meat technology
Founder and publisher:
Institute of Meat Hygiene and Technology, Belgrade

UDK: 637.064:616-097(497.11)"2021/2023"
ID: 158142473
https://doi.org/10.18485/meattech.2024.65.2.1

Original scientific paper

Detection of celery and mustard food allergens in foods of animal origin in Serbia for the period 2021–2023

Vesna V. Janković[1], Branko Velebit[1], Radmila Mitrović[1], Brankica Lakićević[1], Lazar Milojević[1], Dunja Mišić[1] and Slaven Grbić[2]

[1] Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Republic of Serbia
[2] "Slaven" DOO, Ada 19a, 78000 Banja Luka, Republika Srpska, Bosnia and Herzegovina

ARTICLE INFO

Keywords:
Celery
Mustard
Real time PCR
Food allergen
Food safety

ABSTRACT

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. Celery and mustard belong to the group of 14 basic food allergens, and thus, they are potentially hazardous. Therefore, the objective of this mini-review is to evaluate the presence of celery and mustard in foods of animal origin in Serbia for the period 2021–2023. The present study provides a summary of qualitative detection of specific DNA sequences by real time PCR techniques conducted on 179 retail products of animal origin, i.e., coarse-ground cooked sausages, cooked sausages with meat pieces, fermented sausages, smoked products, chicken meat, dairy and meat alternatives, quick-frozen dough products and snacks. Celery DNA (the mannitol dehydrogenase gene region was used for specific celery identification in samples) was detected in 15 samples, while mustard DNA was detected in 14 samples.

1. Introduction

Food allergies are becoming a major global health concern and a serious food safety issue for both private and public health systems (*Muraro et al*., 2022). Consumers must be provided accurate and understandable information regarding the allergenic profile of foods (*Codex Alimentarius Commission FAO/WHO,* 2020).

The term food allergy is used to refer to an immune response directed toward foods (*Sampath et al*. 2021). Food allergies can cause a wide range of symptoms on the skin and in the gastrointestinal and respiratory systems, and can result in anaphylactic shock, which can be fatal *(Renz et al*., 2018). The symptoms usually appear quickly, a few minutes after the triggering food is consumed, and in severe cases, they might result in a deadly reaction (*Ho, Wong, & Chang*, 2014). Over the past three decades, food allergies have become more common in both developed and developing nations. It is believed that they impact up to 8% of young children and 2–3% of adults in Western countries (i.e., Europe, North America, and Australia) (*Sicherer & Sampson*, 2014, *Sampath et al*., 2021). Since it was considered to be an unusual condition until recently, there is insufficient information available in other geographical locations (*EFSA*, 2014; *Loh & Tang*, 2018). The primary approach to controlling food allergies is to avoid or stay clear of foods that trigger an allergic reaction. Patients with food allergies rely on precise information from the allergy statement provided in the ingredient list to prevent adverse effects. Effective avoidance is a complex issue that affects patients and their families, public health authorities, the food sector and governments (*Gargano et al*., 2024).

In Serbia, the *Rulebook on declaring, labeling and advertising of food* (2017–2024) and the *Rulebook on the health safety of dietary foods* (2017–2024) provide allergen legislation considering 14 food ingredients

***Corresponding author:** Vesna Janković, *vesna.jankovic@inmes.rs*

Paper received: October 4th 2024. Paper accepted 28th October 2024.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia.

that can cause allergic reactions or intolerance, method of declaration, and the recommended method for detection (gluten) when this is used as an ingredient in manufactured foods. Precautionary allergen labels (PAL), sometimes referred to as "may contain" labels, are additional labels that customers with food allergies can encounter on product packaging. The allergens that could be present in the product as a result of accidental cross-contamination during food manufacturing are, by definition, the subject of these precautionary labels (*Food Standards Agency*, 2021). PAL are currently unregulated by law and are optional in most countries, including Serbia (*Popov-Raljić et al*., 2022). Because there is no legislation governing when and how to use PAL, their current use—or misuse—is unclear.

