

Protein Quantification, Sandwich ELISA, and Real-Time PCR Used to Monitor Industrial Cleaning Procedures for Contamination with Peanut and Celery Allergens

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In the United States, peanut is one of the main sources of food allergens. Similarly, celery is a common allergenic food in Western Europe. Severe allergic reactions to both foods are common. Unexpected allergic reactions can occur after the consumption of celery- and peanut-free foods as a result of inadvertent cross-contaminations during manufacturing. Therefore, in cooperation with a flavor manufacturer, we monitored the cleaning process of slurry preparation equipment with regard to contaminations of follow-up products with celery and peanut compounds. Washing water samples taken after different cleaning steps and follow-up products were analyzed for the presence of celery and peanut traces with a celery-specific real-time polymerase chain reaction (PCR) and a peanut-specific sandwich enzyme-linked immunosorbent assay (ELISA). PCR and ELISA were compared with a nonspecific protein assay to evaluate whether the detection of protein traces can be a fast and cost-effective method for monitoring the effectiveness of wet cleaning procedures. Additionally, the allergenic potential of the celery and peanut mush, which were used as source material, were measured by a mediator release assay using a rat basophilic leukemia (RBL) cell line. In conclusion, the quantification of total protein in washing water was suitable for monitoring the cleaning process. Our study also revealed evidence that, in cases where wet cleaning is applicable, allergenic traces can be removed with high efficiency.

estimated that approximately 1–2% of the adult population suffers from a clinically proven food allergy. With up to 8%, the prevalence of food allergy in children is significantly higher (1–6). Ninety percent of serious anaphylactic reactions occurring after the consumption of foods can be traced back to peanuts, fish, crustacean, shellfish, milk, eggs, soybeans, tree nuts, or wheat (7, 8). It is estimated that in the United States about 30 000 anaphylactic reactions to food are treated in emergency departments and approximately 150–200 deaths occur each year (9).

Most reported severe anaphylactic reactions are related to the ingestion of peanut or tree nuts (10–12). The prevalence of peanut allergy is estimated to be 0.6–1.0% (13–17). Furthermore, in 2 double-blind placebo-controlled food challenge (DBPCFC) studies, 200 µg peanut was sufficient to trigger an allergic reaction, confirming its high allergenic potential (18, 19). For these reasons, peanut allergy is a serious problem for the affected individual, and with the lack of an effective therapy these patients must strictly avoid peanut-containing foods, which can be a serious problem because of the occasional presence of undeclared peanut traces. For example, it is described that in 48% of peanut-allergic individuals, subsequent reactions caused by inadvertent consumption of peanuts occur within 1 year from the last allergic event as a result of accidental intake of peanut (20).

In Europe, a high prevalence of allergy against celery is observed (19). Celery allergy is often associated with a primary sensitization against birch or mugwort pollen. This cross-reactivity is caused by structural similarities between allergens from these species and celery proteins (21–24). Other birch-associated cross-reactions can occur, e.g., against hazelnut, carrot, apple, and cherry. In Germany, about 70% of subjects with pollen-associated food allergy tested positive for celery by skin prick test or enzyme allergosorbent test (EAST; 24). Similar studies were performed in Switzerland and France where 30–40% of food-allergic patients were sensitized against celery (25). Furthermore, severe allergic reactions were described after the ingestion of celery products (25–27). In a DBPCFC study, it was demonstrated that 0.7 g fresh celery was sufficient to trigger objective allergic reactions. However, threshold doses for celery intake could not be defined because in most of the tested individuals

Food allergies are a common problem in industrialized Western countries. In the United States and Europe, it is

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allergic symptoms already occurred after consumption of 0.7 g celery, the lowest investigated dose (28). Therefore, the threshold dose for celery is expected to be substantially lower.

Unexpected contaminations of foods with allergens or mislabeling are common problems for allergic consumers. These so-called hidden allergens can result from contaminated raw materials, cross-contacts with allergen-containing foods, and carryover contaminations caused by insufficient cleaning during manufacturing. Over the past years, we and others have examined several prepackaged food products for the presence of hidden allergens. Up to 64% of the tested foods contained undeclared traces of allergens (29–36). In some cases, contaminations at levels of several grams per kilogram were observed. Such foods constitute a potential risk for allergic persons.

For better protection of food-allergic consumers, the European Union (EU) has modified the labeling regulation for prepackaged foods. In an amended directive for food labeling (2000/13/EC amended by 2003/89/EC), it is specified that labeling of food ingredients with an allergenic potential is obligatory without any exception. For foods that contain any of the so-called “big eight” allergens (egg, fish, milk, peanut, crustacean, soy, tree nuts, and gluten-containing cereals), labeling is obligatory. In addition, mustard, celery, sesame seed, and the pseudoallergen sulfite (>10 mg/kg) are included in the EU list for labeling.

