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Survey of undeclared soy allergen levels in the most frequently recalled food categories with or without precautionary labelling

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ABSTRACT

A comprehensive study was designed to determine the frequency and levels of soy allergen in packaged bakery and snack food products. A representative sample of products with no soy allergen disclosed on the label was analysed using two widely used enzyme-linked immunosorbent assay (ELISA) methods. Samples were chosen that either had no soy identified on the product label or which had a soy precautionary statement. Among 558 bakery and snack products, soy protein was detected in 17% of the products using the Neogen (NE) kit and 11% of the products using the Elisa Systems (ES) kit. The disagreement rates between kits were 8.8% for bakery products and 3.3% for snack products. Overall soy protein was detected at higher frequency in bakery products than in snack foods. Among 284 bakery samples, soy protein was detected in 25% of the samples with no precautionary statement and 19% of the samples which had a precautionary statement. Among 274 snack samples, soy protein was detected in 11% of the samples with no precautionary statement and 9% of the samples which had a precautionary statement. The sample repeatability was at an acceptable level (< 9%) for each method and food commodity. The reproducibility between kits was 23% for bakery foods and 36% for snack foods. None of the bakery (21) and snack (6) products without precautionary labelling (measured level > 5 ppm) had a higher level of soy protein per serving compared with the eliciting dose₁₀ (ED₁₀) of 10.6 mg for soy allergic patients. But the level of soy protein per serving may be clinically relevant to a subpopulation of soy allergic patients if a more stringent eliciting dose is applied. These findings emphasise that suitable detection methodologies and references doses are crucial for labelling accuracy and the safety of soy allergic consumers.

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Introduction

Food allergen control practices, including proper labelling and industry preventive controls, are necessary to ensure the safety of allergic consumers. Most developed and developing countries have implemented laws that mandate the labelling of major food allergens when used as ingredients in packaged foods. Newly adopted legislation in European countries also mandates allergen disclosure on non-prepacked foods from bakeries, butchers and catering outlets (Remington et al. 2013a; Allen et al. 2014a). In the US, the 2004 Food Allergen Labeling and Consumer Protection Act (FALCPA) requires that manufactures declare eight foods or food groups (egg, milk, peanut, soy, fish, crustacean shellfish, tree nuts and wheat) when used as ingredients

in foods regulated by the USFDA. Voluntary precautionary labelling has increasingly appeared on food labels due to concerns by food manufacturers over the unintentional presence of trace amounts of allergens in foods and difficulty in defining a clinical threshold.

Despite the best efforts of regulatory agencies and food manufacturers, the incidence and severity of food allergic reactions have been increasing (Gupta et al. 2011). Additionally, since the implementation of FALCPA, there has been a trend over time of increasing numbers of allergen-related recalls by the USFDA (Gendel & Zhu 2013). A total of 43.4% of US food product recalls from 2009–14 were related to undeclared allergens (see <http://www.fda.gov/downloads/Food/ComplianceEnforcement/RFR/UCM502117>).

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htm), and the majority of these recalls were due to poor label control (67%) or failures in industry control practices, including cross-contact. Cross-contact, the unintentional introduction of a food allergen into a food, can happen at many points during the manufacturing process and include the use of shared equipment, inadequate cleaning or the use of mislabelled raw ingredients during production. Cross-contact can also occur due to the comingling of raw agriculture products during the harvest, storage and transport phases of food production. Soy is often comingled in raw agriculture products such as wheat and corn (Remington, Taylor, et al. 2013).

Prior survey studies have been conducted to determine the presence of allergen residues in food products and whether these correlate with the presence of precautionary labelling on the package. In one study, only 6.5% of products with precautionary labelling actually contained any level of the indicated allergenic ingredient (Food Safety Authority of Ireland 2011). Pele et al. (2007) showed that a high percentage of chocolate and cookie products with precautionary labelling had detectable levels of peanut and hazelnut. But in other studies, the vast majority of food products with precautionary allergen statements did not have detectable levels of the specified allergen (Hefle et al. 2007; Zurzolo et al. 2013; Remington, Baumert, et al. 2013a). Lot-to-lot variation was also observed in food products with precautionary labelling (Robertson et al. 2013). In all studies it was clear that whether a product tested positive for a regulated food allergen depended on the food category and the allergen under investigation. Results of these types of studies strengthen the scepticism that some allergic consumers have in the accuracy of product labels. The food products that pose the most risk to allergic patients are, of course, those that contain detectable levels of a regulated allergen but which have not declared that allergen or provided a precautionary statement on the package label.

The use of soy in food industries has expanded enormously due to its high protein content and desirable physiochemical properties (Koppelman et al. 2004). A significant number of food allergen recalls are related to soy and, based on USFDA recall data, are often distributed in bakery and snack food commodities (Gendel & Zhu 2013). The current study surveyed soy allergen in a large number (558) and variety of bakery and snack packaged foods with or without precautionary labelling, providing a snapshot of the risk among products that lack precautionary labelling compared with those with precautionary

labelling. Previous studies reported that the selected ELISA methods performed well in terms of sensitivity, specificity, cross-reactivity and food matrix interference (L'Hocine et al. 2007; Platteau et al. 2011; Scharf et al. 2013; Remington, Baumert, et al. 2013a).

