

## Detection method based on real-time polymerase chain reaction for celery (*Apium graveolens*) in beverages and dehydrated soups

LUBICA PIKNOVÁ – VERONIKA JANSKÁ – PETER SIEKEL

### Summary

According to European legislation, food ingredients with potential allergenic properties, including celery (*Apium graveolens*), have to be declared. In order to provide an appropriate detection method for specific matrices, a method based on real-time polymerase chain reaction (PCR) for celery in beverages and dehydrated soups was developed and validated. Three varieties of celery frequently processed in food production were used for artificial contamination of vegetable and fruit beverages as well as instant soups in order to prepare model samples. Detection limits of 2.8–9.2 pg celery DNA per sample (200 mg of food sample) for all three varieties were reached. Inclusivity and exclusivity of the real-time PCR system was 100 %. A practical detection limit of 10 mg·kg<sup>-1</sup> was determined for artificially contaminated beverages and soups. Real samples of fresh pressed vegetable and fruit juices with and without the celery component were analysed with identical results stated by the manufacturer.

### Keywords

allergen; detection; *Apium graveolens*; celery; real-time polymerase chain reaction

Celery (*Apium graveolens*) belongs to the *Apiaceae* family (or *Umbelliferae*), three varieties being mostly used in food production: *Apium graveolens* L. var. *rapaceum* (Mill.) Gaud. (celeriac, celery root), *Apium graveolens* L. var. *secalinum* Alef. (Chinese, leaf or cutting celery) and *Apium graveolens* L. var. *dulce* (Mill.) Pers. (celery). Celery is consumed raw, cooked or dried as a spice [1]. However, IgE-mediated reactions to celery are common in food allergic adults in Europe and it can induce allergic reactions of immediate type, from oral contact, skin contact (urticaria), to anaphylactic shock, if consumed. Sensitization to celery is frequently associated with birch and/or mugwort pollinosis, hence the term „birch-mugwort-celery-syndrome“ was established. Birch pollen and celery allergy are highly incidental in Central Europe, while celery allergy is most frequently related to mugwort pollen in Southern Europe [2].

The major celery antigen Api g 1 belongs to Bet v 1 family. Other identified allergens are Api g 2, Api g 4 and Api g 6 from prolamin and

profilin allergen families. The cross-reactive carbohydrate determinants also seem to be allergenic [1]. Allergic symptoms occurred after ingestion of celery within 30 min to 1 h in 46 % and as little as 700 mg of celery induced allergic symptoms in 48 % of patients [2].

As the only way of allergic reaction prevention, for sensitive persons, is avoiding of consumption, all allergenic food components have to be declared. Celery and products thereof must always be declared according to the European legislation presented in Directive 2003/89/EC and Regulation (EU) No 1169/2011 [3, 4]. Control of declared food components requires appropriate diagnostic methods. Besides direct immunochemical methods, which detect the allergenic component(s), most of the detection methods are based on polymerase chain reaction (PCR) [5–9] or multiplex PCR [10–16].

Production and consumption of fresh fruit and vegetable juices and beverages became very popular in European countries, including Slovakia. Production lines should be cleaned and sanitized to be

Lubica Piknová, Veronika Janská, Peter Siekel, National Agricultural and Food Centre, Food Research Institute, P. O. Box 25, Priemyselná 4, 824 75 Bratislava, Slovakia.

Correspondence author:

Lubica Piknová, e-mail: piknova@vup.sk

free from allergens if several products of different composition are produced. Sanitation procedures of production facilities needs to be safe also from the allergen point of view as in the same manufacture several products of different composition are produced. Effectiveness of cleaning has to be checked by appropriate methods.

