

Detection of the food allergen celery via loop-mediated isothermal amplification technique

Celine Zahradnik · Roland Martzy · Robert L. Mach ·
Rudolf Krška · Andreas H. Farnleitner · Kurt Brunner

Received: 20 February 2014 / Revised: 24 April 2014 / Accepted: 2 May 2014 / Published online: 1 June 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract Since 2005, celery and celery products have to be labeled according to Directive 2003/89/EC due to their allergenic potential. In order to provide a DNA-based, rapid and simple detection method suitable for high-throughput analysis, a loop-mediated isothermal amplification (LAMP) assay for the detection of celery (*Apium graveolens*) was developed. The assay was tested for specificity for celery since closely related species also hold food relevance. The limit of detection (LOD) for spiked food samples was found to be as low as 7.8 mg of dry celery powder per kilogram. An evaluation of different amplification and detection platforms was performed to show reliable detection independent from the instrument used for amplification (thermal cycler or heating block) and detection mechanisms (real-time fluorescence detection, agarose gel electrophoresis or nucleic acid staining). The analysis of 10 commercial food samples representing diverse and complex food matrices, and a false-negative rate of 0 %

for approximately 24 target copies or 0.08 ng celery DNA for three selected food matrices show that LAMP has the potential to be used as an alternative strategy for the detection of allergenic celery. The performance of the developed LAMP assay turned out to be equal or superior to the best available PCR assay for the detection of celery in food products.

Keywords Loop-mediated isothermal amplification · Celery · Food allergen · Isothermal amplification · Visual detection

Introduction

Celery (*Apium graveolens* L.) is a widely used ingredient in seasonings, sauces, bouillons, and instant meals. Allergy to celery is one of the most common food allergies in Europe causing digestive disorders, respiratory distress, and skin reactions when ingested [1, 2]. In severe cases, exposure can even induce anaphylaxis of the affected individual. Doses triggering such a response typically are around 0.7 g of fresh celery; however, lower amounts may also elicit a reaction [3]. Celery is highly associated with an allergization to birch and mugwort, termed birch-mugwort-celery syndrome [4, 5]. Known allergens of celery are the 16 kD heat-labile protein Api g1, which is homologous to the profilin Api g4 and the glycoprotein Api g5, and the protein Bet v1 from birch. Several studies have proven Api g1 to be the most relevant allergen in celery causing allergic reactions [6–8].

Avoiding the allergen-containing food is the only option to prevent an allergic reaction which may often be difficult due to the contamination of food products with allergenic ingredients. Contaminants can be introduced through food-processing machines, storage, and shipping or are added deliberately for flavoring purposes. Directive 2003/89/EC constituted by the European Union states the mandatory labeling

Published in the topical collection *Advances in Food Analysis* with guest editors Michael W.F. Nielen, Jana Hajslova, and Rudolf Krška.

Electronic supplementary material The online version of this article (doi:10.1007/s00216-014-7873-x) contains supplementary material, which is available to authorized users.

C. Zahradnik · R. Martzy · K. Brunner (✉)
Institute of Chemical Engineering, IFA-Tulln, Center for Analytical Chemistry, Vienna University of Technology, Konrad Lorenz Str. 20, 3430 Tulln, Austria
e-mail: kurt.brunner@tuwien.ac.at

R. L. Mach · A. H. Farnleitner
Institute of Chemical Engineering, Gene Technology Group, Vienna University of Technology, Gumpendorfer Str. 1a, 1060 Vienna, Austria

R. Krška
Department IFA-Tulln, Center for Analytical Chemistry, University of Natural Resources and Applied Life Sciences Vienna, Konrad Lorenz Str. 20, 3430 Tulln, Austria

of 12 groups of ingredients with known allergenic potential, including celery and celery products [9].