The amount of the product consumed and the eliciting dose (ED) of allergen for a particular individual are a more reliable measurement of the associated risk to an allergic consumer than the actual amount of allergen present in food (Allen et al. 2014a). Special attention was paid to foods without declared soy on the label to assess the risk to allergic consumers when choosing these products. The most effective strategy for preventing allergic reactions from products that may contain soy allergen is strict dietary avoidance. However, due to the ubiquitous nature of food products with precautionary labelling and the wide usage of soy in processed foods, soy-allergic consumers have limited food choices (Allen et al. 2014b). The findings from our study will further the understanding of the prevalence of soy in packaged foods with or without precautionary labelling and the limitations of existing detection methodologies for soy allergen.

Materials and methods

Packaged bakery and snack samples

A convenience sample of 558 packaged bakery and snack foods with or without precautionary labelling regarding soy were purchased from seven local grocery stores in Maryland. The samples selected were food products with no soy disclosed as an intended ingredient on the label. Bakery products included products that were fresh, refrigerated or frozen. Many food products were ready to eat or needed some further preparation, such as baking. Snack products were mostly prepackaged and shelf stable. About 27% ($n = 151$) of total products had precautionary labelling indicating the possible presence of soy allergen traces. Precautionary labelling was found on 73 out of 284 (26%) bakery products and in 78 out of 274 (28%) snack products. Several different precautionary statements were identified on the labels: may contain, may be present, processed in a facility that also processes, made on equipment with, etc. The food categories of bakery and snacks were chosen following the classification and definitions used in the USFDA (2014) Reportable Food Registry (RFR) report (see <http://www.fda.gov/downloads/Food/ComplianceEnforcement/RFR/UCM502117.htm>). Purchased bakery products were divided into five major categories and snacks into seven. The food categories and number of products tested in each category are indicated

Table 1. Food categories used for sample selection.

Food commodity	Food category	Food type/product description
Bakery (284)	Bread (171)	Dough/fresh and frozen breads, bagels, rolls, buns, croissant, biscuits/tortillas/wraps/naan/flats/pockets/pita/mix/crusts
	Pastry (16)	Pies/puffs/twists/scones
	Cookie (46)	Cookies/biscuits/thins/short bread/crisps
	Cake (18)	Cakes/brownie/muffins/mix
	Other (33)	Bread sticks/wafers/toasts/lady fingers/bread chips
Snack (274)	Chips (100)	Potato /grain /veggie/corn/rice/tortilla/pita/plantain/bean
	Pretzels (17)	Flavoured/originals/nuggets/rods/twists/thins
	Crackers (72)	Originals/flavoured/grain/bread/grahams/rice/potato/novelty
	Bar (10)	Granola/energy/fruit
	Trail mix (10)	Traditional/flavoured/nut mixed
	Popcorn (21)	Original/flavoured
	Other (44)	Curls/mixed nuts/sticks/rings/puffs/crisps/snack foods/novelty

Note: Number of products tested in each category are indicated in parentheses.

in Table 1. Bread constituted a greater proportion of the bakery samples because it was more widely available and chips and crackers comprised the major fractions of the purchased snack products. All products were analysed for the presence of undeclared soy protein using two commercial ELISA assay kits. The results were then compared with the information on the food label related to a soy precautionary statement.

Sample preparation

A representative amount of sample from a single package was homogenised by grinding using a blender (LB10, Waring Laboratory). Pulverized dry ice was added to the grinding chamber for samples containing nuts, chocolate, high levels of fat and other challenging ingredients. Substantial efforts were made to produce homogeneous mixtures and samples were stored at -20°C prior to further use.

Soy analysis by ELISA

The two commercial test kits used in this study were (1) Veratox soy allergen quantitative test kits from Neogen NE Corp. (Lansing, MI, USA) (product 8410) with an LOQ of 2.5 ppm ($\mu\text{g g}^{-1}$) soy flour (equivalent to 1.18 ppm soy protein); and (2) ES soy protein residue kits (BioMerieux, Durham, NC, USA) (product code ESSOYPRD-48) with an LOQ of 2.5 ppm soy protein. The characteristics of the ES and NE test kits are listed in Table A1 in the supplemental data online. Two subsamples were measured (5 g each for NE; 1 g each for ES) and extracted according to the kit instructions for each sample. For each subsample, two aliquots were used for soy allergen quantitation (four total measures per sample). The results were quantitated using a Spectramax M5 ELISA plate reader equipped with Softmax Pro 6.1 software (Molecular Devices Corp., Sunnyvale, CA, USA). Standard curves were

created using the manufacturer's recommended curve fit, or, if none was recommended, by using a four-parameter logistic calibration curve

Statistical analysis

All data analysis was performed using the SAS 9.2 (r) (SAS Institute Inc., Cary, NC, USA) PROC SUMMARY and PROC GCHART procedures; *p*-values were obtained from the Cochran–Mantel–Haenszel statistics (Agresti, 2002). Repeatability is described by the relative SD under repeatability conditions. Repeatability SD was measured as the average SD of the measurements made on the replicates of the same subsamples by the same kit. Repeatability was calculated by dividing the repeatability SD by the mean. Reproducibility SD was measured as the SD of the kit averages calculated within sample. Reproducibility was calculated by dividing the reproducibility SD by the mean. The reproducibility and repeatability of $\leq 25\%$ was considered as acceptable (Hougs et al. 2011; Luber et al. 2014). The calculation of the disagreement rate included the number of food samples that tested positive by one or both kits irrespective of quantitated soy protein level.

