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Research Article

Peach Fruit as Allergen in Food: A Survey for Allergic Consumer Protection in Northern Italy

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Abstract

Food allergy is an immune-mediated reaction to foods: dietary avoidance and food allergen labelling are the main strategies for ensuring allergen consumer safety. In the present study evaluated the performance of a real-time PCR commercial test and we assessed the presence of undeclared peach fruit in food products. A sampling plan was set considering an expected prevalence around 2%, precision was fixed at 5%. The test was confirmed to be able to detect traces of peach DNA (0.0003 ng/µL) in the food matrix. Among the 32 food samples, six ice cream samples (6/32; 18.8%) resulted positive for the presence of hidden peach fruit. We observed that about 18% of food samples were contaminated by peach fruit as an undeclared allergen, far higher than the average of 2-3% reported for various food products sampled and contaminated by different kinds of food allergens. None of the pre-packaged food products tested positive for peach traces, probably due to greater attention to the monitoring plan, the own-check procedures, and the management of the ingredients. In contrast, all the samples that tested positive came from three different cafés or ice-cream shops. Our results stress the importance of raising awareness among food business operators and allergic consumers to report cases of allergic reactions and the risk of contamination by different food products.

Keyword: Food Allergens; Peach Fruit; Consumer Protection

Introduction

Food allergy is an immune-mediated reaction to foods; it manifests with symptoms of variable severity and duration in different organs and systems. The prevalence of food allergies is estimated to be 3% for Europe, the United States, and Australia-New Zealand, and about 1% for Europe alone (EFSA 2014). Young people are more often affected than adults, with a prevalence of up to 8% in children (Worm et al. 2010).

At present, dietary avoidance and food allergen labelling are the main strategies for ensuring allergen consumer safety. European legislation requires the provision of allergen information on food labels (European Parliament, 2011), and currently identifies 14 declarable allergens: cereals containing gluten, crustaceans, molluscs, eggs, fish, peanuts, tree nuts, soybeans, milk, celery, mustard, sesame, lupin, and sulphur dioxide at levels above 10 mg/kg, or 10 mg/liter, expressed as SO2.

Most allergic reactions in children worldwide are due to exposure to five foods: egg, peanut, cow's milk, fish and various nuts; whereas in adults, nearly 50% of allergic reactions are due to fruits of the Rosaceae family, to which also peach fruit tree belongs (EFSA 2014). Peach allergy is the main cause of vegetable food allergy in the Mediterranean area. Despite its frequency in causing allergy, peach fruit is not currently listed as an allergenic ingredient in Europe. The allergen Pru p 3, a peach lipid transfer protein, has been well studied: it is recognized as the major allergen, and it is mainly found in the peel (Boyano-Martínez et al., 2013). Nonspecific lipid transfer pro-

teins are extremely stable, structurally highly conserved plant defense proteins, present throughout the whole plant kingdom (Fernandez-Rivaz, 2009)

Published survey data on the presence of undeclared or hidden allergens in foods are scarce. Techniques currently available for identifying food allergens are based on detection of either protein(s) or nucleic acids. The ELISA assay is the most frequently employed analytical tool to detect allergenic proteins, and it has been recognized as the gold standard method. ELISA is commonly used for qualitative or quantitative allergenic protein detection with the advantages of high specificity, high sensitivity, and rapid processing (Cheng et al 2013). Detection methods targeting nucleic acids are another approach in food allergen detection. Although several food allergens are not suitable for PCR analysis (e.g. eggs and milk), conventional PCR and quantitative real-time PCR techniques can be used for other foods, such as nuts, peanuts and fish or crustaceans. In addition, several DNA-based methods for the detection of traces of allergenic compounds in complex food matrices through real-time PCR have been published, mostly based on the use of labeled probes (Garino et al., 2016).

What data are available usually come from case studies of foodborne allergies or anaphylactic shock after exposure to an allergen. To fill this gap, we assessed the presence of undeclared peach fruit in food products. The aim of the study was to investigate the analytical characteristics of a laboratory method to detect peach DNA as an allergenic protein marker. In addition, a survey was conducted to determine the presence of peach as ingredient or as contaminant in food products for which the label did not declare peach among the ingredients.