Among allergenic substances whose presence in food must be indicated on the label are celery (*Apium graveolens*) and mustard (*Sinapis alba*). These two minor food ingredients are attracting increasing attention because of their popularity as seasoning material worldwide. Celery is an important member of the *Apiaceae* family that is cultivated worldwide. Major identified celery antigens are Api g 1 and Api g 7. Even small amounts of celery can immediately lead to allergic reactions in sensitive individuals. According to the German Federal Institute for Risk Assessment (VITAL, 2019) the eliciting dose 'ED01' and 'ED05' values of 0.05 mg and 1.3 mg protein, respectively, are now the new reference doses for celery. For mustard, 0.05 mg protein has now been derived as a reference dose for 'ED01', and 0.4 mg protein for 'ED05'. The mustard plant belongs to the *Cruciferae* (*Brassicaceae*) family that includes other vegetables, such as radish, rutabaga, cabbage, broccoli, turnip, watercress, horseradish, castor oil plant and rapeseed. Mustard contains three main cultivated species: *Sinapis alba* (yellow mustard), *Brassica nigra* (black mustard) and *Brassica juncea* (oriental mustard). Considering *Sinapis alba*, the main allergens are sin a1, sin a 2, sin a 3 and sin a 4 (*Tanno et al*., 2023).

2. Materials and Methods

A total of 179 food products of animal origin were randomly selected from retail food stores in Serbia. Sampled products were digitally recorded and assigned to one of eight product categories: coarse-ground cooked sausages, cooked sausages with meat pieces, fermented sausages, smoked products, chicken meat, dairy and meat alternatives, quick-frozen dough and snacks. The product name, manufacturer, country of origin, product ingredients list, and any information regarding substances or products causing allergies or intolerances outlined in Annex II of the relevant European regulation (*Regulation (EU) No 1169/2011*, 2011) were among the information gathered from food products.

Additionally, all selected products were analysed using validated methods for the food allergens, celery and mustard. Analysis were carried out according to *SRPS EN ISO 15634-2:2019* (2019) and *SRPS EN ISO 5:2023* (2023), with results being expressed as < or >10 mg/kg. DNA from the food products was extracted using a 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 DNAs with a OD260 nm/OD2800 nm ratio of 1.8 to 2.0 from all samples, indicating the high quality of the extracted DNA.

Extracted DNA materials were used as templates for amplification, identification, and qualitative detection using real-time PCR. Real-time PCR assays were performed with Aria MX (Agilent Technologies). The program included 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. Real-time PCR detection of celery was based on a 101 bp (base pair) sequence from mannitol dehydrogenase gene (GenBank acc. no. AF067082) from celery (*Apium graveolens*) and for mustard (*Sinapis alba*) by determining the gene MADS-D.

3. Results and Discussion

Of the products analysed, 114 of 179 (63.69%) were declared as causing allergies in the ingredient lists. The most frequently listed allergen was soya (27.37 %)(Figure 1). PAL statements were provided for 67 (37.43%) of the food products (Figure 2).

Results for the food allergens in the 179 foods of animal origin are presented in Table 1.

Celery DNA (the mannitol dehydrogenase gene region was used for specific celery identification in samples) was detected in 15 samples, while mustard DNA was detected in 14 samples. Of the 15 samples in which celery DNA was detected, celery was listed on the declaration (list of ingredients) of four products, while celery was mentioned in the PAL statement of 10 products. However, one product (a breakfast grain) in which celery was found did not have this allergen included in the list of ingredients, nor did it have a PAL statement for celery.