In this study, the method of celery detection in beverages, starting from the preparation of raw material to Taqman real-time PCR detection, was provided. The model samples of artificially contaminated non-celery beverages were prepared, as well as real samples were used. Targeting development of comprehensive method also another matrix, instant vegetable soup, was used, as it is one of mostly produced celery-containing products in food industry. The sequences for real-time PCR primers and probe oriented to the mannitol dehydrogenase gene were utilized [17]. The concentration of extracted DNA was determined fluorimetrically and amplifiability was checked by the 18S rRNA-oriented real-time PCR method [18]. The comprehensive detection method encompassed the sample preparation, DNA extraction, optimization and intralaboratory validation of real-time PCR analysis.

## MATERIALS AND METHODS

### Materials for artificial contamination

Celery (*Apium graveolens* L. var. *dulce*), celeriac (*Apium graveolens* L. var. *rapaceum*) and leaf celery (*Apium graveolens* L. var. *secalinum* Alef.) from the market in Bratislava (Slovakia) were used for artificial contamination. Matrices from all three celery varieties for artificial contamination of beverages and instant soups were manually grated using stainless steel cutter and dried as follows: celery and celeriac over 72 h, leaf celery overnight. Matrices were dried at laboratory temperature, being covered with filtration paper to avoid contamination. Dried materials were homogenized for approx. 10 min in a mortar with a pestle, till the powder was obtained.

### Preparation of model samples

Beverages were obtained from fruit and vegetable beverages producer in Slovakia and contaminated using celery root, celeriac or leaf celery powder. Samples of beverages with 100 000, 10 000, 1 000, 100, 10, 1 and 0.1 mg·kg<sup>-1</sup> of a celery component were prepared. Artificially contaminated samples of beverages were incubated overnight at laboratory temperature and DNA was then extracted. Instant soups (Tiha, Thuan An, Vietnam)

were homogenized in HGB2WTS3 mixer with a stainless steel container (Waring, Torrington, Connecticut, USA) for approx. 5 min. Aliquots of the celery powder from all three varieties, alternatively, were added to homogenized instant soups to gain identical celery component contents as it was in the case of artificially contaminated drinks (100 000, 10 000, 1 000, 100, 10, 1 and 0.1 mg·kg<sup>-1</sup>).

### Real food samples

Real samples of green smoothie pure vegetable and fruit (containing celery), 100% juice from pressed vegetable mixture with celery, 100% juice from pressed vegetable mixture without celery, 100% fruit and vegetable juice, and 100% juice from pressed fruit mixture were kindly provided by the Slovak producer of fresh juices.

### DNA extraction

DNA from leaf celery for determination of the detection limit as well as DNA for determination of selectivity (leaves from parsley, parsnip, dill, coriander; all from the market in Bratislava, Slovakia) were extracted by chaotropic solid-phase extraction (SPE) using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). DNA for detection limit experiments from celeriac and celery and DNA from food for selectivity testing (potato, carrot, kohlrabi, leek, onion, garlic, paprika, pumpkin, soya, maize, wheat, chives, cumin, beef, chicken, pork; all from the market in Bratislava, Slovakia) were extracted by chaotropic SPE using Nucleospin Food Kit (Macherey Nagel, Düren, Germany). The latter method was used also for extraction of DNA from real food samples. Both kits were used according to manufacturers' manuals. Blank for the control of reagents and possible contamination was included in every extraction experiment. Amplifiability of the extracted DNA was checked by universal eukaryotic real-time PCR [18]. DNA concentration was determined fluorimetrically using Quant-iT PicoGreen Kit (Invitrogen Molecular Probes, Eugene, Oregon, USA) with fluorescence measured by Tecan Safire2 fluorimeter (Tecan, Grödig bei Salzburg, Austria).

