500 g for 15 min (except for detection of casein using the Veratox kit). The protein extracts were diluted using the recommended buffer by the kit manufacturer.

Total soluble protein concentration was measured using the method of Bradford [21] with a Bio-Rad Protein assay dye using bovine serum albumin (BSA) as the protein standard. Total protein content was expressed as total nitrogen multiplied by a conversion factor of 5.7. Nitrogen was determined by using an FP-428 LECO apparatus (LECO Corp., Saint Joseph, MI, USA). The instrument was calibrated with ethylene diamine tetraacetic acid (EDTA) as a nitrogen standard. Total soluble protein recovery was calculated as follows:

$$\text{Total soluble protein recovery} = \frac{\text{Extracted soluble protein}}{\text{Total protein content}} \times 100$$

Different extraction buffer systems were used for protein extraction as recommended for each ELISA kit. With respect to the Veratox kit, PBS at pH 7.4 and an allergen extraction additive specific for each tested allergen were employed for the three tested allergens. The buffers recommended for the Biokits and ELISA kits were Tris buffer containing sodium chloride and gelatine at pH 8.2 for soy and egg allergens and carbonate/bicarbonate buffer at pH 9.6 for casein.

	Final allergen concentration (ppm)		
Sample	Casein	Soy	Egg
Blank flour	0	0	0
Blank cookie dough	0	0	0
Blank cookie	0	0	0
Incurred flour	1000	1000	1000
Incurred cookie dough	514	514	514
Cookies cooked at 350°F	600	600	600
Cookies cooked at 400°F	616	616	616
Cookies cooked at 450°F	619	619	619

Table 1: Samples employed and their respective final concentration of each allergen.

Target allergen	Limit of detection (ppm)	Quantification range (ppm)	Results expressedt as ppm of	Extraction buffer, pH	Supplier and kit name
Casein	1	1.6-25.6	Casein	Carbonate/bicarbonate buffer at pH 9.6	Neogen (BioKits)
	2.5	2.5-15	Non fat dried milk	PBS, pH 7.4 and specific allergen extraction additive	Neogen (Veratox)
Soy protein	0.3	1.25-20	Soy protein	Tris buffer containing sodium chloride and gelatine, pH 8.2	Neogen (BioKits)
	2.5	2.5-25	Soy flour	PBS, pH 7.4 and allergen extraction additive	Neogen (Veratox)
Egg	0.1	0.5-10	Egg white protein	Tris buffer containing sodium chloride and gelatine, pH 8.2	Neogen (BioKits)
	2.5	2.5-25	Whole dried egg	PBS, pH 7.4 and allergen extraction additive	Neogen (Veratox)

Table 2: ELISA test kits employed.

Quantitative data were obtained by measuring the optical density at the wavelength specified by the manufacturer using a microplate spectrophotometer Epoch (Biotek, Winooski, Vermont, USA). All standard curves were generated with the standard solutions provided with the test kits.

Data analysis was undertaken using the microplate and data collection analysis software Gen5 (Biotek, Winooski, Vermont, USA). Calibration curves were constructed from three replicates at each point of the standard curves.

ELISA measurements were obtained in duplicates for each sample and all experiments were performed twice. In order to determine the percentage recovery, the following conversion factors were calculated from the ELISA results of each standard allergen listed in the materials section: the non fat dried milk was estimated to contain 33.3% casein, the total dried egg was estimated to contain 30% egg white protein and the soy flour was estimated to contain 37.3% protein.

Multiple allergen detection using Flow Cytometry

Protein preparation: Protein extracts was prepared by mixing 2 g of sample with 40 mL of phosphate buffer saline (PBS) pH 7.4. The mixture was shaken for 15 min in a stirring water bath at 60°C. Subsequently, the mixture was centrifuged at 1500 g for 15 min. The protein extracts were diluted using PBS pH 7.4 and used immediately for analysis.