This new legislation rises the demand for specific and sensitive methods for the detection of allergens in food products. Although the characterization of celery allergens via mass spectrometry (MS) has been reported [10], currently, the two most prominent analytical techniques for allergen detection in food are protein-based enzyme-linked immunosorbent assays (ELISA) and nucleic acid-based polymerase chain reaction (PCR) assays. At present, no ELISA assay is available for the specific detection of celery due to cross-reactions with parsley (*Petroselinum crispum* (Mill.) Nyman & A.W. Hill) [11], carrot (*Daucus carota* (Hoffm.) Schübl. & G. Martens), and other closely related members of the Apiaceae family. So far, several real-time PCR assays have been developed targeting the gene encoding sequence for Api g 1. However, due to the high risk of cross-reactions, targeting the celery-specific mannitol dehydrogenase is recommended [12, 13]. Although two quantitative real-time PCR (qPCR) assays for the detection of celery have been developed [13, 14], real-time PCR is still a laborious procedure, requiring sophisticated equipment such as thermal cyclers and trained personnel making it unsuitable for a simple on-site high-throughput detection method for celery in food matrices.

An alternative DNA amplification method termed loop-mediated isothermal amplification (LAMP) was developed in 2000 [15]. The use of three primer sets binding six distinct regions of the target sequence and a *Bst* polymerase with strand displacement activity [16] initially forms a dumbbell-like structure which then undergoes cyclic amplification producing various sized DNA strands with alternately inverted repeats of the target sequence [15, 17]. Based on the lack of a denaturation step, the assay can be carried out under isothermal conditions eliminating the need for a thermal cycler and thus making this method highly cost- and time-efficient as well as field-applicable and easy-to-perform. The high efficiency and sensitivity of a LAMP assay allows detection with the naked eye through the use of an intercalating dye such as SYBR Green I [18].

A recently published assay for the detection of celery based on LAMP [19] uses a primer set showing high-sequence homologies for parsley, dill (*Anethum graveolens*), and fennel (*Foeniculum vulgare*) in nBLAST (National Center for Biotechnology Information NCBI), and for carrot and parsley as experimentally proven by the authors of this study (see Electronic Supplementary Material, Fig. S1 and Table S1). Since all of these plants are widely used, this assay is not a suitable tool for the detection of celery in food-related products.

Here, we report the development of a highly specific LAMP-based assay for the detection of celery in food products targeting a diagnostic fragment of the celery mannitol dehydrogenase gene, which was demonstrated to be specific in an earlier publication [20]. We evaluated species-specificity

and sensitivity by testing several different primer sets and comparing it to conventional real-time PCR. To investigate the potential of the assay for the rapid analysis of celery in food, ten different commercially available products were tested with LAMP and PCR. Celery-negative samples were spiked with celery powder to ensure reliable detection despite the potential of inhibition due to complex food matrices. The LAMP amplification products were detected by real-time fluorescence monitoring, via agarose gel electrophoresis and by staining LAMP products with SYBR Green I for visual detection and similarly compared with a previously published real-time PCR assay for the detection of celery in food [13].

Materials and methods

Plant material and samples

Celery root (*A. graveolens* var. *rapaceum*), parsley (*P. crispum* (Mill.) Nyman & A.W. Hill), carrot (*D. carota* (Hoffm.) Schübl. & G. Martens), anise (*Pimpinella anisum* L.), dill (*A. graveolens* L.), fennel (*F. vulgare* Mill.), coriander (*Coriandrum sativum* L.), caraway (*Carum carvi* L.), cumin (*Cuminum cyminum* L.), lovage (*Levisticum officinale* W.D.J. Koch), parsnip (*Pastinaca sativa* L.), Kotanyi steak seasoning, Billa mushroom instant soup, Wasa sesame crisp bread, Bad Ischler 7 herbs salt, Maggi vegetable bouillon cube, Inzersdorfer wellness turkey spread, Kotanyi herbal mix, Hipp vegetable baby food, Rio Mare tuna pastry and Lorenz Naturals potato chips with garlic, and herbs were obtained from local Austrian supermarkets. All samples except for the commercial food products were extracted from fresh material.