### *Apium graveolens* L. specific real-time PCR

Real-time PCR assays were performed in microplates with individual reaction volumes of 25 µl containing 5 µl of DNA, 1× reaction buffer supplied with DNA polymerase, 2.5 mmol·l<sup>-1</sup> MgCl<sub>2</sub>, 200 µmol·l<sup>-1</sup> dNTP mixture (Applied Biosystems, Foster City, California, USA) and 1.5 U of Cheetah Hot Start Taq DNA polymerase (Biotium, Hayward, California, USA). Primers Ag637F (AGC CTG TTT CCC GTA CGA

GAT) and Ag749R (CTC ATC ACA CCG TAA TCC AAA CAT) were used in concentrations of 500 nmol<sup>-1</sup>, and the probe Ag690P (5'-FAM-TAC ACG CTC ATC GTG ACT CAG CA-BHQ1) was used at a concentration of 200 nmol<sup>-1</sup> (oligonucleotides synthesized by Eurofins MWG Operon (Ebersberg, Germany). The reaction was carried out in a real-time PCR thermal cycler ABI 7900 (Applied Biosystems) using a temperature programme as follows: initial denaturation at 95 °C for 2 min, 45 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 60 s. Fluorescence data were collected and processed using internal software of the instrument.

## RESULTS AND DISCUSSION

The aim of this work was development of sample preparation, optimization and intralaboratory validation of the real-time PCR method for celery detection and its adaptation to the beverages and dehydrated soup analyses. The specific real-time PCR system was oriented to the target gene encoding for mannitol dehydrogenase of celery. The amplification product had a length of 113 bp, the real-time PCR exhibited 100% selectivity when tested all three variants of celery (celery, celeriac and leaf celery) and 100% selectivity when 20 different plant and animal samples were tested (Tab. 1). Selectivity test was implemented using DNA extracted in two replicates and each PCR was run on in 6 replicate measurements. Species from *Apiaceae* family and food, which could be used along with celery, were chosen for selectivity testing.

The procedure of grating, pressing and filtration the juice from celery root was found to be laborious and time-consuming. The procedure of grating, drying and powdering the celery root was found to have identical yield in DNA extraction (data not shown) and was easier implemented. Thus, the latter was chosen for preparation of samples artificially contaminated by celery, beverages as well as dehydrated soups. Amplifiability of DNA was confirmed by universal eukaryotic real-time PCR oriented to 18S rRNA gene.

DNA-based detection limit was determined by analysis of series of four times diluted samples of DNA extracted from celery, leaf celery and celeriac, measured in 4 replicates. Standard curves were generated by plotting the average cycle threshold values against the logarithm of corresponding DNA concentration in nanograms per microlitre (Fig. 1).

Limit of detection (*LOD*) was expressed in

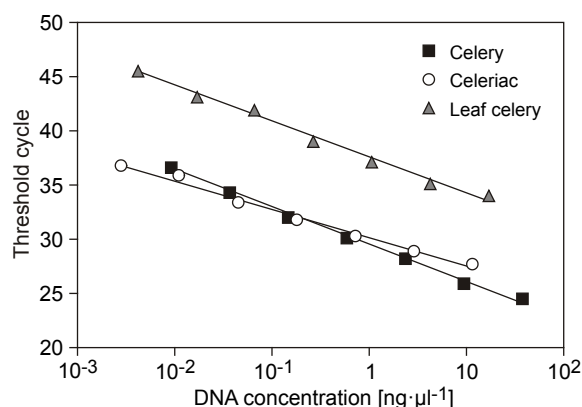
terms of the lowest concentration reliably detectable in all 4 replicate analyses. Standard curves for celery (slope  $k = -3.39 \pm 0.08$ , coefficient of determination  $R^2 = 0.99$ ) and celeriac ( $k = -2.61 \pm 0.1$ ,  $R^2 = 0.97$ ) were linear and nearly identical. However, the calibration curve for leaf celery was also linear ( $k = -3.29 \pm 0.08$ ,  $R^2 = 0.99$ ). The absolute *LOD* was 2.8 pg (1.6 haploid genome copy) for celeriac, 4.2 pg (2.4 haploid genome copy) for celery and 9.2 pg (5.3 haploid genome copy) for leaf celery, as the haploid genome size for *Apium graveolens* is 1.73 pg ( $1.69 \times 10^9$  bp) [19]. The determined values of *LOD* were found to be comparable with other real-time PCR methods reported previously [7, 9, 12].

