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Original Research Article

Peanut traces in packaged food products consumed by allergic individuals: Results of the MIRABEL project

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ABSTRACT

In the frame of the French research project MIRABEL, 899 food samples which contained no peanut ingredients according to the labeling list were analyzed for the presence of peanut allergen traces. Samples covered a broad range of products from ten major food categories. In a stepwise procedure, samples were screened using a sensitive lateral flow assay with a limit of detection of 2 ppm total peanut or 0.5 ppm peanut protein. Positive as well as suspect samples (139/899) were confirmed by real-time PCR with the same sensitivity. Positives in both approaches were quantified by two different commercial ELISA tests. 1% (9/899) out of all samples did contain measurable peanut DNA and protein traces above the detection limit of the applied methods. Six samples had a content of total peanut protein <5 mg/kg, two samples contained between 8 and 10 mg/kg and one sample a maximum of about 20 mg/kg. An excellent correlation was found between Ct-values obtained by PCR and ppm peanut calculated by ELISA. It is concluded that, in the light of future thresholds for labeling of relevant allergens, the methods used for peanut detection in this study are able to detect contaminations as low as 2 ppm.

1. Introduction

Peanut (*Arachis hypogaea*) is one of the fourteen major food allergen species that have to be labeled when used as ingredients in the recipes of packaged and non-prepackaged foods, according to the European legislation (European Directive 1169/2011/EC). Peanut is, like soybean, a member of the leguminous plant family and may elicit severe reactions in sensitized persons. It has long been known that peanut consumption is a frequent cause of lethal anaphylactic reactions (Yunginger et al., 1988; Sampson et al., 1992).

The reported prevalence of allergies against peanut depends on the country and on the age of patients under study, but it is roughly estimated to be about 0.5–1.1% in the US population and also in some European countries (EFSA, 2004). Among food allergens, peanut is associated with the highest prevalence, which is estimated to be 0.3–0.75% of the French population (Morisset et al., 2005). It appears that children are more frequently and increasingly affected than adults (Grundy et al., 2002; Hourihane, 2011). Combining the observations of Rancé et al. (2005) and Moneret-Vautrin (2008), peanut allergy prevalence in France is estimated to be 0.3% in adults aged 18 to 79 years, but 0.6% of children aged 3 to 17 years. Children are particularly endangered since peanut may be hidden in chocolate, snacks and biscuits. Once manifested, peanut allergy tends to persist for the whole lifetime (Husain and Schwartz, 2012). Peanut allergic adults and in particular parents of sensitized children are confronted with the problem of carefully avoiding any unintended contact with a potentially life-threatening allergen. A continuously updated epidemiological literature survey spanning from 1994 until present is published on the 'allergome' database website (www.allergome.org).

In the frame of the French research project MIRABEL (Crépet et al., 2015) led by the French Agency for Food, Environmental and

Abbreviations: CTAB, cetyltrimethyl ammonium bromide; COV, coefficient of variation; DNA, desoxyribonucleic acid; ED, effective dose; ELISA, enzyme-linked immunosorbent assay; LFA, lateral flow assay; LOAEL, lowest observed adverse effect level; LOD, level of detection; PCR, polymerase chain reaction; SD, standard deviation.

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Occupational Health & Safety (ANSES), about nine hundred food samples not labeled for peanut as an ingredient were collected from the French market and investigated for the presence of peanut traces. Aims of the project were (i) to evaluate the consumption behavior and attitude respecting product price and labeling of peanut allergic patients, (ii) to analyze the real market situation regarding the presence and amount of unintended peanut traces for labeled (with precautionary labels related to allergen traces on package such as "may contain traces of peanut") and unlabeled products, (iii) to assess and quantify the all over risk against the background of analytical results, medical data and individual consumption data, based on a Bayesian statistical model, and (iv) finally to derive a cost-/benefit analysis and management strategies from all data (Crépet et al., 2015).

The investigation of 899 market samples for the presence of peanut traces, which is presented in this work, was an essential core part of the integrated framework MIRABEL, addressing aims (ii) and (iii) of the MIRABEL project outlined above. To meet the real life situation, products were not taken randomly but ranked into different categories and subcategories considering their labeling types (labeled/unlabeled) to reflect consumption habits of peanut allergic patients, as derived from the MIRABEL consumption survey. In order to optimize the sample plan to monitor allergen traces in products consumed by allergic patients, a Bayesian network was developed and applied in MIRABEL project (Elegbede et al., 2015). Samples were allocated to ten groups of food: breakfast cereals; cereal bars; bread and bakery products; appetizer products; pizzas; cream desserts, mousse or fresh desserts; biscuits and pastry; chocolate bars or chocolate spread; other chocolate products; and ice cream and sorbets. These ten major food categories were further divided into subcategories according to their ingredients and flavouring (supplementary information, Table A). Moreover, the brands of the collected products were the major ones consumed by the allergic patients in the MIRABEL consumption survey.