Coupling of protein to the magnetic COOH beads: The stock bead concentration employed was 1.25 x 10^7 beads per ml. For each coupling reaction, 1.25 x 10^6 beads were transferred to a 1.7 ml black micro centrifuge tube for coupling. Stock uncoupled microspheres were re-suspended in sodium phosphate buffer to replace the buffer used for storing the beads by vortexing at high speed for 30 sec, followed by sonication for 1 min, magnetic separation for 30 to 60 sec, supernatant removal without disturbing the beads and resuspension in 100 µl sodium phosphate 0.1 M (pH 6.2).

Beads were activated using freshly prepared N-hydroxysulfosuccinimide (Sulfo-NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) immediately before use. A solution of Sulfo-NHS (50 mg/ml) was prepared in activation buffer and 10 µl of it was added to each magnetic bead set and vortexed gently. Likewise, a 50 mg/ml solution of EDC was prepared and 10 µl was added to each bead set and vortexed. The suspensions were incubated in the dark for 20 min at room temperature during which time the beads were agitated with gentle mixing on a shaker. The microspheres were washed twice with PBS at pH 7.4 by vortexing, sonication and magnetic separation, as described earlier.

Allergen(s) coupling to the activated beads: Activated beads were resuspended in 100 µL of PBS at pH 7.4 and vortexed at medium speed for 30 sec. The protein solution (20 µg) prepared as aforementioned was added to the activated beads and the total volume was brought to 500 µL with PBS, pH 7.4. The suspensions were vortexed and incubated in the dark for 20±1 min at room temperature with mixing on a shaker. The coupled beads were washed twice in PBS, resuspended in 250 µL of blocking buffer PBS, 1% BSA, 0.05% azide, pH 7.4 (PBS-BN) and vortexed at medium speed for 15 sec. Coupling reaction tubes were agitated on a shaker in the dark for 30 min at room temperature. After blocking, the blocking buffer was replaced with PBS-BN by washing twice and the coupled beads were resuspended in 500 µL of storage buffer (PBS-BN) by vortexing for 20 sec. The bead concentration was determined using a hemocytometer. The total microspheres were calculated by counting the microspheres in the center square (1 mm x 1 mm x 0.1 mm) using the equation:

Total microspheres per 1 ml = microsphere counts in the center square x 10^4 x dilution factor

The coupled beads were refrigerated at 2-8°C in the dark.

Confirmation of protein coupling: The validation method for coupling was based on the detection of the coupled protein with phycoerythrin (PE)-conjugated secondary antibody. The concentration of the beads was adjusted to 1 x 10^6 beads/mL using a hemocytometer. All detection antibodies were conjugated to phycoerythrin (PE) using a kit and following the manufacturer's recommendations (Prozyme, USA). The produced PE conjugated antibody (reporter antibody) was used to estimate the density of the antibody coupled to the beads. Each coupling reaction was repeated in triplicate in order to confirm the reproducibility of the data.

The PE-labelled antibody was diluted to 2 µg/mL with staining buffer. 50 µL of the 2 µg/mI diluted RE-labelled antibody were added to 50 µL of the sample or standard and incubated for 30 min with shaking at room temperature followed by adding approximately 5,000 coupled beads for each detection run. Beads were vortexed at medium speed for 15 sec. Beads were agitated with a shaker at room temperature for 30 min. After washing twice using Wash station II (Bio-Rad, USA) beads were resuspended in 125 µL of storage buffer. Each sample (125 µ1) was vortexed and transferred to a single well of a flat-bottom 96-well plate. Coupled beads were analyzed using a Bio-Plex system by reading 50 µL on the Bio-Plex 200 Luminex analyzer (Bio-Rad) according to the system manual.

Statistical analyses

Experimental results were recorded as mean ± coefficient of variation. Data were analyzed using SAS for Windows (version 9.1)

following an analysis of variance (ANOVA) one-way linear model. Mean comparisons were performed using the Duncan test, and the significance level of $p \leq 0.05$ was considered to indicate significance.