Parameters of practical analytical applicability were determined using model samples of artificially contaminated vegetable and fruit juices and a dehydrated soup. The commodities used as model samples were found to be free of celery before the samples were used in experiments. The vegetable and fruit juices represent a complicated

**Tab. 1.** Selectivity of real-time PCR method for detection of celery.

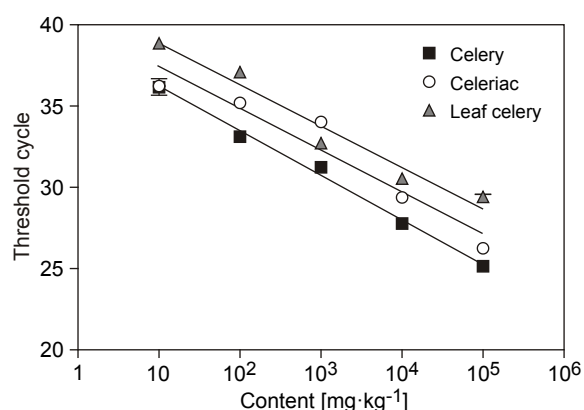
Species	Latin nomenclature	Result
Celery	<i>Apium graveolens</i> var. <i>dulce</i>	+
Celeriac	<i>Apium graveolens</i> var. <i>rapaceum</i>	+
Leaf celery	<i>Apium graveolens</i> var. <i>secalinum</i>	+
Carrot	<i>Daucus carota</i>	-
Parsley	<i>Petroselinum crispum</i>	-
Parsnip	<i>Pastinaca sativa</i>	-
Potato	<i>Solanum tuberosum</i>	-
Onion	<i>Allium cepa</i>	-
Leek	<i>Allium porrum</i>	-
Garlic	<i>Allium sativum</i>	-
Chives	<i>Allium schoenoprasum</i>	-
Pumpkin	<i>Cucurbita pepo</i>	-
Kohlrabi	<i>Brassica oleracea</i>	-
Paprika	<i>Capsicum annum</i>	-
Maize	<i>Zea mays</i>	-
Soy	<i>Glycine max</i>	-
Wheat	<i>Triticum aestivum</i>	-
Coriander	<i>Coriandrum sativum</i>	-
Cumin	<i>Carum carvi</i>	-
Dill	<i>Anethum graveolens</i>	-
Beef	<i>Bos taurus</i>	-
Pork	<i>Sus scrofa domesticus</i>	-
Chicken	<i>Gallus gallus domesticus</i>	-

(+) – positive PCR result; (-) – negative PCR result.

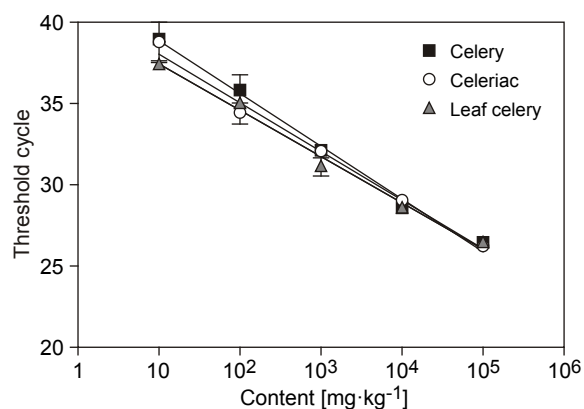


**Fig. 1.** Calibration lines for the celery-specific real-time PCR.

Three celery varieties were analysed.



**Fig. 2.** Practical detection limit of the celery-specific real-time PCR using an artificially contaminated fruit and vegetable juice.



**Fig. 3.** Practical detection limit of the celery-specific real-time PCR using artificially contaminated dehydrated soup.

matrix for DNA extraction, which are however of interest due to the increasing trend in their consumption. Developing a method for juice analysis reflects the problems with these products being produced in the same equipment as celery-free juices and the necessity to monitor the efficiency of sanitary procedures. The model sample of dehydrated soup was used as it is the most often consumed celery-containing product available on the market in Slovakia.