Results

Soy protein quantitation in bakery and snack products

The two ELISA methods used for our study are different with respect to extraction procedures, calibration material, LOQ, antibody specificity and reporting units (L'Hocine et al. 2007; Scharf et al. 2013; Remington, Baumert, et al. 2013a; Gomaa & Boye 2015). The ES and NE methods express results as parts per million (ppm) of soy protein and soy flour, respectively. For direct comparison between the two kit results, the soy

Table 2. Results of soy protein analysis of bakery products using the ES kit (LOQ = 2.5 ppm) and NE kit (LOQ = 1.18 ppm).

No precautionary labelling						Precautionary labelling							
NE						NE							
		< LOQ	LOQ–5 ppm	5–20 ppm	> 20 ppm	Total ES results			< LOQ	LOQ–5 ppm	5–20 ppm	> 20 ppm	Total ES results
ES	< LOQ	159	19	1	0	179	ES	< LOQ	59	4	0	0	63
	LOQ–5 ppm	0	12	4	0	16		LOQ–5 ppm	0	2	2	0	4
	5–20 ppm	1	1	9	2	13		5–20 ppm	0	0	5	0	5
	> 20 ppm	0	0	1	2	3		> 20 ppm	0	0	1	0	1
Total NE results		160	32	15	4	211	Total NE results		59	6	8	0	73

Note: Results are categorised into four range levels based on quantitated soy protein levels. Results for the NE kit are presented in columns; results for the ES kits are presented in rows. Products shaded in grey indicate agreement between results of both test kits. The total number of tested products are in bold.

levels measured with the NE kit were calculated as soy protein by multiplying results by 47.01%, per the kit instructions. The LOQs of the ES (2.5 ppm soy protein) and NE (1.18 ppm soy protein) kits were used as parameters for analysis of the data obtained from purchased food samples.

When analysing the presence of allergen traces in foods with or without precautionary statements, the presence of detectable allergen does not always correlate with the package labels. (Pele et al. 2007; Crotty & Taylor 2010; Ford et al. 2010; Spanjersberg et al. 2010; Robertson et al. 2013; Zurzolo et al. 2013; Remington, Baumert, et al. 2013a). Soy allergen in processed foods has not been surveyed on a large scale even though it was the third most recalled allergen from 2007 to 2012, responsible for 153 recalls of USFDA-regulated foods (Gendel & Zhu 2013). Therefore, food products without soy disclosed on the ingredient list and with no precautionary statement were of particular interest in this study.

The products testing positive for soy protein ($\geq$ LOQ) were divided into three detection ranges, based on the quantitated levels of soy protein using the ELISA kits: LOQ–5 ppm, 5–20 ppm and > 20 ppm. Not all products tested positive for soy protein with both kits. Table 2 summarises the frequency and quantitated levels of soy protein found in bakery products with or without soy precautionary labelling. A total of 159 (75%) of the 211 bakery items with no precautionary labelling and 59 out of 73 (81%) bakery items with precautionary labelling were negative ($< \text{LOQ}$) for soy protein with both kits. Among all bakery products, soy protein was detected in 10 products by the ES kit and in 14 products by the NE kit with precautionary labelling. Among products without precautionary labelling, soy protein was detected in 32 products by the ES kit and 51 products by the NE kit. Of the bakery products with no precautionary labelling, 16 tested positive with the ES kit and 32 with the NE kit at the LOQ–5 ppm soy protein level. Six products with precautionary

labelling (four by the ES kit and six by the NE kit) also tested positive for soy protein at the LOQ–5 ppm level. The NE kit was found to be more sensitive than the ES kit since soy protein was detected in a greater number of bakery products in the LOQ–5 ppm (lowest) detection range. An additive in the extraction buffer of the NE kit may have enhanced the extraction and detection of modified soy proteins in these processed foods samples. Among products with no precautionary labelling, 13 tested positive for soy protein when using the ES kit and 15 were positive with the NE kit in the 5–20 ppm range. Among the products with precautionary labelling five tested positive in this range using the ES kit and eight when using the NE kit. In the > 20 ppm range, there were a total of five bakery products without precautionary labelling (three by the ES kit and four by the NE kit) and one with precautionary labelling (one by the ES kit and none by the NE kit) which tested positive for soy protein. The LOQs of the test kits affected the classification of the detection range of 23 bakery products in which soy protein was detected at very low levels. However, there were minimal differences between actual measured amounts in these products using the different kits. Nineteen bakery products without precautionary labelling and four bakery products with precautionary labelling tested negative ($< \text{LOQ}$) with the ES kit but their detected soy protein level fell in the LOQ–5 ppm range using the NE kit.