DNA extraction

For specificity tests and for generating a calibration curve, fresh celery root as well as all other plant materials were lyophilized for 48 h and then ground to a fine powder using a mortar and pestle. Extraction of nucleic acids was performed using a CTAB extraction protocol as previously described [21]. Briefly, 100 mg of plant material were used for extraction using a 10 % CTAB buffer and chloroform/isoamylalcohol (24:1) solution. Subsequent to an ethanol precipitation, the obtained DNA pellet was washed twice with 70 % ethanol and then resuspended in 10 mM Tris buffer. Sensitivity tests, false-negative rate, and the analysis of commercial food samples were performed using DNA extracted with the SureFood® PREP Allergen Kit (Congen, Berlin, Germany) according to the manufacturer's instructions. DNA concentration and purity were determined photometrically using NanoVue Plus (VWR, Radnor, USA).

Table 1 List of LAMP primers and their sequences targeting the celery mannitol dehydrogenase gene from celery (GenBank acc. no. AF067082)

LAMP primer	Sequence 5'–3'
FIP	GCTGCAAAATCAAGCATTCTTGTGCAGGGAGA AAGCTTCTTGG
BIP	AACAGCTGATGTTGAAGTTATTCCTAAATCTGT ATCGAACATCTGAC
F3	CATTGTTATACTAAATGTGAGTCAC
B3	CGTATTTGCAATGTCGATGAC
LoopF	CTTTATCCCACCATTAATAGTGCCT
LoopB	ATGGACTATGTGAACACCGCA

Primers and probes

All oligonucleotides were synthesized by Eurofins MWG (Ebersberg, Germany). Real-time PCR primers and probe were used as published by Hupfer et al. [13]. LAMP primers were designed using the Primer Explorer V4 software (Eiken Chemical Co., Ltd.; Tokyo, Japan) on basis of the sequence for celery mannitol dehydrogenase, GenBank acc. no. AF067082. The LAMP primer sequences are given in Table 1.

Real-time PCR reaction

DNA extract (2 µL) either undiluted or diluted 1:4 was added to 13 µL of reaction mix containing Kapa™ Probe® Fast (Peqlab, Erlangen, Germany), primers and probe (in a final concentration of 0.3 µmol/L each). Amplification reactions were performed on a 7500 Fast Real-Time PCR system (Applied Biosystems, New York, USA) according to the following thermal cycling protocol: initial steps of 2 min at 95 °C,

followed by 60 cycles of 15 s at 95 °C and 1 min at 60 °C. Unless noted otherwise, amplification reactions were carried out in five replicates.

LAMP reaction

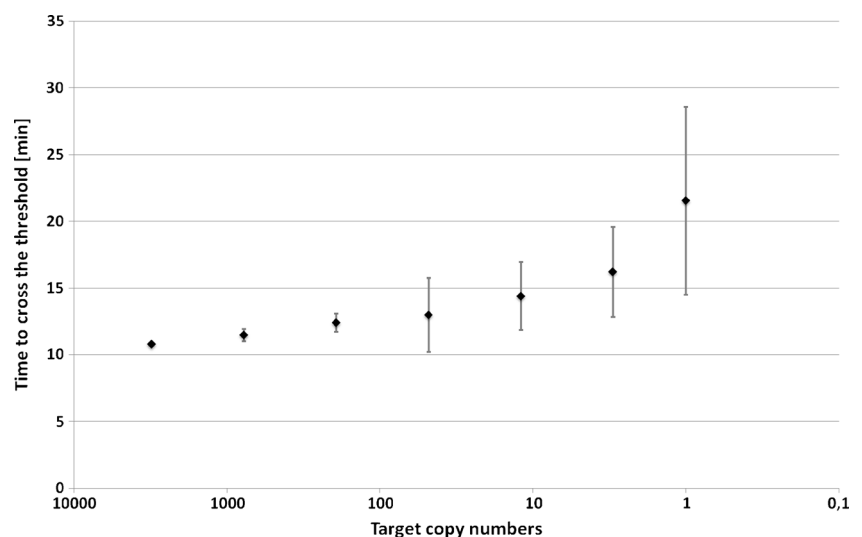
LAMP reactions were carried out in a total reaction volume of 25 µL containing 1.4 µM each FIP and BIP, 0.2 µM each F3 and B3, 0.8 µM each LoopF and LoopB, 1.4 µM of each dNTP (Peqlab, Erlangen, Germany), 0.8 M betaine solution (Sigma-Aldrich, St. Louis, USA), 8 U *Bst* DNA polymerase (New England Biolabs, Ipswich, USA), 20 mM Tris-HCl (pH 8.5), 10 mM KCl, 10 mM (NH₄)₂SO₄, 6 mM MgSO₄, 0.1 % Triton X-100 (Sigma-Aldrich, St. Louis, USA), 1 µL Syto® 9 Green Fluorescent Nucleic Acid Stain (Life Technologies, Carlsbad, USA) [22], and 2 µL undiluted DNA extract. The reaction mixture was incubated for 60 min at 65 °C. Amplification reactions were carried out in five replicates. Aliquots of 10 µL of LAMP products were used for agarose gel electrophoresis. For the detection with the naked eye 1 × SYBR® Green I Nucleic Acid Stain (Life Technologies) was added to aliquots of 15 µL of LAMP products and viewed under UV light.