Results of beverages samples in Fig. 2 (celery  $k = -2.74 \pm 0.09$ ,  $R^2 = 0.99$ ; celeriac  $k = -2.58 \pm 0.21$ ,  $R^2 = 0.92$ ; leaf celery  $k = -2.54 \pm 0.15$ ,  $R^2 = 0.96$ ) and instant soup samples in Fig. 3 (celery  $k = -3.23 \pm 0.22$ ,  $R^2 = 0.94$ ; celeriac  $k = -3.05 \pm 0.14$ ,  $R^2 = 0.97$ ; leaf celery  $k = -2.83 \pm 0.12$ ,  $R^2 = 0.97$ ) present the determined calibration lines. The practical detection limit of  $10 \text{ mg} \cdot \text{kg}^{-1}$  was achieved for all used celery varieties in beverages and in soups. This is in accordance with other celery-specific real-time PCR-based methods published previously [7, 9, 12].

Real samples of beverages were also analysed, with the aim to verify the developed method for celery detection. All samples had been pasteurized. Celery was declared on the package of two samples of beverages. The developed method for celery detection was able to detect celery in both samples declared to contain it. We assume that the method is suitable for celery detection in fruit and vegetable freshly pressed beverages and also in other food products, such as dehydrated soups.

#### Acknowledgements

This research was carried out in frames of the project 471/2014-310 „Authentication of products and cross-contamination characterization of plant allergens in production processes applying molecular-biological methods“ of the Ministry of Agriculture and Regional Development of the Slovak Republic. It is a result of implementation of the project of ITMS 26240120013 “Establishment of a HiTech Centre for Research on Formation, Elimination and Assessment of Contaminants in Food” supported by the Research & Development Operational Programme funded by the ERDF.

#### REFERENCES

1. Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes. EFSA Journal, 12, 2014, pp. 158–163. DOI: 10.2903/j.efsa.2014.3894.
2. Ballmer-Weber, B. – Besler, M. – Hoffmann-Sommergruber, K. – Vieths S. – Wuthrich, B.: Allergen data collection: Celery (*Apium graveolens*).