Table 3 shows results of the soy protein analysis of snack products. Of the 196 snack products with no precautionary labelling and 78 products with precautionary labelling, 175 (89%) and 71 (91%) products were negative for soy protein, respectively, when tested with both ELISA kits. A total of seven (seven by both ES and NE kits) and 21 (12 by the ES kit and 21 by the NE kit) snack products tested positive for soy protein in products with and without precautionary labelling, respectively. In the LOQ–5 ppm range, soy protein was detected in a greater number of snack products (20)

Table 3. Results of soy protein analysis of snack products using the ES kit (LOQ = 2.5 ppm) and NE kit (LOQ = 1.18 ppm).

No precautionary labelling						Precautionary labelling					
NE						NE					
		<LOQ	LOQ–5 ppm	5–20 ppm	> 20 ppm			<LOQ	LOQ–5 ppm	5–20 ppm	> 20 ppm
		Total ES results						Total ES results			
ES	< LOQ	175	9	0	0	ES	< LOQ	71	0	0	0
	LOQ–5 ppm	0	5	0	0		LOQ–5 ppm	0	3	0	0
	5–20 ppm	0	1	5	1		5–20 ppm	0	2	1	1
	> 20 ppm	0	0	0	0		> 20 ppm	0	0	0	0
	Total NE results	175	15	5	1		Total NE results	71	5	1	1

Note: Results are categorised into four range levels based on quantitated soy protein levels. Results for the NE kit are presented in columns; results for the ES kits are presented in rows. Products shaded in grey indicate agreement between results of both test kits. The total number of tested products are in bold.

using the NE kit than with the ES kit (eight). Among snack products with no precautionary labelling, seven tested positive with the ES kit and five with the NE kit in the 5–20 ppm soy protein range. Among products with precautionary labelling, four tested positive for soy protein in the 5–20 ppm range using the ES kit, and one when using the NE kit. In the > 20 ppm range, there was one snack product without precautionary labelling (none by the ES kit and one by the NE kit) and one with precautionary labelling (none by the ES kit and one by the NE kit) which tested positive for soy protein. The quantitated soy protein levels of a few ($n = 5$) snack products varied between test kits. Nine snack products without precautionary labelling tested negative (< LOQ) by ES but in the LOQ–5 ppm range using the NE kit. The NE and ES kit-specific parameters (antibody specificity, sample size, extraction buffer, LOQ and serial calibrators used for creating the standard curve) most likely contribute to the differences in kit results.

Overall, 11% of bakery and snack products had detectable levels of soy protein when using the ES kit, and 17% when using the NE kit. Soy protein was found to be present in about 13% ($n = 73$) of bakery and snack products with-

out precautionary labelling indicating the potential risk for an adverse reaction, depending on the sensitivity of the soy-allergic consumer. The significant number of products containing soy protein residue could be explained by the frequent comingling of soy with wheat or other raw agricultural commodities during harvest, transport and storage. A recent market survey found 1.6–236 ppm undeclared soy protein in 62.8% of commercial wheat flour products (Remington, Taylor, et al. 2013b).

Performance of ELISA test kits

Detection of soy residues in bakery and snack products with or without precautionary labelling

Although highly sensitive ELISA methods have been developed for detection and quantitation of soy allergens in food, their performance characteristics (recovery rates, LOQ, repeatability, reproducibility, influence of food matrix, etc.) can be greatly altered by food processing and assay-specific parameters (L'Hocine et al. 2007; Scharf et al. 2013; Morishita et al. 2014; Gomaa & Boye 2015). Figure 1 (a) displays percentages of soy positive bakery and snack samples, irrespective of quantified soy protein level. When examining aggregated results of both kits, soy protein was

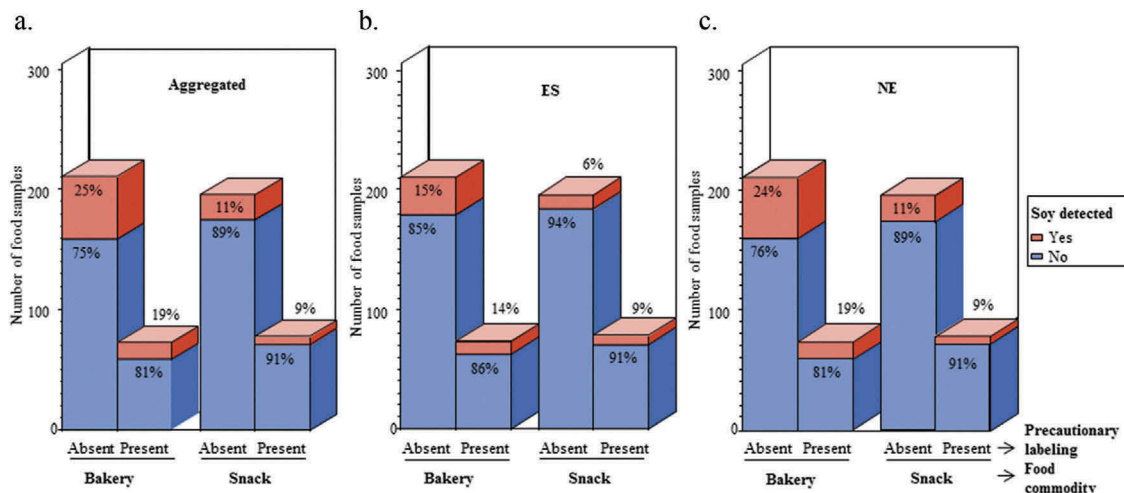


Figure 1. Relative percentage of bakery and snack products with or without a precautionary labelling (soy not declared as an ingredient) yielding positive and negative results for soy residue by test kit.