In Switzerland, a revised regulation for food labeling was implemented in May 2003. Therein the “big eight” allergens plus celery must be labeled, even if they are not part of the recipe, provided that their concentration exceeds 1 g/kg.

Facing such revised regulations in the EU and Switzerland, food manufacturers will have to assess the risk of contamination during production of foods. Consequently, the efficacy of cleaning processes must be validated with methods suitable for detection of allergens.

In this study, we monitored the cleaning process of a slurry preparation equipment that is used to produce intermediate stages of flavorings and is subsequently subjected to spray-drying. Worst-case recipes (WCR) with a high content of celery (40%) and peanut (30 and 20%) were used for this approach. After processing the model recipes, the slurry preparation equipment was cleaned with alkaline and acidic detergent solutions. A celery-specific real-time polymerase chain reaction (PCR) and a peanut-specific sandwich enzyme-linked immunosorbent assay (ELISA) were used to analyze samples of washing water collected after cleaning. The PCR method was chosen because of a lack of availability of commercial or published celery-specific immunological assays. Results of the PCR and the ELISA were compared with the Bradford protein assay to evaluate whether a fast and cost-effective determination of total proteins in washing water can efficiently monitor the efficacy of cleaning processes applied to slurry preparation equipment. Additionally, we evaluated the influence of the processing process on the potential allergenicity of the celery and peanut mush used in the model experiments. For this, a rat basophilic leukemia (RBL) cell mediator release assay (37, 38) was applied as a

murine *in vitro* model to assess the allergenic potential of the tested food. Such data can be important for the food industry with regard to the possibility of modifying the manufacturing process of ingredients to reduce the allergenic potential.

METHOD

Sample Preparation

For DNA preparation, a commercial kit (SureFood Plant X, Congen, Berlin, Germany) was used according to the manufacturer's instructions. Briefly, 150 mg sample or 150 µL rinse water was lysed for 30 min with lysis buffer and proteinase K that were provided with the kit. After centrifugation, the supernatant was clarified by filtration. For optimal DNA binding to the silica matrix, binding buffer was added to the supernatant. After DNA binding to the silica column and subsequent washing steps, the pre-purified DNA was eluted with elution buffer. Again, the DNA was bound to a new silica column, washed, and finally eluted with 50 µL elution buffer. The purified DNA could be stored for 4 weeks at –20°C.

Primers and Probes

All oligonucleotides for real-time PCR were synthesized by MWG Biotech, Ebersberg, Germany. Primers and probe were specific for the *api g 1* gene. Forward Primer (Api-346F): 5'-ACCACTGCCATCTTTCACA-3'; Reverse Primer (Api-490R): 5'-TGACATTACAACAAGACATTCC-3'; Probe (Api-391T): 5'-CTTCTGGAACCACAGCATCACCT-3' (5'FAM / 3'TAMRA). The size of the generated amplicon was 145 base pairs (bp).

Real-Time PCR Procedure

A mastermix sufficient for 200 reactions was prepared by mixing the following compounds in a volume of 9 mL: 1× TaqMan buffer A; 20 µg/mL bovine serum albumin (BSA); 5mM MgCl₂; 200 µM dATP, dCTP, and dGTP; 400 µM dUTP; 300nM oligonucleotides; 200nM TaqMan probe. The mastermix was stored in 20 aliquots of 442.5 µL at 4°C. One aliquot was sufficient for 10 reactions. A 2.5 µL volume of Taq-polymerase (5 U/µL) and 5 µL Uracil-N-glycosylase (UNG, 1 U/µL) were added immediately to each aliquot before pipetting the final PCR reaction. Chemicals and enzymes used for the mastermix were part of the TaqMan PCR Core Reagent Kit (Applied Biosystems, Weiterstadt, Germany). BSA (Calbiochem, Schwalbach, Germany) used in this assay was certified to be free of nuclease and DNase. PCR reactions were prepared by mixing 45 µL mastermix (including Taq-polymerase and UNG) and 5 µL sample DNA in a 96-well optical plate (Applied Biosystems). UNG was used to avoid false-positive results caused by carryover contaminations from previous amplification reactions. DNA samples were tested in duplicate. For sample analysis, 2 separate DNA extractions were performed and amplified in 2 separate reactions. On each 96-well plate, 6 nontemplate controls (NTCs) were used to ensure PCR performance. The following PCR conditions (time-release protocol) were used:

primary incubation step at 50°C for 2 min (UNG digest); second step at 95°C for 2 min (activation of hot start Taq-polymerase); and 55 cycles of 95°C for 30 s and 58°C for 1 min. Fluorescence was read after each cycle in an ABI Prism 7700 (Applied Biosystems).