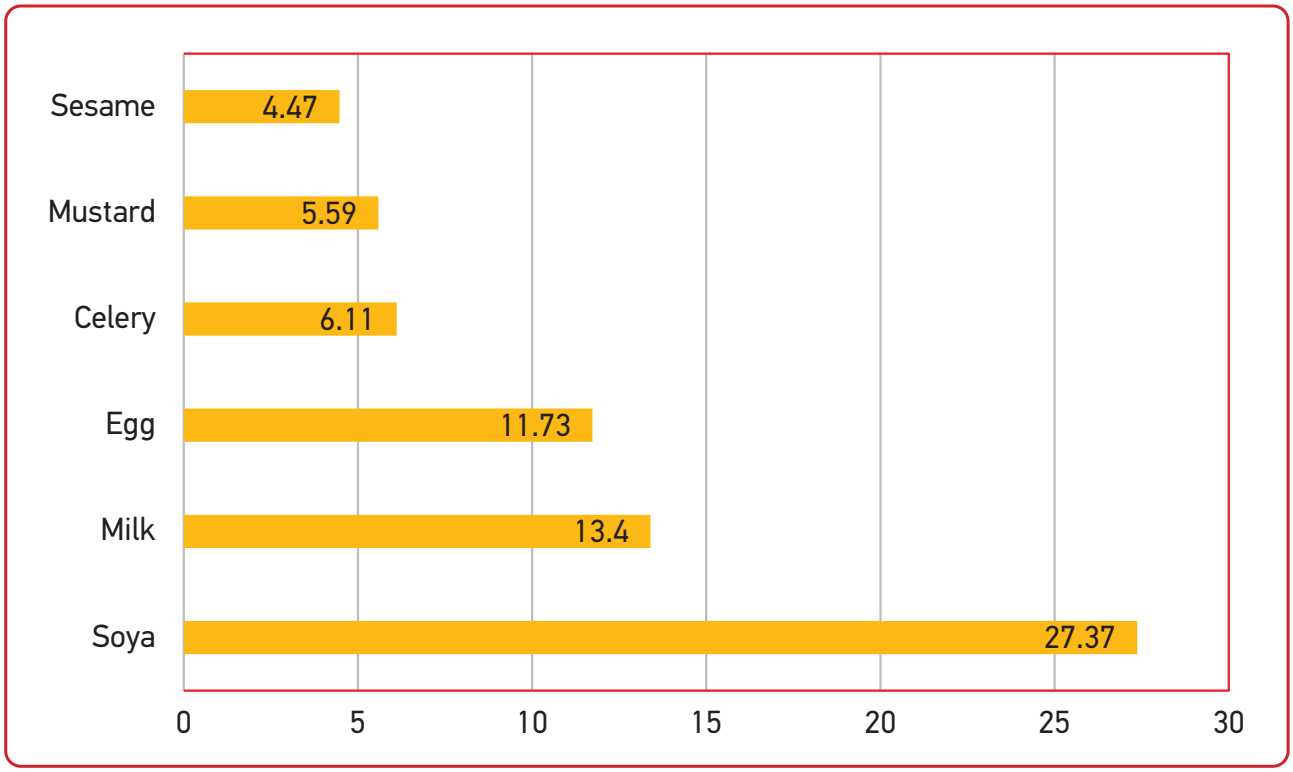

Figure 1. Prevalence of food allergens provided on 179 food labels from foods of animal origin randomly sourced from retail outlets in Serbia

Mustard was detected in seven food products that included mustard in the declaration (list of ingredients), while in five food products, the allergen mustard was mentioned in the PAL statement. In the case of two food products in which mustard DNA was detected, the allergen was not mentioned at all. The obtained results were in accordance with those of other studies (*Vipa et al*., 2021; *James et al*., 2024).

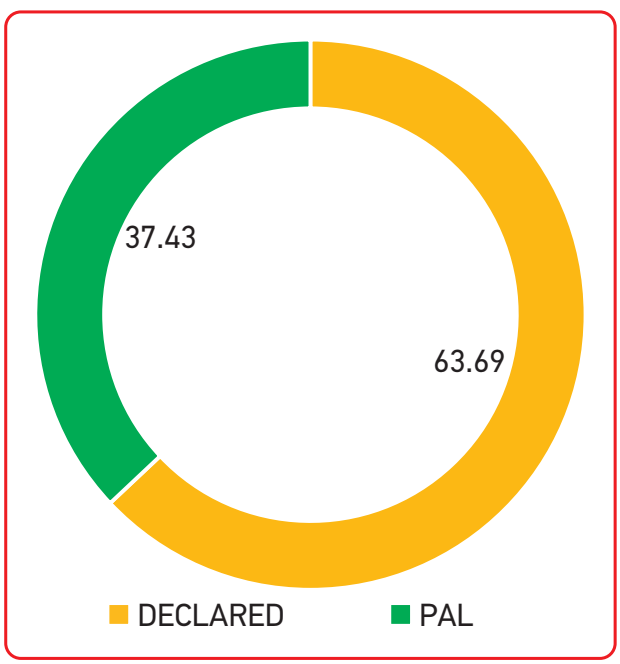

Figure 2. Percentage of sampled foods with food allergen information in food declaration ingredient lists (declared) and PAL statements (PAL)

This study demonstrated how ineffective PAL currently is for people with food allergies. The absence of established reference doses has led to uneven PAL application by the food industry and to uneven levels of contamination that trigger enforcement officers to instigate a food withdrawal, according to a summary of the opinions of all the major stakeholders (including clinicians, patients, the food industry and regulators). The real risk of allergic consumers developing an allergic reaction is not well correlated with the use of PAL or the presence of allergens in foods. As a result, consumers are less likely to trust food labels and to make wise decisions.