detected in 25% of bakery products without precautionary labelling, 19% of bakery products with precautionary labelling, 11% of snack products without precautionary labelling and 9% of snack products with precautionary labelling. The ES kit detected soy protein in 15% of bakery products without precautionary labelling, 14% of bakery products with precautionary labelling, 6% of snack products without precautionary labelling and 9% with precautionary labelling (Figure 1(b)). Soy protein was detected in 24% of bakery products without precautionary labelling, 19% of bakery products with precautionary labelling, 11% of snack products without precautionary labelling and 9% of snack products with precautionary labelling when using the NE kit (Figure 1(c)). The soy protein detection rate using NE kit was similar to the combined kit results (cf. Figures 1(a, c)) because the NE kit detected soy protein in more products than the ES kit.

The numbers of soy protein-positive products for different categories of bakery and snack products for each kit are illustrated in Figure 2. There was no significant difference ($p = 0.1$) observed for per cent positive samples across individual categories of bakery commodities (Figure 2(a)). When comparing the number of bakery samples testing positive for soy allergen, there was a significant difference between kits

($p = 0.01$). The same was not true for the snack products. There was no significant difference ($p = 0.15$) between results of ELISA kits when comparing the individual snack food categories. However, a significant difference ($p < 0.0001$) was found when comparing results between individual categories of snacks, regardless of the test kit utilised. Crackers exhibited the highest number of positive snack samples for soy protein (18% by the ES kit and 26% by the NE kit), followed by pretzels (12% by ES, 14% by NE). The remaining food categories had 7% or less soy protein-positive products.

Analytical variation

Subsample repeatability within a kit and reproducibility between kits were calculated as performance characteristics of the test kits for this study. The calculated subsample repeatability revealed that it was in an acceptable level of $\leq 25\%$ (Luber et al. 2014). The repeatability of bakery products was 8.64% for the ES kit and 8.93% for the NE kit. For snack products, the repeatability was 4.90% for the ES kit and 6.34% for the NE kit (Table 4). Reproducibility represents the difference in the measurements of soy protein in a positive food sample between the two ELISA kits. Reproducibility was only calculated for those products that tested positive with both test kits. The reproducibility

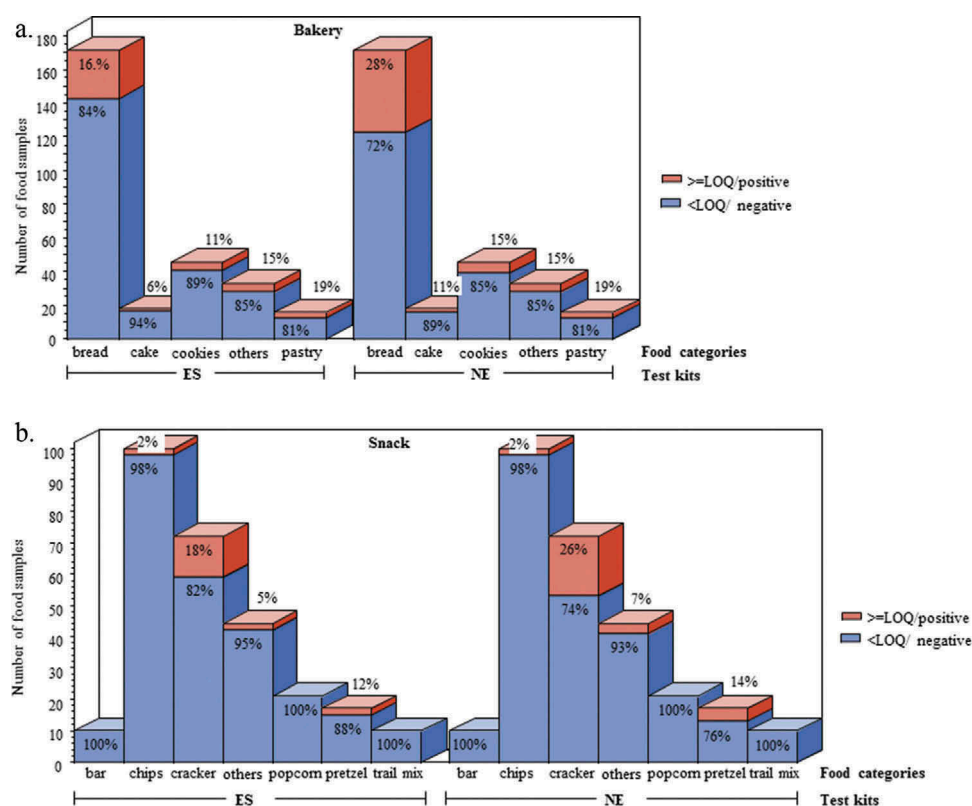


Figure 2. (a) The relative percentage of samples testing positive for soy was significantly different between test kits ($p = 0.01$) but was not significantly ($p = 0.1$) different across food categories when testing a variety of bakery products. (b) The number of snack samples testing positive for soy was not significantly different between test kits ($p = 0.15$) but was significantly different across the food categories ($p < 0.0001$) of snack products.