Results

Design and specificity of LAMP primers

To ensure efficient and specific amplification of celery DNA, five primer sets were designed and tested. All subsequent experiments were performed using the primer set given in Table 1, which showed the highest fluorescence intensity and the lowest Ct value without amplifying DNA other than

Fig. 1 Calibration curve shows standard deviation (SD) increasing from 0.01 % for 3,093 target copies to 55 % for three target copies. The increasing error for decreasing target copies clearly demonstrates that this assay cannot be used for a reliable quantification of trace amounts of celery DNA



celery and lacks relevant homologies with other closely related species as checked by NCBI Blast search. To rule out cross-reactive detection of other members of the Apiaceae family, genomic DNA from herbs and spices commonly used as food ingredients (see plant material & samples) was extracted and analyzed. All tested species did not produce an amplicon in LAMP which demonstrated the specificity of the assay (data not shown).

Sensitivity and limit of detection

To evaluate the potential of the assay for quantitative analysis, DNA was extracted from fresh celery roots, nucleic acid concentrations were determined photometrically in order to prepare serial dilutions ranging from 10 to 0.0039 ng/ μ L. Based on the estimation that celery contains 3.0 pg DNA per haploid nucleus [23] and one base pair has a molecular weight of 650 Da, the target copy number can be calculated. Therefore, 10 ng/ μ L celery DNA correspond to a dilution of 3,093 target copies. Each concentration was analyzed in 20 replicates. A calibration curve was generated by plotting the average threshold time against the corresponding DNA amounts (Fig. 1). The European Network of GMO Laboratories requires a correlation coefficient higher than $R^2 \geq 0.98$ for the real-time PCR quantification of genetically modified organisms [24]. The assay failed to meet this requirement due to its deviating Ct values, even though 20 out of 20 replicates gave a positive signal for an estimated concentration of three target copies. Although development of quantitative real-time LAMP assays for several targets using intercalating dyes has been reported, it is pointed out that the quantitative capability relates to ranges of 1×10^4 – 1×10^{10} target copies [25–27]. This clearly shows that the designed celery LAMP assay is not suitable for the accurate quantification of trace amounts of DNA.

Two food samples, previously tested negative for celery DNA, were spiked with defined amounts of dry celery powder as given in Table 2 and were then extracted two times each. Tuna pastry and crisp bread were both selected due to their high contents of salts and carbohydrates or proteins which can act as inhibitors of amplification reactions [28]. Each sample was analyzed by PCR and LAMP in five replicates. Table 2 shows that 7.81 mg celery/kg sample still could be detected with 100 % positive replicates in tuna pastry whereas 500 mg/kg are required for five positive replicates for crisp bread. This variation between the two food matrices might be due to the high amount of carbohydrates typical for crisp bread which can compromise the accurate amplification of DNA. However, both samples representing both matrices could still be identified as positive even if a low amount of 1.95 mg celery powder was spiked to 1 kg food.