Sandwich ELISA Procedure

The peanut specific-sandwich ELISA was performed as described elsewhere (39). Briefly, cavities of a 96-well plate were coated overnight at 4°C with sheep antiserum raised against peanut. Blocking was performed for 1 h at 37°C followed by 4 washes with 200 µL washing buffer. The samples of washing water from the slurry preparation equipment were diluted 1:5 in incubation buffer, and 150 µL was applied to the plate. Duplicate washing water samples were analyzed. After incubation for 1 h at 37°C, ELISA plates were again washed 4 times. A 150 µL volume of rabbit antiserum was applied and incubated for 1 h at 37°C followed by a washing step. Then, 150 µL horseradish peroxidase-labeled detection antibody from goat raised against rabbit IgG was added for 1 h at 37°C. After washing, 150 µL substrate buffer was applied and incubated for 15–30 min at room temperature. Substrate reaction was stopped by adding sulfuric acid, and the coloration was measured with a microplate reader. Logarithmic regression of standard curves was performed with the 4-Parameter-Logistic model. The limit of quantitation was 5 ng/mL peanut protein.

Agarose Gel Electrophoresis

For analyzing the PCR products by gel electrophoresis, 5 µL of the PCR reactions were mixed with 1 µL sample buffer, containing 15% ficoll, 0.06% bromophenol blue, and 0.06% xylencyanol. Samples were loaded onto a 3% agarose gel. Gels were run with 1× triethylamine (TAE) in a RAGE RGX-60 chamber (TEBU, Frankfurt/Main, Germany) for 10 min at 200 V. Gels were stained with ethidium bromide (20 µg/mL) and visualized on a UV transilluminator.

Manufacturing of Celery and Peanut Mush

The celery mush used for the WCR was prepared by chopping and squeezing fresh celery root (celeriac). Afterwards, the celery mush was treated with pectinases and heated for 6 s at 140°C and subsequently evaporated for 60 s at a temperature between 50° and 60°C. The commercially available peanut mush was produced by grinding oil-roasted peanuts. No further treatment was applied.

Protein Extraction

Protein extracts for the rat basophilic mediator release test were produced as follows: prior to protein extraction 40 g raw and cooked celery (boiled in water for 20 min) and from roasted peanuts (140°C, 20 min) were frozen with liquid nitrogen and homogenized in an analytical mill (Grindomix GM 200, Retsch, Haan, Germany). Homogenization was not necessary in the case of the celery and peanut mush. For protein extraction, 2 g homogenized celery material was suspended in 20 mL phosphate-buffered saline (PBS, pH 7.4;

10mM NaH₂PO₄; 70mM Na₂HPO₄; and 150mM NaCl) and stirred overnight at 4°C. Protein from the peanut mush was extracted by suspending 1 g mush in 20 mL 25mM Tris-tricine buffer (pH 8.6) at 45°C for 2 h. For clarification, the extracts were centrifuged at 20 000 × g. The supernatant was stored in aliquots at –20°C.

RBL Cell Mediator Release Assay

To estimate the allergenic potential of the celery and peanut mush, we used an in vitro mediator release assay. The RBL cell assay was performed as previously described (37, 38). Briefly, RBL cells (subline 2H3) were passively sensitized with murine IgE raised against extracts from cooked celery root (100°C, 30 min) and roasted peanuts (140°C, 45 min). Afterwards, cells were incubated with serial dilutions of extracts from fresh celery and roasted peanuts that were used as reference. Specific cross-linking of receptor-bound IgE by allergens elicits mediator-release by RBL cells. For visualization *p*-nitrophenyl-*N*-acetyl β-D-glucosaminide, a substrate for the mediator β-hexosaminidase, was added. The resulting color was read in a photometer at 405 nm. The mediator release induced by the tested celery and peanut samples was related to the maximal release induced by an anti-IgE antibody. To determine the spontaneous mediator release, the RBL cells were incubated with Tyrode's buffer. Unspecific release caused by peanut or celery extract in the absence of IgE antibody was excluded in separate experiments. The percentage of the mediator release induced by the allergen was calculated as follows:

$$\text{Release, \%} = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{spontaneous release}}}{\text{OD}_{\text{maximal release}} - \text{OD}_{\text{spontaneous release}}} \times 100 [\%]$$

Bradford Protein Assay

A commercial Bradford assay (Pierce Coomassie Plus, Perbio Science, Bonn, Germany) was used for protein determination. Assays were performed according to the manufacturer's instructions. The assay setup was suitable to quantify protein concentrations ranging from 1 to 25 µg/mL. BSA was used as protein standard.