Results

Effect of baking temperature on protein extraction

The quantity of total soluble protein present in the samples extracted using the different buffer systems used in this study (i.e., sodium carbonate buffer, PBS and Tris buffer) is provided in Figure 1. The data did not show significant differences between the dry mix sample and non cooked samples (dough) for all tested buffer systems. Data also showed that : (i) sodium carbonate buffer was the least effective in extracting soluble protein in that it had the lowest quantity of determined soluble protein (i.e., 30.8% of the total protein content) for the dry mix sample and 7.6-2.2% for the baked cookies; (ii) PBS extraction buffer including extraction additive (provided with Veratox kits for each allergen) was the most effective buffer for extraction of soluble proteins from the dry mix samples with a soluble protein recovery of 61.4% and 60%, respectively, compared with 48% for Tris buffer and 30.8% for sodium phosphate buffer; and (iii) Tris buffer had the highest soluble protein recoveries among the three buffers for the cooked samples with respect to the three temperatures studied with a soluble protein recovery of 46%, 44%, 32% for the baked cookies at 350°F, 400°F, 450°F, respectively.

These findings clearly indicate that thermal processing reduced protein solubility. Furthermore, the protein extraction data showed that the soluble protein recovery as a percentage of the total protein content decreased with increasing processing temperature for the three buffers which can further limit the accuracy of determination of allergen quantities. These results are in agreement with previous findings [5,6,22-24].

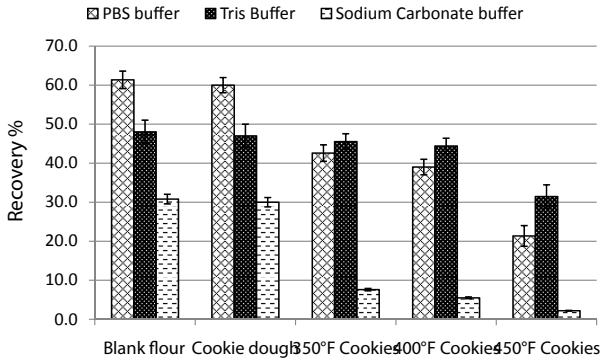
Detection of allergens by ELISA and flow cytometry

Casein allergens: Figure 2 presents the percentage recoveries of casein as detected by ELISA. The results show that the Veratox and Biokits test kits performed well in detecting casein allergens in the dry mix and non cooked cookie dough. Allergen recoveries were 119% and 115% using the Veratox kit and105% and 108% using the Biokits, for the dry mix and cookie dough, respectively. The kits, however, performed poorly with respect to the baked cookies. Recoveries detected using the Veratox kit decreased from 74.5% for the cookies baked at 350°F to 35.3% for the cookies baked at 450°F. For the Biokits, the recoveries were even lower, decreasing from 55% for the cookies baked at 350 °F to 20% for the cookies baked at 450°F. Both kits performed well on the casein-free samples and no false positives were determined for the casein-free dry mix sample, cookie dough, and baked cookies. In another study [25], the effect of food processing on the detection of milk proteins in incurred peanut butter cookies baked at various times was compared using different ELISA kits (Tepnel, Neogen, r-Biopharm and Morinaga). The author also reported low and variable results in the ability of all tested ELISA kits to detect heat-treated milk proteins in baked cookies. Similarly, casein was reported to have a recovery of only 50% and 20% in a cookie food matrix after baking at 180°C (356°F) for 12 and 30 min, respectively, [23].

The flow cytometry method yielded percentage recoveries of casein allergens of 121% and 108% in the dry mix and non cooked cookie dough samples, respectively (Figure 2). However, as with the ELISA kits, the recovery decreased with increasing baking temperature and was 96%, 74% and 43% for cookies baked at 350°F, 400°F and 450°F. No false positives were detected.

