



Detection of peanut allergens in retail food items in Serbia (2021-2024)

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ABSTRACT

Food allergies are a significant and growing public health concern globally, impacting millions of individuals and placing immense pressure on healthcare systems and personal well-being. Peanuts are one of the 14 fundamental food allergens, making them potentially harmful. As a result, the goal of this mini review is to assess the prevalence of peanuts in commercial food products in Serbia between 2021 and 2024. The current study summarizes the qualitative detection of specific DNA sequences using real-time PCR techniques on 212 commercial food goods, including frozen desserts, biscuits and related products, cream products, snacks, and sausages. Undeclared peanut allergen DNA occurred across different food categories, with positive results observed in frozen desserts (n=3), biscuits (n=4), and snacks (n=2). Peanut allergen DNA was detected in six samples with a precautionary allergen label (PAL) statement, while in the case of three samples in which peanut allergen DNA was detected, the presence of the allergen was not stated at all.

1. Introduction

Food Allergy is a growing global public health concern. The presence of undeclared allergenic ingredients or the presence of traces of allergens due to contamination during food processing poses a great health risk to sensitised individuals (Janković *et al.*, 2024).

These disorders are identified by unfavourable immune responses to specific food proteins and can cause a wide range of symptoms, from slight irritation of the skin and intestinal problems to severe, life-threatening anaphylactic reactions. The growing frequency of food allergies, particularly in industri-

alized nations, highlights the crucial need for effective prevention, diagnostic, and management techniques (Sicherer & Sampson, 2018).

Peanut allergy is a significant public health concern, affecting a substantial portion of the population worldwide, with prevalence rates varying across different regions. It is characterized by an adverse immune response to proteins found in peanuts, which can manifest in a range of symptoms from mild reactions like hives and itching to severe, life-threatening anaphylaxis. Unlike some other food allergies, peanut allergy often persists throughout life, necessitating strict avoidance and careful management (Food Allergy Research & Education, 2024).

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The allergic reaction is primarily mediated by immunoglobulin E (IgE) antibodies, which recognize specific peanut proteins, known as allergens. These allergens, such as Ara h 1, Ara h 2, and Ara h 3, are encoded by specific genes within the peanut genome (Palladino and Breiteneder, 2018). Upon exposure, IgE binds to these proteins, triggering a cascade of immune responses that lead to the symptoms of an allergic reaction.

Peanut allergy prevalence varies considerably across Europe. Studies have shown rates ranging from 0.2% to 7.7% in different European populations (Spolidoro et al., 2023). Data on peanut allergy prevalence in Serbia is more limited, but available research suggests it is within the range observed in other European countries (Davidovic et al., 2022).

2. Materials and methods

This study investigated the qualitative presence of peanut (*Arachis hypogaea*) DNA in a range of commercial food products available in Belgrade, Serbia, between 2021 and 2024. A total of 212 food samples were analysed, encompassing five categories: frozen desserts (n=44), biscuits and related products (n=73), cream products (n=25), snacks (n=63), and sausages (n=7). These samples were randomly selected from various retail outlets, including supermarkets and local markets, within the Belgrade area.

2.1 Sample collection and preparation

Commercial food goods belonging to the specified categories were purchased between January 2021 and December 2024. Upon collection, samples were labelled and stored according to the manufacturer's instructions until processing.

2.2 DNA extraction

All selected products were analysed using validated methods for food allergen peanut. Analysis was carried out according to the SRPS EN ISO 15634-4:2023. The DNA from product samples was extracted using protocol with cetyltrimethylammonium bromide – CTAB. The DNA was quantified by spectrophotometry using a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The applied CTAB extraction method resulted in DNA preparations with an OD₂₆₀ nm/OD₂₈₀

nm ratio of 1.8 to 2.0 in all samples, indicating the high quality of the extracted DNA.

2.3 Real-Time PCR for qualitative detection of peanut DNA

The pure DNA preparations were used as templates for amplifying, identifying, and qualitative detection using real-time PCR. Real-time PCR assays were performed with Aria MX (Agilent Technologies). The program used an initial denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 15 s and primer annealing and elongation at 60°C for 1 min. The qualitative detection of peanut-specific DNA sequences was performed using real-time PCR, Aria MX. Real-time PCR was performed to detect a specific DNA sequence from the Ara h 2 gene of peanut.

3. Discussion

The findings of this study, which involved the qualitative screening of 212 commercial food products from Belgrade, Serbia, between 2021 and 2024, revealed the presence of peanut DNA in nine products across the tested categories. Notably, the detection of peanut DNA in these nine products suggest the presence of undeclared peanut material, as the study focused on identifying its presence irrespective of labelling information. Undeclared peanut allergen DNA across different food categories was detected in frozen desserts (n=3), biscuits (n=4), and snacks (n=2). Peanut allergen DNA was detected in six products with a precautionary allergen label (PAL) statement, while in the case of three products in which this peanut DNA was detected, the potential presence of the allergen was not stated at all.

Comparing our findings to other studies investigating undeclared allergens in food products is crucial for contextualization. While the prevalence of undeclared peanut DNA in our sample set (9 out of 212, approximately 4.2%) appears relatively low, it still represents a tangible risk to allergic consumers in Belgrade. Studies from other regions have reported varying rates of undeclared allergens, influenced by factors such as food regulations, manufacturing practices, and the specific food categories analysed (Allen et al., 2014, Sprickelman et al., 2016, EFSA, 2014; Sampath et al., 2021). In Europe, the prevalence of undeclared allergens has been a concern, with studies highlighting issues in various food products, including peanuts (Vadas & Perelman, 2003, Martínez-

Pineda & Yagüe-Ruiz, 2022). For Serbia, while comprehensive data may be less extensive, some studies indicate the presence of undeclared allergens is also a concern, reflecting the need for stringent control measures and robust analytical methods like the one described here (Davidović *et al.*, 2022).

4. Conclusion

The presence of undeclared peanut allergens in food is a major global health hazard that requires ongoing attention and proactive measures. The discovery of undeclared peanut allergen DNA in Belgrade, Serbia, exposes the risk of peanut allergen exposure from the local food supply and emphasizes the importance of food businesses implementing strong allergy management practices. The issue of undeclared peanut allergens is further complicated

by the inconsistent and often unreliable use of PAL. Our study, which detected peanut DNA in products not labelled with PAL, supports concerns about the inadequacy of relying on PAL alone to protect allergic consumers.

The Voluntary Incidental Trace Allergen Labelling (VITAL) program takes a formal, risk-assessment-based approach to PAL, with the goal of providing more consistent information to both businesses and consumers. Our findings show that, even with systems like VITAL, a comprehensive plan is necessary. This strategy must involve strong allergen management methods by food producers, stricter regulatory oversight, better labelling, comprehensive consumer education, and continuing monitoring for undeclared allergens. Finally, limiting or eliminating allergen contamination throughout the food supply chain is critical for preserving the safety and well-being of people with peanut allergies.

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