Materials and Method

Sampling Plan

A sampling plan was set considering an expected prevalence around 2% (Bianchi et al., 2016); precision was fixed at 5%. Food samples were collected at a retail market between May and September 2016. According to this sampling plan, a total of 32 food samples were necessary to be analyzed in order to obtain statistically significant results. The 32 food products to be included in the sampling plan were equally divided into four food categories, all belonging to the commodities and delicatessens group. Based on our experience with and knowledge of food processing plants and food recipes, food products were included if considered to be likely subject to peach fruit contamination during processing or if a similar product or recipe with peach listed as ingredient is known to be commercially available on the market by the same brand. The food categories were fruit jams and marmalades, ice fruit-teas, fruit juices, and ice creams. At the moment of collection the whole selling packaging were sampled: the entirety of the packs was checked, the samples were purchased and then stored at the temperature appropriate for product type. Finally samples were delivered to the Food Safety Control Department of the Istituto Zooprofilattico Sperimentale Del Piemonte, Liguria e Valle d'Aosta, Turin, Italy, which is the Regional Centre for Food Intolerance and Allergies (CREALIA). Analyses were performed within 10 working days from sample collection and before the expiry date of the sample. To ensure high homogeneity and allergen dispersion in the sample, the entire packaging unit was blended and brought to opportune weight for analyses.

Test Performance Evaluation

Food samples were analyzed for the presence of peach fruit as allergen using the PEACHKIT real-time PCR (Incura, Codogno Lodi, Italy). The test detects peach DNA through a real-time PCR assay; the kit includes all reagents required for the detection of DNA of *Prunus persica*. According to the kit manufacturer, the test's limit of detection (LOD) is expressed as 0.28 pg of *Prunus persica* DNA in 100 ng of reaction. In order to evaluate the intralaboratory LOD of the test and to determine inclusivity and exclusivity of the kit, spiked samples were prepared *ad hoc* by personnel not involved in subsequent parts of the study.

A peach fruit (*Prunus persica* var. *Percoca*) was bought at the local market. The peel and core were removed, and the pulp was minced with a blender to obtain a fluid of pure peach. DNA was extracted from the pulp using Ion Force DNA Extraction FAST (Generon, Modena. Italy) according to the kit manufacturer's instructions. DNA concentration was calculated by spectrophotometry and reported as 2.47 ng/μL.

A pear fruit (*Pyrus communis*) purée was purchased at the local market and analyzed: extraction was performed using Ion Force DNA Extraction FAST (Generon); the PEACHKIT real-time PCR (Incura) was used to check for the absence of peach traces. The pear purée was spiked with minced pure peach to obtain theoretical peach concentrations of 0.3 ng/μL (L1), 0.03 ng/μL (L2), 0.003 ng/μL (L3), 0.0015 ng/μL (L4), and 0.0003 ng/μL (L5).

In order to determine the exclusivity of the kit, samples of apple (*Malus domestica*), pear (*Pyrus communis*), apricot (*Prunus armeniaca*), and banana (*Musa acuminata*) was purchased at the local market. A portion of 10 g was separately blended and then extracted and analyzed according to the kit manufacturer's instructions. In order to determine the inclusivity of the test, four different varieties of peach fruit were purchased: *Prunus persica* var *Percoca*, *Prunus persica* var *Sbergiu*, *Prunus persica* var *Platycarpa*, and *Prunus persica* var *Springcrest*. A portion of 10 g of each fruit was separately blended and then extracted and analyzed according to the kit manufacturer's instructions.

Sample Preparation and Analysis

All 32 samples' DNA was extracted in duplicate using Ion Force DNA Extraction FAST (Generon, Modena, Italy) according to the kit manufacturer's instructions.

Real time PCR was conducted on 64 DNA samples using the using the PEACHKIT real-time PCR (Incura, Codogno Lodi, Italy). In each test tube, 5 µL of extracted DNA (5 to 25 ng) and 20 µL of mix test peach were added; negative control and a positive control tubes were prepared adding to the mix test peach, 5 µL of water and 5 µL DNA positive control (provided by the kit), respectively.

In each inhibition tube, 5 µL of extracted DNA (5 to 25 ng) and 20 µL of mix test inhibition were added; an inhibition positive control tube was prepared mixing 20 µL of mix test inhibition to 5 µL of water.

To perform analysis Applied Biosystems® 7500 Real-Time PCR Systems (Waltham, MA USA) was used: the reaction was as follow: 95 °C for 5 minutes; 95 °c for 30 seconds and 50 °C for 1 minute for a total of 40 cycles. In order to analyse the results the positive control samples is supposed to have a Ct ranging from 19 to 21 cycles. Food sampled was considered positive when Ct was lower than 35 cycles.