Detecting egg allergens: The recoveries of egg allergens in the dry mix samples were 110.6% and 90.9% for the Veratox and Biokits, respectively (Figure 3). Both ELISA kits performed poorly in detecting egg allergens in the baked cookies and recoveries were less than 10% at the three different temperatures studied. The Veratox kit was unable to detect egg allergens in cookies baked at 400°F and 450°F, and yielded a false negative at 450°F. Similarly, the recoveries of egg allergens detected using the Biokits were less than 5% at 400°F and 450°F. Both kits again performed well on the egg-free samples and no false positives were determined for the dry mix sample and in the non-cooked and baked cookies (Figure 3). Faeste et al. [26] obtained similar results when they tested three different commercial ELISA kits for the quantification of egg allergens. Their results showed that the BioKit and RIDASCREEN egg assay kits failed to detect any egg allergens in the tested processed matrix. However, the egg protein ELISA Kit No. 140OA from Morinaga Institute of Biological Science was able to detect egg allergens in the processed food matrix because of the extraction buffer used (i.e., SDS and 2-ME extraction buffer) and the protocol employed. Other studies have also reported that heat treatment decreased the antigenicity of the egg allergens ovomucoid [27] and ovotransferrin [6].

The recoveries of egg allergens in the dry mix and cookie dough samples using the flow cytometry method were high (103% and 93%, respectively) (Figure 3). Similar to the ELISA kits, the flow cytometry



The value is an average of triplicate measurements. The total protein content was calculated according to the equation N content x 5.7. The total soluble protein content was determined with Bio-Rad protein assay kit.

Figure 1: Total soluble protein recovery in blank dry cookie mix flour, non-cooked dough and cookies baked at different temperatures.

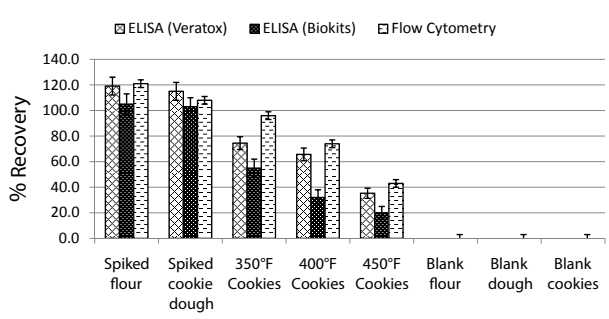


Figure 2: Casein recovery in an incurred dry cookie mix flour, non-cooked dough and baked cookies using two different ELISA kits and flow cytometry.

method also showed poor detectability of the egg allergens in the baked cookies at the three different temperatures studied with egg allergen recoveries ranging from 13% for cookies baked at 350°F to a low of 2% at 450°F. No false positives were determined using the dry mix, cookie dough and baked cookie samples.

Detecting soy allergens: The percentage recoveries of soy in the dry mix sample and cookie matrices as detected by ELISA are presented in Figure 4. Soy allergen recoveries in the non-cooked samples were high for the Veratox and Biokits at 121.5% and 107.5%, respectively. Recoveries were, however, less than 20% for the cooked samples, ranging from 18.7% to 7.5% at 350°F and 450°F, respectively, for the Veratox kit, and from 8.4% to 3.7% at 350°F and 450°F, respectively, for the Biokits. As with the other two allergens, both ELISA kits performed well on the soy-free samples and no false positives were determined for the dry mix sample and in the non-cooked and baked cookies.

The percentage recoveries of soy as detected by flow cytometry are also presented in Figure 4. Soy allergen recoveries in the dry mix and cookie dough samples were 96% and 98%, respectively. Recoveries were, however, much lower for the baked cookies, ranging from 26.5% at 350°F to 9.0% at 450°F. No false positives were determined for the dry mix, cookie dough and baked cookie samples.

Discussion

In general, the reduced solubility of thermally processed proteins could be attributed to chemical reactions such as the Maillard reaction and other protein-protein and protein-non protein interactions [28]. During processing, proteins can form oligomers, become denatured, degraded, aggregated, cross-linked, fragmented and re-assembled and these changes most often result in reduced solubility [29]. Thus, in addition to reduced availability, processing can result in reduced accessibility which can alter the overall IgE binding profiles of a particular extract [30]. Thermal effects are therefore an important consideration when detecting allergens.