Table 2 Results of spiking experiments with LAMP for two food matrices. One hundred grams of food sample were spiked with 5 g of celery powder and then diluted with the same homogenized sample matrix to obtain low concentrations of celery. Both matrices were extracted twice and analyzed in five replicates per extraction

Celery content (mg celery/kg)	Wasa sesame crisp bread positive reactions		Rio Mare tuna pastry positive reactions	
	Extraction 1	Extraction 2	Extraction 1	Extraction 2
1.95	2/5	2/5	4/5	3/5
7.81	2/5	2/5	5/5	5/5
31.25	2/5	2/5	5/5	5/5
125	3/5	2/5	5/5	5/5
500	5/5	5/5	5/5	5/5
1.000	5/5	5/5	5/5	5/5
5.000	5/5	5/5	5/5	5/5
10.000	5/5	5/5	5/5	5/5
50.000	4/5	5/5	5/5	5/5

False-negative rate

The determination of a false-negative rate is used to identify the lowest concentration at which the assay detects celery positive samples correctly. Below this concentration, a certain percentage of celery DNA containing replicates appear to be negative. DNA isolated from crisp bread, turkey spread, and baby food, previously tested negative for the presence of celery, was spiked with a decreasing number of celery DNA target copies. Figure 2 shows that decreasing the estimated target copy number below 24, leads to false negative replicates. If 24 or more copies per assay are present, the false-negative rate drops down to 0 % for all three food matrices. These results indicate that LAMP is quite insensitive to co-isolated amplification inhibitors and enables analysis of

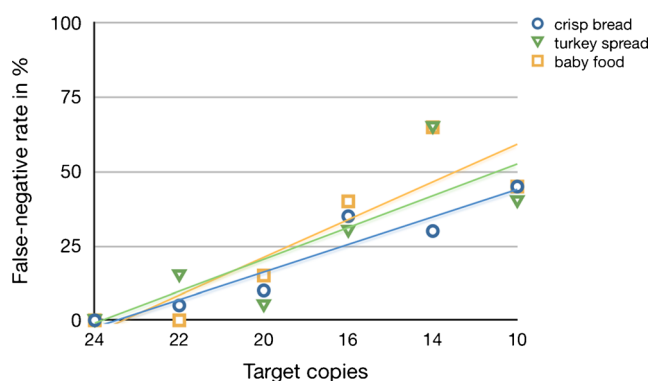


Fig. 2 DNA extracted from celery-free food samples was spiked with different amounts of celery DNA. The false-negative rate was 0 % for 24 or more target copies for all tested matrices and increased to 50 % when analyzing concentrations lower than 16 copies. Number of replicates $n=20$

Table 3 Ten food samples from the supermarket were analyzed for celery DNA with PCR and LAMP

Sample ID	Product	Declaration	extraction 1		extraction 2		extraction 3	
			PCR	LAMP	PCR	LAMP	PCR	LAMP
1	Steak seasoning	(+)	+++++	+++++	+++++	+++++	+++++	+++++
2	Mushroom soup	(+)	++----	+-----	+++--	+++--	++----	++++-
3	Crisp bread	–	-----	-----	-----	-----	-----	-----
4	Herbs salt	+	+++++	+++++	+++++	+++++	+++++	+++++
5	Bouillon cube	+	+-----	+++--	-----	-----	++----	++++-
6	Turkey spread	+	-----	-----	-----	-----	-----	-----
7	Herbal mix	–	-----	+-----	-----	-----	-----	+-----
8	Baby food	–	-----	-----	-----	-----	-----	-----
9	Tuna pastry	–	-----	-----	-----	-----	-----	-----
10	Potato chips	–	-----	-----	-----	-----	-----	-----

Labeling on the food packages: + contains celery, (+) may contain traces of celery, – contains no celery or not declared

samples without extensive clean-up procedures before introducing isolated DNA into the amplification reaction [29, 30].