Table 4. Performance of the test kits used for the quantification of soy protein from bakery and snack products.

Food commodities	Repeatability between subsamples within kit (%) ^a		Reproducibility between kits (%) ^b	Disagreement rate (%)
	ES	NE		
Bakery	8.64	8.93	22.61	8.8
Snack	4.9	6.34	35.59	3.3

Note: ^aRepeatability standard deviation/mean; ^bReproducibility standard deviation/mean

was lower for bakery products (22.61%) compared with snacks (35.59%). This could be because the quantitated levels of soy protein in most of the snack products were at the lowest detection range (LOQ=5 ppm), where greater method variability is expected. The disagreement rate between kits was 8.8% for bakery products and 3.3% for snack products.

Risk associated with levels of soy protein detected (> 5 ppm) in bakery and snack products per suggested serving size

To protect allergic consumers, ED₀₁, ED₀₅, ED₁₀ (eliciting/provoking doses which trigger objective symptoms in 1%, 5% and 10% of the allergic population, respectively), minimal eliciting doses (MED), and reference doses (amount of allergic protein that would provide a defined level of protection) have been established using dose-distribution modelling of data from clinical challenges (Taylor et al. 2014; Allen et al. 2014a, 2014b; Zhu et al. 2015). Based on these models, action levels for precautionary labelling by food manufacturers have been proposed. These are the levels of allergen above which precautionary labelling on manufactured products would be recommended due to the high risk of unavoidable cross-contact. For those products with a low risk, the use of a precautionary label would be discouraged, thereby greatly increasing food choices for allergic consumers. The Allergen Bureau of Australia & New Zealand created such a programme in 2007 called the Voluntary Incidental Trace Allergen Labeling (VITAL) (Taylor et al. 2014). To increase the confidence level of allergic consumers in food product labelling, VITAL 2.0 has established recommended precautionary labelling action levels for 11 food allergens, based on ED₀₁ or the 95% lower confidence interval of the ED₀₅, depending on the availability of statistically significant clinical datasets (Taylor et al. 2014; Allen et al. 2014b). The recommended VITAL Reference Dose for soy protein is 1.0 mg (Taylor et al. 2014).

Zhu et al. (2015) estimated the ED₁₀ for soy allergic patients to be 10.6 mg soy protein using the Weibull distribution model. Klein-Entink et al. (2014) used log-normal, log-logistic and Weibull dose-distribution models and a slightly larger dataset for the estimation of soy threshold distributions. In this study, the Weibull threshold distribution model, the most conservative, estimated a reference ED₁₀ of 12 mg of soy protein. It is worth emphasising, however, that EDs for soy allergic individuals are based on limited patient challenge data (Klein-Entink et al. 2014; Allen et al. 2014b; Zhu et al. 2015).

The risk to consumers with food allergies is that they may consume products with undeclared allergens or ignore precautionary labelling. The allergen levels per serving in these foods may reach levels that could induce an allergic response (Spanjersberg et al. 2010). It was important to consider the potential clinical relevance of soy protein levels in the purchased bakery and snack products. Based on serving size, exposure levels from the consumption of 29 bakery and 10 snack products with soy protein > 5 ppm were determined (Table 5). Products where soy protein levels were < 5 ppm were not included because the soy protein levels per serving of these products were considered too low to induce an allergic reaction, based on available threshold data. Of the 29 bakery products, 21 did not have precautionary labelling. Of the 10 snack products, six did not have precautionary labelling. Based on the exposure per serving, all these products had quantitated levels of soy protein below the ED₁₀ of 10.6 mg, as modelled by Zhu et al. (2015). While larger patient datasets would provide better estimates of soy threshold distributions, several of the products in Table 5 could potentially pose a threat to a more sensitive soy allergic subpopulation, particularly if larger amounts of product than the indicated serving size were consumed. However, in examining USFDA recall data from 2005 to 2010, no recalls were prompted by consumer reactions to undeclared soy (Malyukova et al. 2012).

