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Short communication

Hidden peanut allergens detected in various foods: findings and legal measures

Background: Undeclared allergens in foodstuffs represent a major health problem for sensitized persons. Until recently, most food control authorities were not in the position to monitor hidden allergens and to take legal measures against their presence in foodstuffs.
Methods: In this study, we employed human sera-based immunoassay techniques, enabling semiquantitative detection and identification of peanut allergens in a variety of foodstuffs.
Results: This study showed the presence of undeclared allergens in products belonging to various food categories, such as cereals, cookies, cakes, and snacks. The detection limit for peanut contamination was in most instances less than 50 mg peanut material per kg, i.e., less than about 5 mg peanut allergens per kg. We legally objected to products with more than one part per thousand or 1000 mg/kg of peanut contamination.
Conclusions: In most cases, food producers, confronted with our results, were able to detect and eliminate the sources of the contamination. They implemented measures to prevent the presence of hidden peanut allergens in their products, increasing food safety for sensitized persons and overall food quality.

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In industrialized countries, food allergies represent an important health problem. Food allergies affect up to 2% of the adult population and up to 8% of children (1). In a sensitized individual, intake of even minute amounts of allergens can provoke gastrointestinal, respiratory, and skin symptoms, and can even lead to severe systemic reactions (anaphylaxis) that are potentially fatal (2–4). Over the past years, an increase in food allergen-induced life-threatening syndromes was observed in hospital admissions (5). Although there are some promising advances in the immunotherapy of food allergies, the only reliable and effective way to prevent symptoms consists of strict allergen avoidance (6).

Sensitized persons, urged to avoid certain allergens, are highly dependent on reliable product labels and on the availability of foods not containing the respective allergens as contaminants (7). In Switzerland, potentially allergenic foodstuffs used as ingredients have to be declared, except for ingredients of compounds not exceeding 25% of composed foods. Yet, many producers in Switzerland voluntarily also declare those ingredients. Still, a wide range of products is suspected or even reported to contain undeclared allergens and thus to cause adverse reactions (8–10). Those allergens are either deliberately used as ingredients or are present as contaminants. Consequently, the detection of hidden allergens in foodstuffs and taking measures against their presence are novel and important tasks for the food industry and the food control authorities.

The legal action of food control authorities against contaminants in foods is based on the concept of concentration limits. Yet, this concept is not easily applicable to food allergens since there are considerable inter- and even intraindividual variations in sensitivity to allergens (11). Thus, as far as allergenic contaminants are concerned, there are no legal concentration limits to date.

Because of their extraordinary significance, we chose peanut allergens as pilot allergens in this study (12). Peanut (*Arachis hypogaea* L.) kernels contain on average 25% (23–27%) proteins (13), of which approximately 20% represent the major allergen Ara h 1 (vicilin) with a molecular mass of 63.5 kDa, and approximately 10% represent Ara h 2 (conglutin-like protein) with a molecular mass of 17 kDa (14, 15). Other relevant major allergens contained in peanuts are Ara h 3 (16) and Ara h 4 (17). Those major allergens are seed-storage proteins, well characterized concerning primary structure and major IgE-binding epitopes, and they are recognized by more than 50% of peanut-allergic patients.

In this study, we aimed to implement a reliable and sensitive allergen-monitoring system that can be rou-

tinely employed in a food control authority setting, and to test a range of products for undeclared peanut allergens. On the one hand, we considered clinical data and food production aspects on the other hand, and we defined an action level of one part per thousand or 1000 mg/kg of undeclared peanut material in the examined products. This action level represented a reference point in order to enable legal measures. On this basis, in cooperation with the food producers, we aimed to take measures to avoid henceforth the presence of hidden food allergens.

Material and methods

Sera

Sera were provided by the allergy unit from patients with a history of severe allergic reactions to peanuts. The selected patients showed a high level of sensitization to peanut allergen with a positive CAP result of at least class 4 (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) and no significant reactivity to other food allergens. Both single and pooled sera were used in the assays without significant changes to the results.

Extraction of allergens from foodstuffs

Food samples were mashed in 6 M urea buffer and stored at 4°C overnight. The samples were then centrifuged (10 min at 6000 rpm), and aliquots of the supernatants were stored at −20°C until analysis. To reduce high fat content, the separated fat portion was removed from previously frozen samples. Standards were produced accordingly, using commercially available roasted peanuts at a concentration of 0.5 g/ml.