A cascade of methods was applied for efficient analysis of the high number of samples. In the first step, all samples were screened for the presence of peanut with a sensitive and rapid immunological lateral flow assay (LFA). Secondly, positive as well as ambiguous and suspect samples were confirmed using a real-time PCR method with the same sensitivity as the LFA. Finally peanut traces were quantified with two different commercial ELISA (enzyme-linked immunosorbent assay) kits.

2. Material and methods

2.1. Food samples

Between January and March 2013, 899 food samples were collected on the French market according to a previously developed and recently published model (Elegbede et al., 2015). The full sample list including the number of investigated lots is presented in the supplementary information (supplementary information, Table A). Due to the sample collection some slight differences appear between the optimized number of samples provided by the Elegbede et al. (2015) model and the number of samples actually collected: the recommended number of 900 samples was reduced by one item (one appetizer product 'Curly Fromage'), because peanut was indicated as a regular ingredient on the product label. Additionally, some products were not well classified into the right categories by the patients and were thereafter reallocated to the appropriate category. That is why, for the categories 'cream desserts, mousse or fresh desserts' and 'ice creams and sorbets', larger differences can be observed between the optimized number of samples as provided by the model and the number of samples actually collected. According to Elegbede et al. (2015) the optimal sample size for the category 'cream desserts, etc.' would have been 53 (versus 30 in this study) and for the category 'ice cream and sorbets' 37 (versus 50 in this study).

All samples were immediately stored at −20 °C until analysis. Samples were given a unique sample code, the status of allergen labeling, a product- and subcategory code, and a unique lot number. The 899 samples consisted of individual lots of different brands belonging to 10 major product groups further divided into subcategories (supplementary information, Table A). From each of the 899 individual lots a minimum of three packages were collected to form at least 300 g of product. 633 (∼70%) samples were not particularly labeled for peanut and 266 samples (∼30%) were labeled for peanut traces.

2.2. Reference material

Reference material (dark chocolate spiked with 0 and 2 ppm roasted peanut) was derived from the national research project 'Development of rapid tests and screening methods for on-site detection of food allergens in product development and control' (grant no. 132-281 6400508; German Ministry of Food and Agriculture, BMEL) and produced by IfP (Institute for Product Quality, Berlin, Germany).

2.3. Homogenization

One third of each individual package of different weight was taken (e.g. 3 1/3 chocolate bars out of 10 chocolate bars in one package, or 170 g of a 500 g package of breakfast cereals) to yield ≥300 g and all subsamples per lot were pooled for homogenization. Dry and coarse samples (e.g. cereals, biscuits, appetizers) were crushed and homogenized by mixing for at least 2 min at 2000 rpm in a Grindomix GM300 laboratory mill (Retsch, Haan, Germany). More or less soft samples (e.g. cake) or those that were covered by ingredients of greasy texture (e.g. pizza) were first shock-frozen in liquid nitrogen and subsequently processed by milling. This procedure was also applied to chocolate bars (e.g. with caramel), other chocolate products and ice cream with tree nut particles. Chocolate tablets without visible particles, chocolate spread and ice cream were melted in a water bath (45 °C) and then homogenized by agitating. Cacao powder was mixed for at least two minutes in a three-dimensional shaker (TURBULA, Bachofen AG, Muttenz, Switzerland). 2× 100 mL or g of each homogenate was filled into two separate sample vials. The A-sample was directly analyzed, while a B-sample was retained at −20 °C for repeated analysis or cross-check.

2.4. Lateral flow assay (LFA)

All food samples were screened for peanut traces using a peanut lateral flow immunoassay kit (ImmunoFast, No. IF1002, IfP, Berlin) according to the manufacturer's instructions. The limit of detection (LOD) of the test is specified as 2 ppm total peanut. The rapid stripe test includes an extraction buffer and is developed within 5 min. Incubation times were kept equal in all measurements. Two independent extractions from 200 mg or μL of homogenized sample were prepared. The optical density of the developed tests was monitored with a lateral flow immunoassay reader (OPTricon, Berlin, Germany) and calibrated against the reference materials. Samples yielding positive or suspect scores at least in one of two independent measurements were further investigated by real-time polymerase chain reaction (PCR). In addition, 5% of the double-negative samples in the LFA were randomly selected and also subjected to real-time PCR analysis.