Analysis of commercial food samples

Ten food samples obtained from local supermarkets were selected with regard to a broad spectrum of matrices rich in carbohydrates, proteins, fats and other components known to inhibit amplification reactions [26, 27]. Three different batches of each product were used for the extraction of DNA. For each extraction, both, PCR and LAMP reactions were performed in five replicates (Table 3). The analyte could be detected in four samples that were declared to contain celery. Interestingly, celery DNA was found in the herbal mix which was not declared to contain celery, while turkey spread, positively declared, could not be confirmed to contain celery neither with PCR nor with LAMP. This might be due to DNA fragmentation caused by extensive food processing like

heat treatment or harsh chemical conditions during the production. The remaining four samples crisp bread, baby food, tuna pastry and potato chips did not indicate the presence of celery in compliance with their declaration. Figure 3 shows that the numbers of positive and negative signals for the analysis of these samples correlate closely between PCR and LAMP. For mushroom soup and bouillon cube, the LAMP reactions showed more positive replicates than PCR. LAMP resulted in a positive amplification signal for the herbal mix, whereas with PCR no signal could be detected.

Evaluation of different amplification and detection platforms

Several tests were performed to show the independence of this method from experimental conditions. Table 4 shows that at least 95 % of replicates containing approximately 40 target copies (0.13 ng/μL) of celery DNA can be detected when

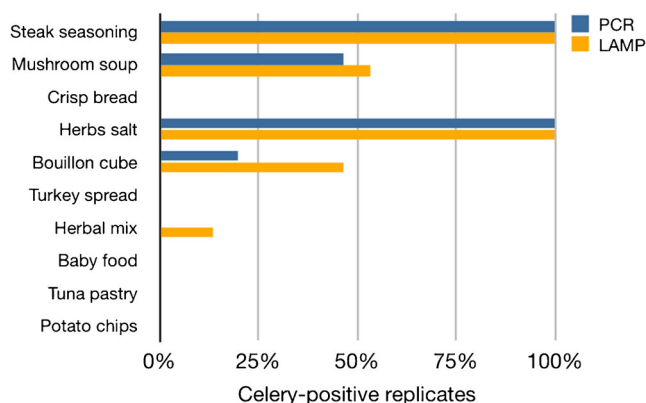


Fig. 3 Both methods, PCR (blue) and LAMP (orange), give similar numbers of positive replicates. In some cases LAMP shows to be even more sensitive than the corresponding PCR assay

Table 4 Comparison of positive amplification signals obtained with one thermal cycler (Applied Biosystems 7500 Fast Real-time PCR) and two different heating blocks (Eppendorf Thermomixer plus and Peqlab Digital Block Heater HX-1), resulting in at least 19 of 20 positive replicates. Three different methods for the detection of the amplified DNA were chosen—Syto® 9 in a real-time PCR cycler, agarose gel electrophoresis and staining with SYBR Green I, 40 target copies were applied per reaction and the number of replicates was 20

Detection method amplification instrument	Real-time fluorescence detection	Agarose gel electrophoresis	SYBR Green I staining
Applied Biosystems 7500 Fast Real-Time PCR System	20/20	20/20	20/20
Heating block Eppendorf	Not possible	19/20	19/20
Heating block Peqlab	Not possible	19/20	19/20

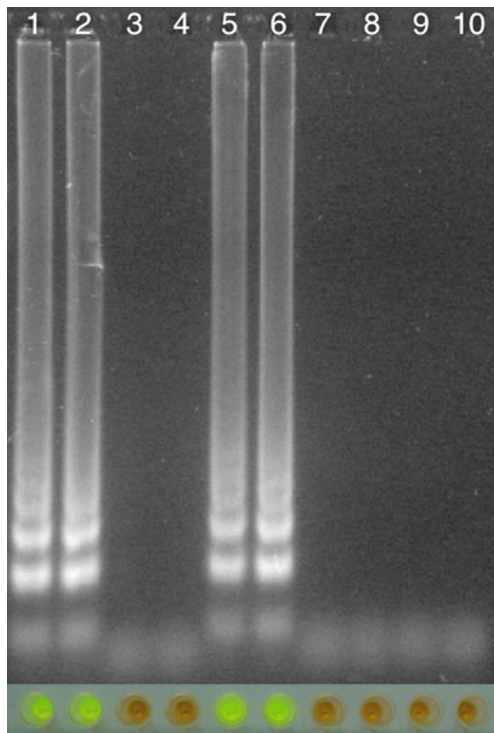


Fig. 4 Top agarose gel electrophoresis, bottom staining with SYBR Green I (green target present, orange no target present); 1, 2, 5, and 6 celery-positive sample (1 ng celery DNA/ μ L); 3 and 4 celery-negative sample, 7–10 no template control