Dot blots

Volumes of 4 µl of samples, standards, and negative samples (urea buffer and a set of various food allergens) were dotted on nitrocellulose membrane (NCM) (Protran BA84, Schleicher & Schuell, Riehen, Germany) and developed as described earlier (18). Briefly, NCM were incubated in phosphate-buffered saline (PBS) containing 10% (w/v) skimmed milk powder for 1 h. After this, NCM were washed once in 0.1% Tween 20 in PBS (TPBS) and twice in PBS only, 5 min each, with gentle horizontal shaking. The blots were then incubated for 1 h with sera (diluted 1:4 with 0.5% bovine serum albumin [BSA] in PBS) from patients with documented peanut allergy. After washing with TPBS and PBS as above, bound IgE was detected with goat IgG specific for the ε-chain of human IgE and labeled with peroxidase (SIGMA) by incubation for 1 h. The blots were washed with TPBS and PBS as above and developed in chromogenic 4-chloro-1-naphthol (SIGMA) substrate in methanol. Quantification was performed by comparing the intensity of the sample dot with the dot intensities of the standard dilution series that were included in each individual assay. The detection limit of this test system was, depending on serum strength and matrix, approximately 50 mg peanut material per kg foodstuff.

SDS–PAGE

Food extract samples, standards, and negative samples in Premixed Laemmli Sample Buffer (BioRad), were separated each on two identical 10% homogeneous gels with 4% stacking gels. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of the two identical gels was carried out on a Mini-PROTEAN 3 Cell/Mini Trans-Blot Module (BioRad) at constant 200 V, according to Laemmli (19). Immediately after electrophoresis, the separated proteins on one of the gels were transferred on the Mini-PROTEAN 3 Cell/Mini Trans-Blot Module (BioRad) at constant 100 V to NCM (20). The other identical gel was developed in Coomassie brilliant blue solution (BioRad) to stain the protein bands. The molecular masses of the protein bands were calculated in relation to prestained broad range protein standards (Bio-Rad).

Immunoblotting

Blotted membranes of SDS–PAGE gels were incubated with patient sera and peroxidase-labeled goat IgG specific for the ε-chain of human IgE and developed as described above.

Results

To date, a total of 61 potentially problematic products with and without declared peanut contents was examined. Hidden peanut allergens were detected in a variety of foodstuffs, especially cereals, cereal bars, cookies, and various types of snacks. Of 46 products without declared peanuts, 27 did indeed not contain peanut allergens. Nineteen products were shown to contain hidden peanut allergens (Table 1).

Detected peanut contamination ranged between 50 mg/kg and 1% of the product. The detection limit for peanut contamination was in most instances less than 50 mg/kg, depending on the reactivity of the employed serum and matrix examined. Remarkably, many products with hidden peanut allergens were produced by a manufacturer who also produces a related product with declared peanuts (Table 2). For example, variety 2 of the cereal bar from company A contained hidden peanut allergens, as confirmed with two different lots (lot a and lot b). Lot c was indeed peanut-free, as was the examined variety 3. A related cereal bar (variety 1, "with nuts and peanuts") contained a total of 46% nuts and peanuts. Of the 19 peanut-allergen positive products, eight were shown to contain peanut amounts above the action level of one part per thousand and were legally objected to.

Discussion

In industrialized countries, food allergies represent a health problem of considerable and yet increasing relevance. There are promising, but still preliminary perspectives in prophylactic and therapeutic treatment of allergies (21–23). To date, the only effective method to prevent adverse reactions to food allergens is strict allergen avoidance, requiring correct and integral declaration of products and the availability of products not inadvertently contaminated with allergens (24). As, to date, this is not completely provided, hidden allergens in foodstuffs represent a serious health hazard for sensitized persons (3). Consequently, detection of undeclared allergens in foodstuffs is a novel and important task for food control authorities.

In this study, we set up a reliable and sensitive test

Table 1. Product categories with detected declared and hidden peanut material

Product category	Number of samples examined	Containing declared peanuts	Containing hidden peanuts
Cereals	5	2	2
Cereal bars	5	2	2
Cookies	10	0	4
Cakes	6	0	0
Snacks	26	4	11

system that can be routinely employed for food control purposes. This system yielded plausible, consistent, and reproducible results. Our methods are based on antibodies from patients with well-defined allergy. This means that, in comparison with mono- or polyclonal animal antibody-based methods (see Ref. 25), our methods may be limited by the availability of sera from correctly diagnosed patients. In addition, it might be difficult to standardize completely the method, and certain analytical measures are necessary to achieve satisfactory specificity. However, the use of human sera is most interesting as it enables an analytical approach that is very representative of the mechanisms in the human organism and thus potentially provides answers to questions that might not be answerable by mono- or polyclonal animal antibody-based methods. Since the protein composition of different species of roasted peanuts processed by the food industry is quite comparable (15), we could choose one commercially available cultivar as a representative standard for roasted peanuts of a variety of species.

The results of the peanut allergen monitoring showed that in certain product categories (cereals, cereal bars, cookies, and various types of snacks), there is a remarkable quantity of products with hidden allergens. Of 46 selected samples, 19 were shown to contain undeclared peanut material. As mentioned, many products with hidden peanut allergens have related products with declared peanuts.