Conclusions

This was one of the largest known studies to address the frequency of soy allergen in bakery and snack foods. Significant numbers of bakery and snack products tested positive for soy protein where there was no declaration of a soy ingredient on the food label. A number of products with precautionary labelling also contained soy protein. The soy content per serving of some of products could potentially pose a risk of allergic reaction, depending on the sensitivity level of soy allergic consumer. It is conceivable that the high number of products testing positive for soy may be due to co-mingling of soy in the wheat ingredients used in many bakery and snack supermarket products. The

Table 5. Exposure levels from the consumption of products containing soy allergen (> 5 ppm). None of the bakery and snack products with or without precautionary labelling had higher levels of soy protein per serving than the ED₁₀ (10.6 mg)^a for soy allergic patients. Products with no precautionary labelling that have exposure levels exceeding the VITAL 2.0 Reference Dose of 1.0 mg protein (95% lower confidence interval of the ED₀₅)^b with one or both kits are shaded.

Food samples	ES (soy protein): LOQ 2.5 (mg g ⁻¹)		NE (soy protein): LOQ 1.18 (mg g ⁻¹)		Precautionary labelling
	Average measured concentration (µg g ⁻¹)	Exposure per serving (mg)	Average measured concentration (µg g ⁻¹)	Exposure per serving (mg)	
Bakery 1	3.65	0.19	5.24	0.28	no
Bakery 2	23	0.64	15.09	0.42	no
Bakery 3	2.18	0.06	5.17	0.15	no
Bakery 4	13.17	0.54	8.98	0.37	no
Bakery 5	17.68	1.3	11.17	0.8	no
Bakery 6	37.5	2.63	60.93	4.27	no
Bakery 7	11.68	0.51	14.59	0.64	no
Bakery 8	44.5	1.51	45.92	1.56	no
Bakery 9	17.16	0.48	21.67	0.61	no
Bakery 10	14.72	0.9	11.19	0.68	no
Bakery 11	9.14	0.32	<LOQ	<LOQ	no
Bakery 12	4.15	0.24	5.7	0.28	no
Bakery 13	18.51	0.52	22.98	0.64	no
Bakery 14	4.32	0.5	6.37	0.8	no
Bakery 15	6.01	0.26	3.9	0.2	no
Bakery 16	13.81	0.79	18.27	1.04	no
Bakery 17	7.63	0.2	8.65	0.22	no
Bakery 18	4.94	0.18	8.58	0.32	no
Bakery 19	6.67	0.34	6.31	0.32	no
Bakery 20	9.73	0.27	6.84	0.18	no
Bakery 21	6.96	0.2	8.58	0.24	no
Snack 1	12.2	0.37	22.34	0.67	no
Snack 2	14.43	0.45	12.3	0.38	no
Snack 3	12.94	0.39	15.16	0.45	no
Snack 4	7.44	0.21	4.59	0.13	no
Snack 5	8.32	0.13	5.47	0.09	no
Snack 6	9.52	0.29	8.32	0.25	no
Bakery 22	18.24	0.57	8.53	0.26	yes
Bakery 23	13.47	1	12.7	0.9	yes
Bakery 24	4.06	0.16	5.43	0.21	yes
Bakery 25	11.08	1.1	12.2	1.2	yes
Bakery 26	10.27	0.32	14.35	0.54	yes
Bakery 27	6.69	0.44	7.34	0.48	yes
Bakery 28	42.57	1.51	19.42	0.7	yes
Bakery 29	4.28	0.15	9.48	0.34	yes
Snack 7	5.14	0.15	2.61	0.08	yes
Snack 8	6.37	0.22	20.43	0.58	yes
Snack 9	7.53	0.14	12.22	0.22	yes
Snack 10	6.9	0.2	3.43	0.1	yes

Sources: ^aZhu et al. (2015); ^bTaylor et al. (2014).

data found in this study will add to the understanding of labelling compliance with respect to soy and the importance of accurate detection methods for food allergens.

Disclosure statement

No potential conflict of interest was reported by the authors. The opinions and conclusions expressed in this article are solely the views of the authors and do not necessarily reflect those of the US Food and Drug Administration (USFDA).

References

- Agresti A. 2002. Categorical data analysis. 2nd ed. New York: Wiley.
- Allen KJ, Turner PJ, Pawankar R, Taylor S, Sicherer S, Lack G, Rosario N, Ebisawa M, Wong G, Mills EN, et al. 2014a. Precautionary labelling of foods for allergen content: are we ready for a global framework? World Allergy Organ J. 7:10.
- Allen KJ, Remington BC, Baumert JL, Crevel RW, Houben GF, Brooke-Taylor S, Kruizinga AG, Taylor SL. 2014b. Allergen reference doses for precautionary labeling (VITAL 2.0): clinical implications. J Allergy Clin Immunol. 133:156–164.
- Crotty MP, Taylor SL. 2010. Risks associated with foods having advisory milk labeling. J Allergy Clin Immunol. 125:935–937.
- [FSAI] Food Safety Authority of Ireland. 2011 June. Food Allergens & Labelling Survey. Available from: www.fsai.ie/resources_publications/allergen_labelling_2011. https://www.fsai.ie/resources_publications.html
- Ford LS, Taylor SL, Robert Pacenza BA, Niemann LM, Debra M, Lambrecht BS, Sicherer SH. 2010. Food allergen advisory labeling and product contamination with egg, milk, and peanut. J Allergy Clin Immunol. 126:384–385.
- Gendel SM, Zhu J. 2013. Analysis of U.S. food and drug administration food allergen recalls after implementation of the food allergen labeling and consumer protection act. J Food Prot. 76:1933–1938.