- Internet Symposium on Food Allergens, 2 (3), 2000, pp. 145–167. ISSN: 1437-0573. <<http://www.food-allergens.de/password/PDF-downloads/complete-data/celery.pdf>>
3. Directive 2003/89/EC of the European Parliament and the Council of 10 November 2003 amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs. Official Journal of European Communities, 46, 2003, L308, pp. 15–18. ISSN: 1725-2555. <<http://data.europa.eu/eli/dir/2003/89/oj>>
  4. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. Official Journal of European Union, 54, 2011, L304, pp. 18–63. ISSN: 1977-0677. DOI: 10.3000/19770677.L\_2011.304.eng.
  5. Dovičovičová, L. – Olexová, L. – Pangallo, D. – Siekel, P. – Kuchta, T.: Polymerase chain reaction (PCR) for the detection of celery (*Apium graveolens*) in food. European Food Research and Technology, 218, 2004, pp. 493–495. DOI: 10.1007/s00217-004-0899-0.
  6. Stephan, O. – Weisz, N. – Vieths, S. – Weiser, T. – Rabe, B. – Vatterott, W.: Protein quantification, sandwich ELISA, and real-time PCR used to monitor industrial cleaning procedures for contamination with peanut and celery allergens. Journal of AOAC International, 87, 2004, pp. 1448–1457. ISSN: 1060-3271 (print), 1944-7922 (Online).
  7. Hupfer, C. – Waiblinger, H.-U. – Busch, U.: Development and validation of a real-time PCR detection method for celery in food. European Food Research and Technology, 225, 2006, pp. 329–335. DOI: 10.1007/s00217-006-0418-6.
  8. Wu, Y. – Chen, Y. – Wang, B. – Gao, Y. – Bai, L. – Wang, H.: SYBR green real-time PCR used to detect celery in food. Journal of AOAC International, 93, 2010, pp. 1530–1536. ISSN: 1060-3271 (print), 1944-7922 (Online).
  9. Fuchs, M. – Cichna-Markl, M. – Hohegger, R.: Development and validation of novel real-time PCR method for the detection of celery (*Apium graveolens*) in food. Food Chemistry, 130, 2012, pp. 189–195. DOI: 10.1016/j.foodchem.2011.07.005.
  10. Köppel, R. – Dvorak, V. – Zimmerli, F. – Breitenmoser, A. – Eugster, A. – Waiblinger, H. U.: Two tetraplex real-time PCR for the detection and quantification of DNA from eight allergens in food. European Food Research and Technology, 230, 2009, pp. 367–374. DOI: 10.1007/s00217-009-1164-3.
  11. Köppel, R. – van Velsen-Zimmerli, F. – Bucher, T.: Two quantitative hexaplex real-time PCR systems for the detection and quantification of DNA from twelve allergens in food. European Food Research and Technology, 235, 2012, pp. 843–852. DOI: 10.1007/s00217-012-1806-8.
  12. Mustorp, S. – Engdahl-Axelsson, C. – Holck, A.: Detection of celery (*Apium graveolens*), mustard (*Sinapis alba*, *Brassica juncea*, *Brassica nigra*) and sesame (*Sesamum indicum*) in food by real-time PCR. European Food Research and Technology, 226, 2008, pp. 771–778. DOI: 10.1007/s00217-007-0589-9.
  13. Coisson, J. D. – Cereti, E. – Garino, C. – D'Andrea, M. – Recupero, M. – Restani, P. – Arlorio, M.: Microchip capillary electrophoresis (Lab-on-chip®) improves detection of celery (*Apium graveolens* L.) and sesame (*Sesamum indicum*) in food. Food Research International, 43, 2010, pp. 1237–1243. DOI: 10.1016/j.foodres.2010.03.008.
  14. Pafundo, S. – Gulli, M. – Marmioli, N.: Comparison of DNA extraction methods and development of duplex PCR and real-time PCR to detect tomato, carrot, and celery in food. Journal of Agricultural and Food Chemistry, 59, 2011, pp. 10414–10424. DOI: 10.1021/jf202382s.
  15. Fuchs, M. – Cichna-Markl, M. – Hohegger, R.: Development and validation of a duplex real-time PCR method for the simultaneous detection of celery and white mustard in food. Food Chemistry, 141, 2013, pp. 229–235. DOI: 10.1016/j.foodchem.2013.02.088.
  16. Luber, F. – Demmel, A. – Pankofer, K. – Busch, U. – Engel, K.-H.: Simultaneous quantification of the food allergens soy bean, celery, white mustard and brown mustard via combination of tetraplex real-time PCR and standard addition. Food Control, 47, 2014, pp. 246–253. DOI: 10.1016/j.foodcont.2014.06.047.
  17. Kashanian, E. A.: Detection of celery (*Apium graveolens*) in food with real-time PCR. Uppsala: Uppsala Universitet, 2006. <<https://uu.diva-portal.org/smash/get/diva2:131027/FULLTEXT01>> In Swedish.
  18. Brežná, B. – Hudecová, L. – Kuchta, T.: Detection of pea in food by real-time polymerase chain reaction (PCR). European Food Research and Technology, 222, 2006, pp. 6600–603. DOI: 10.1007/s00217-005-0168-x.
  19. Yang, X. – Quiros, C. F.: Characterizing the celery genome with DNA-based genetic markers. Journal of the American Society for Horticultural Science, 120, 1995, pp. 747–751. ISSN: 0003-1062 (print), 2327-9788 (online). <<http://journal.ashspublications.org/content/120/5/747.full.pdf>>

Received 27 July 2016; 1st revised 7 September 2016; accepted 4 October 2016; published online 14 October 2016.