To be able to take legal measures based on the data from this study, we had to determine an action limit for undeclared peanut. A ''zero-tolerance'' for hidden allergens did not seem to be realistic on a short-term perspective. We defined an action level of one part per thousand of undeclared peanut contamination as avoidable in manufacturing processes. One part per thousand peanut means 1 g undeclared peanut or 0.25 g peanut protein (13), corresponding to approximately 0.1 g peanut allergen (14, 15), respectively, per kilogram of the supposedly peanut-free, ready-to-eat product. Hourihane et al. (26) performed a double-blind, placebo-controlled, food-challenge (DBPCFC) study and determined a threshold dose for objective symptoms caused by peanut allergens of 2 mg in adults. Consequently, a sensitized person could consume 20 g of a product with peanut content as high as the action level applied in this study without showing objective allergic symptoms (Fig. 1). Yet, subjective symptoms could occur at lower doses of the product (26). In a further DBPCFC study performed with 125 subjects, 5% of the test persons reacted to 1 mg of peanut allergen, and 36% reacted to a dose below 100 mg (27). Consequently, the 5% most sensitive persons would react to 10 g of a product with peanut content as high as the action level applied in this study.

Eight samples were objected to because they contained amounts above the action level of one part per thousand peanut. In most cases, food producers confronted with our results were able to detect the sources of the contamination. In detail, the reasons for cross-contamination of peanut-free products were as follows:

- contaminated raw materials
- common transport containers for peanuts and other foods

Table 2. Examples of samples with and without declared peanut components; n.d. = <50 mg peanut material/kg food or <0.005%

Product (variety, lot)	Company	Declaration (labeling)	Peanut content	Detected allergens
Cereal bar, variety 1	A	With 46% nuts and peanuts	>20%	Ara h 1, 2, 3
Cereal bar, variety 2, Lot a	A	No peanut components	0.2–0.5%	Ara h 1, 2, 3
Cereal bar, variety 2, Lot b	A	No peanut components	0.05–0.1%	Ara h 1, 2, 3
Cereal bar, variety 2, Lot c	A	No peanut components	n.d.	–
Cereal bar, variety 3	A	No peanut components	n.d.	–
Energy cereal bar, variety 1	B	With peanuts	10–20%	Ara h 1, 2, 3, 4
Energy cereal bar, variety 2	B	No peanut components	0.2–0.5%	Ara h 1, 2, 3, 4
Corn crackers (snacks), variety 1	C	With peanuts	10–20%	Ara h 1, 2
Corn crackers (snacks), variety 2	C	No peanut components	0.2–0.5%	Ara h 1, 2
Peanut snacks	D	With peanuts (30%)	>20%	Ara h 1, 2, 3, 4
Potato snacks	D	No peanut components	0.2–0.5%	Ara h 1, 2

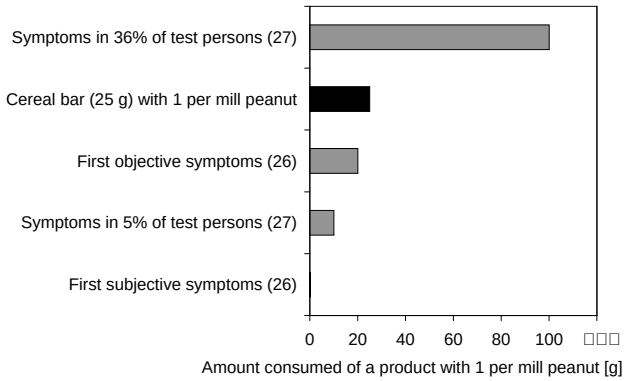

Figure 1. Amounts consumed of product with 1 per mill peanut and described related effects of two DBPCFC studies (26, 27); included as example is cereal bar (25 g) with 1 per mill peanut.

- lack of separate production lines and equipment for peanut-containing and peanut-free foods
- processing of peanut-free products immediately after peanut-containing products
- unsafe rework-management
- insufficient cleaning steps.

According to their respective problems, the food producers implemented measures to prevent the presence of hidden peanut allergens in their products, such as the following:

- auditing of installations and manufacturing practices of providers of raw materials
- use of certified raw materials
- use of separate and thoroughly cleaned transport containers
- separation of production lines
- sensible rework-management
- additional cleaning steps
- scheduling peanut-containing foods to be last in a production series.

With good manufacturing practice, the action level applied in this study should be within reach of the producers. Precautionary labeling should not be used as a substitute for effective measures to prevent the presence of hidden allergens. If too widely used, labels reading ''may contain traces of peanut'' are not helpful information for consumers, since they create confusion rather than provide guidance about which foods are safe for allergic individuals.

The data on hidden peanut allergens presented in this study and the legal measures taken on the basis of the action level cited in this report enabled food industries to detect problematic foodstuffs, to determine the causes of peanut allergen contamination, and to improve the situation. In the near future, investigations will be expanded to cover other hidden food allergens. The action level presented in this study will be reviewed for the respective allergen and accordingly applied to enable legal measures against the presence of undeclared allergens. Modifications in production and logistics implemented by the food industry will provide an increase in food safety for sensitized persons and in overall food quality.

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