In the context of assessing results for qualitative allergen detection, voluntary incidental trace allergen labelling (VITAL) can be instrumental in risk assessment and labelling decisions. VITAL could be applied for:

1. Thresholds for risk assessment: VITAL establishes action levels for allergen presence, meaning detected allergens can be categorised by risk threshold.

Table 1. Presence of mustard and celery food allergens in foods of animal origin

PRODUCT TYPE	NUMBER OF SAMPLES	CELERY > 10 mg/kg	MUSTARD > 10 mg/kg
COARSE-GROUND COOKED SAUSAGES	25	4	4
COOKED SAUSAGES WITH MEAT PIECES	30	5	5
FERMENTED SAUSAGES	21	< 10 mg/kg	< 10 mg/kg
SMOKED PRODUCTS	16	< 10 mg/kg	< 10 mg/kg
CHICKEN MEAT	12	< 10 mg/kg	< 10 mg/kg
DAIRY AND MEAT ALTERNATIVES	28	3	3
QUICK-FROZEN DOUGH	18	< 10 mg/kg	1
SNACKS	29	3	1

2. Qualitative detection interpretation: Since PCR can qualitatively detect DNA from specific allergens, a presence/absence result is obtained, rather than a quantified result. VITAL guidelines for actionable risk can help in the interpretation of these qualitative results. If PCR indicates the presence of a particular allergen, VITAL's thresholds can inform whether this result should lead to labelling based on the typical serving size or consumption pattern.

4. Conclusion

This study is the first to identify the risk that unintentional inclusion of celery or mustard in food products both with and without PAL poses to Serbian consumers who are sensitive to these ingredients. It is apparent from the study that there are no explicit standards for food manufacturers using PAL. Also, the use of VITAL should improve risk management in order to develop risk-based labelling strategies (a trace level below VITAL's Action Level would mean labelling is unnecessary, while higher levels, such as those above Action Level 2, indicate that precautionary labelling is advised). In summary, VITAL can guide interpretation, risk categorisation, and labelling decisions based on PCR detection of allergens, enhancing the practical applicability of PCR results in food safety and allergen management. Prospective novel strategies might assist in resolving the present problems with food labelling for those with severe food allergies. To help food allergic consumers in Serbia, the best practices include standardising detection assays, certifying reference materials, defining acceptable risk levels for food allergies, and harmonising labelling actions.

Utvrđivanje prisustva alergena celera i slačice u hrani životinjskog porekla u Srbiji za period 2021–2023 godine

Vesna V. Janković, Branko Velebit, Radmila Mitrović, Brankica Lakićević, Lazar Milojević, Dunja Mišić i Slaven Grbić

INFORMACIJE O RADU

Ključne reči:
Celer
Slačica
PCR u realnom vremenu
Alergeni u hrani
Bezbednost hrane

APSTRAKT

Alergija na hranu (AH) je predstavlja ozbiljan globalni problem za javno zdravlje. Prisustvo nedeklarisanih alergenih sastojaka ili njihovo prisustvo u tragovima usled kontaminacije tokom prerade hrane predstavlja veliki zdravstveni rizik. AH predstavljaju značajan zdravstveni problem širom sveta i potrošači bi trebalo da imaju pouzdane podatke o prisustvu alergena. Celer i slačica spadaju u grupu od 14 osnovnih alergena u hrani i stoga su potencijalno opasni. Cilj ovog pregleda je procena prisustva celera i slačice u hrani životinjskog porekla u Srbiji za period 2021–2023. godine Ova studija pruža rezime kvalitativne detekcije specifičnih DNK sekvenci PCR tehnikom u realnom vremenu sprovedenom na 179 maloprodajna proizvoda, uključujući grubo mlevene kuvane kobasice, kuvane kobasice sa komadima mesa, fermentisane kobasice, dimljene proizvode, pileće meso, mlečne proizvode i alternative za meso, grickalice i brzo zamrznuti proizvodi od testa.

Disclosure statement: No potential conflict of interest was reported by authors.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant No. 451-03-66/2024-03/200050 from 05.02.2024.

References

Codex Alimentarius Commission FAO/WHO (2020). Code of practice on food allergen management for food business operators CXC 80-2020. Rome: FAO/WHO.

EFSA (2014). Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes.

Food Standards Agency (2021). Precautionary allergen labelling

Gargano, D., Appanna, R., Santonicola, A., De Bartolomeis, F., Stellato, C., Cianferoni, A., Casolaro, V. & Iovino, P. (2024). Food Allergy and Intolerance: A Narrative Review on Nutritional Concerns. *Nutrients,* 13(5), 1638. doi: 10.3390/nu13051638.