changing the instrument for amplification (thermal cycler with real-time detection and a simple heating block) and subsequent detection techniques (agarose gel electrophoresis and nucleic acid staining). Figure 4 shows four celery-positive samples resulting in a typical LAMP gel electrophoresis pattern with various-sized amplification products. Staining with SYBR Green I results in a clearly distinguishable color change from orange to green in accordance with the results from the agarose gel electrophoresis.

Conclusion

The loop-mediated isothermal amplification assay was shown to be a highly specific and sensitive method for the detection of celery in food products. Although it is not suitable for the quantification of low amounts of celery DNA, it represents an accurate and robust technique that can be performed in less than 1 h without special equipment and therefore is a valuable tool for rapid and high-throughput analyses of food samples. Furthermore, the naked eye detection using SYBR Green I eliminates the need for a gel documentation system and allows the on-site visualization of the amplified DNA. This work contributes to a future approach on food analysis by describing a novel assay which meets the current demands for

analytical methods to be rapid, easy-to-perform and to have the potential for on-site applications.

Acknowledgments This work was financially supported by the Federal Country Lower Austria in cooperation with the European Regional Development Fund (ERDF).

References

1. Etesamifar M, Wüthrich B (1998) IgE-vermittelte Nahrungsmittelallergie bei 383 Patienten unter Berücksichtigung des oralen Allergie-Syndroms. *Allergologie* 21:451–457
2. André F, André C, Colin L, Cacaraci F, Cavagna S (1994) Role of new allergens consumption in the increased incidence of food sensitization in France. *Toxicology* 92:77–83
3. Ballmer-Weber BK, Vieths S, Lüttkopf D, Heuschmann P, Wüthrich B (2000) Celery allergy confirmed by double-blind, placebo-controlled food challenge: a clinical study in 32 subjects with a history of adverse reactions to celery root. *J Allergy Clin Immunol* 106:373–378
4. Wüthrich B, Stäger J, Johansson SGO (1990) Celery allergy associated with birch and mugwort pollinosis. *Allergy* 45:566–571
5. Jankiewicz A, Aulepp H, Baltes W, Bögl KW, Dehne LI, Zuberbier T (1996) Allergic sensitisation to native and heated celery root in pollen-sensitive patients investigated by skin test and IgE binding. *Int Arch Allergy Immunol* 111:268–278
6. Breiteneder H, Hoffmann-Sommergruber K, O’Riordain G, Susani M, Ahorn H, Ebner C, Kraft D, Scheiner O (1995) Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17-kDa tree pollen allergens. *Eur J Biochem* 233:484–489
7. Hoffmann-Sommergruber K, Demoly P, Cramer R, Breiteneder H, Ebner C, Laimer Camara da Machao M, Blaser K, Ismail C, Scheiner O, Bousquet J, Günther M (1999) IgE reactivity to Api g 1, a major celery allergen, in a Central European population is based on primary sensitization by Bet v 1. *J Allergy Clin Immunol* 107:478–484
8. Bublin M, Radauer C, Wilson IB, Kraft D, Scheiner O, Breiteneder H, Hoffmann-Sommergruber K (2003) Cross-reactive N-glycans of Api g 5, a high molecular weight glycoprotein allergen from celery, are required for immunoglobulin E binding and activation of effector cells from allergic patients. *FASEB J* 17:1697–1699
9. European Commission (2003) Off J Eur Union 46:15
10. Bollen MA, Garcia A, Cordewener JHG, Wichers HJ, Helsper JPFG, Savelkoul HFJ, van Boekel MAJS (2007) Purification and characterization of natural Bet v 1 from birch pollen and related allergens from carrot and celery. *Mol Nutr Food Res* 51:1527–1536
11. Demmel A, Hahn A (2010) In: Busch U (ed) *Molekularbiologische Methoden in der Lebensmittelanalytik*, 1st edn. Berlin, Springer
12. Stephan O, Weisz N, Vieths S (2004) Protein quantification, sandwich ELISA, and real-time PCR used to monitor industrial cleaning procedures for contamination with peanut and celery allergens. *J AOAC Int* 87(6):1448–1457
13. Hupfer C, Waiblinger HU, Busch U (2007) Development and validation of a real-time PCR detection method for celery in food. *Eur Food Res Technol* 225:329–335
14. Mustorp S, Engdahl-Axelsson C, Svensson U, Holck A (2008) Detection of celery (*Apium graveolens*), mustard (*Sinapis alba*, *Brassica juncea*, *Brassica nigra*) and sesame (*Sesamum indicum*) in food by real-time PCR. *Eur Food Res Technol* 226:771–778
15. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K (2000) Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res* 28(12):I–VII