- Gomaa A, Boye J. 2015. Simultaneous detection of multi-allergens in an incurred food matrix using ELISA, multiplex flow cytometry and liquid chromatography mass spectrometry (LC-MS). *Food Chem.* 175:585–592.
- Gupta RS, Springston EE, Warrier MR, Smith B, Kumar R, Pongracic J, Holl JL. 2011. The prevalence, severity, and distribution of childhood food allergy in the United States. *Pediatrics.* 128:9–17.
- Hefle SL, Furlong TJ, Niemann L, Lemon-Mule H, Sicherer S, Taylor SL. 2007. Consumer attitudes and risks associated with packaged foods having advisory labeling regarding the presence of peanuts. *J Allergy Clin Immunol.* 120:171–176.
- Hougs L, Zel J, Charles-Delobel C, Burns M, Charels D, Ciabatti I. 2011. Verification of analytical methods for GMO testing when implementing interlaboratory validated methods. ENGL working group on “Method Verification” of the Joint Research Centre of the European Commission. Luxembourg: JRC Scientific and Technical Reports. Publications Office of the European Union.
- Klein Entink RH, Remington BC, Blom WM, Rubingh CM, Kruizinga AG, Baumert JL, Taylor SL, Houben GF. 2014. Food allergy population thresholds: an evaluation of the number of oral food challenges and dosing schemes on the accuracy of threshold dose distribution modeling. *Food Chem Toxicol.* 70:134–143.
- Koppelman SJ, Lakemond CM, Vlooswijk R, Hefle SL. 2004. Detection of soy proteins in processed foods: literature overview and new experimental work. *J AOAC Int.* 87:1398–1407.
- L'Hocine L, Boye JI, Munyana C. 2007. Detection and quantification of soy allergens in food: study of two commercial enzyme-linked immunosorbent assays. *J Food Sci.* 72:145–153.
- Luber F, Demmel A, Herbert D, Hosken A, Hupfer C, Huber I, Busch U, Engel KH. 2014. Comparative assessment of DNA-based approaches for the quantification of food allergens. *Food Chem.* 2014:104–111.
- Malyukova I, Gendel SM, Luccioli S. 2012. Milk is the predominant undeclared allergen in US food product recalls. *J Allergy Clin Immunol.* 129:S234.
- Morishita N, Matsumoto T, Morimatsu F, Toyoda M. 2014. Detection of soybean proteins in fermented soybean products by using heating extraction. *J Food Sci.* 79:1049–1054.
- Pele M, Brohée M, Anklam E, Van Hengel AJ. 2007. Peanut and hazelnut traces in cookies and chocolates: relationship between analytical results and declaration of food allergens on product labels. *Food Add Contam.* 24:1334–1344.
- 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 Addit Contam Part A Chem Anal Control Expo Risk Assess.* 28:127–135.
- Remington BC, Baumert JL, Marx DB, Taylor SL. 2013. Quantitative risk assessment of foods containing peanut advisory labeling. *Food Chem Toxicol.* 62:179–187.
- Remington BC, Taylor SL, Marx DB, Petersen BJ, Baumert JL. 2013. Soy in wheat–contamination levels and food allergy risk assessment. *Food Chem Toxicol.* 62:485–491.
- Robertson ON, Hourihane JO, Remington BC, Baumert JL, Taylor SL. 2013. Survey of peanut levels in selected Irish food products bearing peanut allergen advisory labels. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 30:1467–1472.
- Scharf A, Kasel U, Wichmann G, Besler M. 2013. Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (*Glycine max* L.) and wheat gluten (*Triticum aestivum* L.). *J Agric Food Chem.* 61:10261–10272.
- Spanjersberg MQ, Knulst AC, Kruizinga AG, Van Duijn G, Houben GF. 2010. Concentrations of undeclared allergens in food products can reach levels that are relevant for public health. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 27:169–174.
- Taylor SL, Baumert JL, Kruizinga AG, Remington BC, Crevel RW, Brooke-Taylor S, Allen KJ, Allergen Bureau of Australia & New Zealand, Houben G. 2014. Establishment of Reference Doses for residues of allergenic foods: report of the VITAL Expert Panel. *Food Chem Toxicol.* 63:9–17.
- US Food and Drug Administration (USFDA). 2014. The Reportable Food Registry: targeting inspection resources and identifying patterns of adulteration. Available from: <http://www.fda.gov/downloads/Food/ComplianceEnforcement/RFR/UCM502117.htm>
- Zurzolo GA, Koplin JJ, Mathai ML, Taylor SL, Tey D, Allen KJ. 2013. Foods with precautionary allergen labeling in Australia rarely contain detectable allergen. *J Allergy Clin Immunol Pract.* 1:401–403.
- Zhu J, Pouillot R, Kwegyir-Afful EK, Luccioli S, Gendel SM. 2015. A retrospective analysis of allergic reaction severities and minimal eliciting doses for peanut, milk, egg, and soy oral food challenges. *Food Chem Toxicol.* 80:92–100.