Ho, M. H., Wong, W. H., & Chang, C. (2014). Clinical spectrum of food allergies: a comprehensive review. *Clinical Review Allergy Immunology*, 46(3), 225–40. doi: 10.1007/s12016-012-8339-6.

James C. A., Welham, S., & Rose, P. (2024). Evaluation of food allergen information, labelling and unintended food allergen presence in imported prepacked foods and drinks purchased online in the UK. *Food Control*, 162, 110462.

Loh, W., & Tang, M. L. K. (2018). The Epidemiology of Food Allergy in the Global Context. *International Journal of Environmental Research & Public Health*, 15(9), 2043. doi: 10.3390/ijerph15092043.

Muraro, A., de Silva, D., Halken, S., Worm, M., Khaleva, E., Arasi, S., Dunn-Galvin, A., Nwaru, B. I., De Jong, N.W., Del Río, P. R., & Turner, P. J. (2022). Managing food allergy: GA2LEN guideline 2022. *World Allergy Organization Journal*, 15(9), p.100687. 10.1016/j.waojou.2022.100687.

Popov-Raljić, J., Aleksić, M., & Janković, V. (2022). Food allergens — food safety hazard. *Meat Technology*, 63(1), 11–25. https://doi.org/10.18485/meattech.2022.63.1.2.

Regulation (EU) No 1169/2011 (2011). Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011.

Renz, H., Allen, K. J, Sicherer S. H., Sampson, H. A., Lack, G., Beyer, K., & Oettgen H. C. (2018). Food allergy. *Nature Reviews Disease Primers*, 4, 17098. doi: 10.1038/nrdp.2017.98.

Rulebook on declaring, labeling and advertising of food (2017–2024). *Official Gazette of the Republic of Serbia*, No. 19/2017, 16/2018, 17/2020 and 118/2020 17/2022, 23/2022, 30/2022 and 61/2024 – other).

Rulebook on the health safety of dietary foods (2017–2024). *Official Gazette of the Republic of Serbia*, No. 19/2017, 16/2018, 17/2020 and 118/2020 17/2022, 23/2022, 30/2022 and 61/2024 – other.

Sicherer S. H., & Sampson H. A. (2014). Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. Journal of Allergy Clinical Immunology, 133(2), 291–307. doi: 10.1016/j.jaci.2013.11.020.

SRPS EN ISO – 5:2023 (2023). Foodstuffs — Detection of food allergens by molecular biological methods — Part 5: Mustard (*Sinapis alba*) and soya (*Glycine max*) — Qualitative detection of a specific DNA sequence in cooked sausages by real-time PCR.

SRPS EN ISO 15634-2:2019 (2019). Foodstuffs — Detection of food allergens by molecular biological methods — Part 2: Celery (*Apium graveolens*) — Detection of a specific DNA sequence in cooked sausages by real-time PCR.

Surojanametakul Vipa., Srikulnath S., Chamnansin, P., Shibata, H., & Shoji, M. (2021). Investigation of undeclared food allergens in commercial Thai food products update after enforcing food allergen labeling regulation. *Food Control*,120,107554. https://doi.org/10.1016/j.foodcont.2020.107554.

Tanno, L. K. et al. (2023). Optimization of the allergen classification of the *International Classification Of Diseases, 11th Revision* (ICD-11). *Journal of Allergy and Clinical Immunology*, 151(6), 1655 – 1659.

Vanitha Sampath. et al. (2021). Food allergy across the globe. *Journal of Allergy and Clinical Immunology*, 148(6), 1347 – 1364.

Vanitha S., et al. (2021). Food allergy across the globe. *Journal of Allergy and Clinical Immunology,*148(6), 1347–1364. doi: 10.1016/j.jaci.2021.10.018.

VITAL (2019). The Allergen Bureau.

Authors info

Vesna V. Janković https://orcid.org/0009-0003-0095-1498
Branko Velebit https://orcid.org/0000-0002-7577-8074
Radmila Mitrović https://orcid.org/0000-0003-2912-2784
Brankica Lakićević https://orcid.org/0000-0002-0175-5830
Lazar Milojević https://orcid.org/0000-0001-6901-6033
Dunja Mišić https://orcid.org/0009-0006-2213-2612
Slaven Grbić https://orcid.org/0009-0007-8486-5720