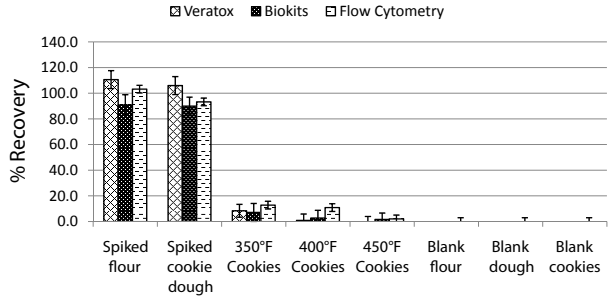


Figure 3: Eggs recovery in an incurred dry cookie mix flour, non-cooked dough and baked cookies using two different ELISA kits and flow cytometry.

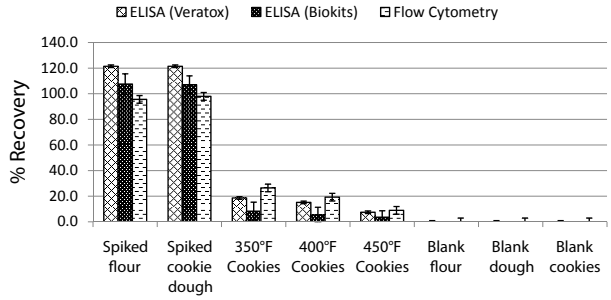


Figure 4: Soy recovery in an incurred dry cookie mix flour, non-cooked dough and baked cookies using two different ELISA kits and flow cytometry.

On average the performance of the ELISA kits was similar to that obtained by flow cytometry with the added advantage that flow cytometry allowed the simultaneous detection of all three allergens with one test. A variety of factors can explain the decreases in recovery observed on baking and on increasing of the baking temperature. Reduced protein solubility following thermal processing due to protein structural changes and the reduced ability of the extraction buffers used to solubilise the heat treated proteins provide some explanations. Diaz-Amigo [31] reported a decrease in detection of egg allergens in baked cookie extracts using ELISA kits due to the reduced quantity of protein extracted with the buffers employed. Other workers have shown changes in secondary structure and sulfhydryl-disulfide interchange reactions on thermal treatment of egg proteins which affected protein antigenicity [6]. A decrease of soy allergen recovery has also been explained by the interactions occurring between soy and flour gluten proteins as a consequence of dough mixing and the formation of high molecular weight complex between soy 11S globulin and prolamin of wheat dough [32]. Monaci et al. [23] attributed such changes to conformational modifications of the epitopes that reduced antibody binding during ELISA analysis, the Maillard reaction, protein modification, and a reduction of extraction efficiency.

Clearly, the variations in the results observed and the particularly low recoveries obtained in the incurred samples raises questions about the ability of legislators and policy enforcers to accurately detect the presence of allergens in foods. For kit manufacturers it continues to highlight the importance of optimizing the protocol for detecting allergens in both raw and cooked samples. Buffer selection in the preparation of ELISA kits and the conditions employed during testing may help to improve the extraction efficiency of processed food proteins [31].

Conclusions

The present research showed that ELISA (using both kits tested) and flow cytometry were accurate in detecting allergens in uncooked samples. For both methods, recoveries were, however, very low for thermally processed samples, especially those processed at very high temperatures. Moreover, the results obtained using flow cytometry were very similar to those obtained by ELISA which makes flow cytometry a promising, time-saving method for the detection of multiple allergens in a food matrix. The study further provides evidence that thermal processing may affect the detection of allergens and their quantification. This information is important for kit manufacturers, food industry and policy makers and enforcers who are interested in the monitoring of allergenic substances in processed foods. It further illustrates the challenge of ensuring that thermally processed foods are accurately labelled in order to minimize potential risks to allergenic consumers. Ongoing research to improve methods for the detection of allergens in processed food samples continue to be needed. For both ELISA and flow cytometry, development of appropriate buffers that maximise the solubilisation of processed allergenic proteins will be useful.

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