Results and Discussion

Test Performance Evaluation

Each of the five spiked sample sets was analyzed in triplicate with the peach DNA detection kit. The presence of peach DNA was correctly identified in all spiked samples, with a threshold cycle (Ct) ranging between 26 and 32. The test was able to detect traces of peach DNA (0.0003 ng/µL) in the food matrix. Analyses to confirm inclusivity gave good results: all samples were tested in triplicate and the kit correctly identified the presence of peach DNA (at a concentration of 0.003 ng/µL), with a Ct ranging between 26 and 28. Samples for exclusivity were tested in triplicate and consistently gave negative results at the same DNA concentration.

Food Samples Results

According to our results, among the 32 food samples, twenty-six samples (8 tea, 8 jams, 8 juices and 2 ice-cream samples) did not contain peach DNA; on the other side, six ice-cream samples (6/32; 18.8%) resulted positive for the presence of hidden peach fruit. The Ct threshold cycle of the six positive samples ranged between 23 and 32, and the Ct of the positive control sample was 23. Two ice cream samples and all the other food samples (26/32; 81.2%) resulted negative for the presence of peach traces. Table 1 reports the food categories for the sampling and the results on peach detection.

Food Category	N. of samples	N. of positive samples
Tea	8	0
Jam and marmalade	8	0
Fruit juices	8	0
Ice cream	8	6 (75%)
Total food samples	**32**	**6 (18.8%)**

Table 1. List of the food categories included in the sampling plan to detect undeclared peach fruit traces. The second column reports the number of positive samples for each category.

This survey focused on peach fruit as a hidden food allergen. In order to determine the presence of peach in food products where it was not included as an ingredient, we evaluated an analytical test to detect peach in traces. There are very few commercially available ready-to-use tests for detecting allergens not included in the list of compulsory ingredients. At the time of this survey, the only kit available on the European market was a real-time PCR test that comprised the reagents needed for the analyses, except for the plastics and consumables and the real-time machine. We found that the test performance for intralaboratory LOD, inclusivity, and exclusivity matched that declared by the manufacturer.

We observed that about 18% of food samples were contaminated by peach fruit as an undeclared allergen, far higher than the average of 2-3% reported for various food products sampled and contaminated by different kinds of food allergens (Bianchi et al., 2016; Decastelli et al., 2012). To explain the difference in prevalence rates in the present study, the two different kinds of sampling sites and food sales need to be distinguished. None of the pre-packaged food products tested positive for peach traces probably due to greater attention to the monitoring plan, the own-check procedures, and the management of the ingredients or the production lines operated in the food processing plants. In contrast, all the samples that tested positive came from three different cafés or ice-cream shops. In all cases, the ice cream was made in a room adjacent to the point of sale and served over the counter by the same personnel.

Conclusion

Since peach fruit is not included in the list of allergenic ingredients on food labels, no official analyses are conducted by competent food safety authorities and food business operators are not required to set own-check plans to verify peach fruit contamination. Nevertheless, the present findings are important for two reasons: first, they reveal gaps in the management of production processes in small food plants and signal that criticalities due to contamination with other allergens could be caught by own-check plans. Second, they stress the impor-

tance of raising awareness among food business operators and allergic consumers to report cases of allergic reactions and the risk of contamination by different food products. In fact, the percentage of peach IgE sensitization in consumers has been reported to be as high as 7.9% (BURNEY et al, 2014). In our study, we choose to check for the presence of peach DNA as peach marker. We didn't check for the presence of PruP3, the peach lipid transfer protein recognized to be the major allergen nor we performed its quantification: for this reasons it is not possible to suppose the amount of peach in the positive samples nor to evaluate the real risk for potential allergic consumers. This is the main concern for the most part of allergenic ingredients as the Eliciting Doses at the moment, are not recognized to have legal significance and even very small amount of allergens' traces are considered unsafe; furthermore as no official test methods are indicated in the regulatory contest all kinds of validated and accreditated analytical methods can be used in the frame of official control.

According to international labelling legislation, peach fruit is only included on the Japanese and Korean lists of foods for which labelling is recommended but not required (Gendel, 2012). This is probably because expert consultation (JECFA, 2000) suggests three criteria for selecting foods to be labelled (cause-effects relationship, prevalence, and systemic reaction). While peanut and other treenuts allergy are usually associated with severe and systemic reactions, fruit is associated with milder symptoms and only occasionally have anaphylactic or systemic reactions been reported (Le et al., 2008). At the moment of writing, peach fruit is not included in the European list of food allergens to be declared and food business operators are not required to indicate the presence of peach traces in their food products.

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