16. Aliotta JM, Pelletier JJ, Ware JL, Moran LS, Brenner JS, Kong H (1996) Thermostable Bst DNA polymerase I lacks a 3'→5' proof-reading exonuclease activity. *Genet Anal Biomol Eng* 12(5–6):185–195
17. Nagamine K, Hase T, Notomi T (2002) Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Mol Cell Probes* 16:223–229
18. Iwamoto T, Sonobe T, Hayashi K (2003) Loop-mediated isothermal amplification for direct detection *Mycobacterium tuberculosis* complex, *M. avium*, and *M. intracellulare* in sputum samples. *J Clin Microbiol* 41(6):2616–2622
19. Focke F, Haase I, Fischer M (2013) Loop-mediated isothermal amplification (LAMP): methods for plant species identification in food. *J Agric Food Chem* 61:2943–2949
20. Dovičovičová L, Olexová L, Pangallo D, Siekel P, Kuchta T (2004) Polymerase chain reaction (PCR) for the detection of celery (*Apium graveolens*) in food. *Eur Food Res Technol* 218:493–495
21. Brunner K, Paris MPK, Paolino G, Bürstmayr H, Lemmens M, Berthiller F, Schuhmacher R, Krska R, Mach RL (2009) A reference-gene-based quantitative PCR method as a tool to determine *Fusarium* resistance in wheat. *Anal Bioanal Chem* 395:1385–1394
22. Monis PT, Giglio S, Saint CP (2005) Comparison of SYTO9 and SYBR Green I for real-time polymerase chain reaction and investigation of the effect of dye concentration on amplification and DNA melting curve analysis. *Anal Biochem* 340(1):24–34
23. Yang X, Quiros CF (1995) Characterizing the celery genome with DNA-based genetic markers. *J Amer Soc Hortic Sci* 120(5):747–751
24. European Network of GMO Laboratories (2008) Definition of minimum performance requirements for analytical methods of GMO testing. http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf
25. Han F, Ge B (2010) Quantitative detection of *Vibrio vulnificus* in raw oysters by real-time loop-mediated isothermal amplification. *Int J Food Microbiol* 142:60–66
26. Aoi Y, Hosogai M, Tsuneda S (2006) Real-time quantitative LAMP (loop-mediated isothermal amplification of DNA) as a simple method for monitoring ammonia-oxidizing bacteria. *J Biotechnol* 125:484–491
27. Mori Y, Kitao M, Tomita M, Notomi T (2004) Real-time turbidimetry of LAMP reaction for quantifying template DNA. *J Biochem Biophys Methods* 59:145–157
28. Cankar K, Štebih D, Dreo T, Žel J, Gruden K (2006) Critical points of DNA quantification by real-time PCR effects of DNA extraction method and sample matrix on quantification of genetically modified organisms. *BMC Biotechnol* 6:37
29. Kaneko H, Kawana T, Fukushima E, Suzutani T (2007) Tolerance of loop-mediated isothermal amplification to a culture medium and biological substances. *J Biochem Biophys Methods* 70:499–501
30. Lee D, La Mura M, Allnutt TR, Powell W (2009) Detection of genetically modified organisms (GMOs) using isothermal amplification of target DNA sequences. *BMC Biotechnol* 9:7