Industrial Equipment, Model Recipes, Cleaning Procedure, and Collection of Samples

For validation of the cleaning process, a commercial scale of the original slurry preparation equipment was used (Figure 1B). To simulate a worst-case scenario, recipes (Table 1) with high contents of celery (WCR-Ce) and peanut (WCR-Pn) were processed. After dispersing (5 min) a WCR, the slurry preparation equipment was superficially cleaned with 50 L water. At this time, the first washing water samples were taken from 3 different sampling points (Figure 1B). Then, the equipment was initially cleaned with P3mip alkaline solution (Henkel, Düsseldorf, Germany) with a conductance of 22 mS/cm. Afterwards, the equipment was extensively washed with water to remove residuals of the cleaning solution until the conductance was <0.5 mS/cm. At this time, the second

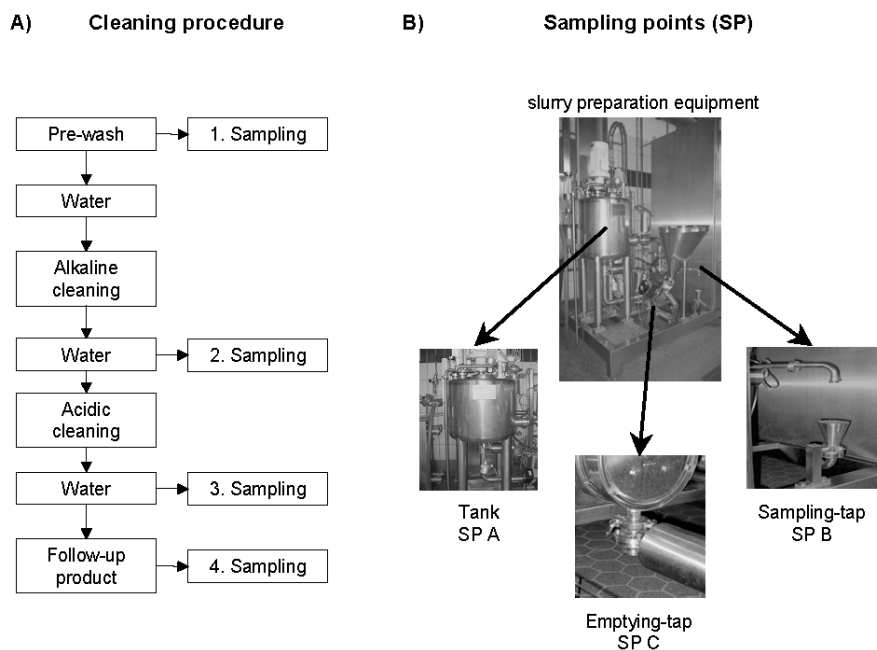


Figure 1. Washing and sampling procedure. After each of the 3 washing steps (water, alkaline solution, acidic solution), rinse-water samples were collected (A) from 3 sampling points (B). This procedure was repeated 4 times.

set of washing water samples was taken at the same sampling points. Afterwards, the cleaning process was continued with an acidic solution (P3-horolith CIP, conductance 5.5 mS/cm, Henkel). Again, the equipment was extensively washed with water to reach a conductance <0.5 mS/cm. The third set of samples was then taken. All washing water samples were analyzed by sandwich ELISA for the presence of peanut, by real-time PCR for the presence of celery, and by the Bradford assay for protein traces. After the cleaning, an artificial follow-up product (50 kg water, 50 kg maltodextrin) was dispersed (5 min) through the equipment and finally analyzed by sandwich ELISA for peanut contaminations and by real-time PCR for celery contaminations. The whole test procedure was repeated 4 times on 4 different days.

Results and Discussion

Sensitivity of the Real-Time PCR

To evaluate the sensitivity of the real-time PCR, we purified DNA from celeriac, celery powder, and celery seeds and from the celery mush that was used in the WCRs described above. These isolates were serially diluted and used as template for the real-time PCR (Figure 2). About 60 pg template DNA from celery roots and approximately 250 pg DNA from celery powder were suitable for amplification. Approximately 30 pg DNA from celery seed were necessary for successful amplification. In the case of the celery mush, we observed decreased sensitivity. At least 500 pg template DNA was necessary for successful amplification. This decreased sensitivity could be the result of a DNA degradation. This assumption was supported by the

appearance of the genomic DNA, displayed in Figure 2, lane 9. In comparison to the other DNA isolates, the proportion of DNA with high molecular weight was clearly reduced in celery mush. These results indicate that the sensitivity of the PCR assay depends on the degree of processing of the celery sources. To estimate the sensitivity of the PCR assay in the matrixes used in this study, serial dilutions of the WCR-Ce and of the celery mush were prepared and 150 µL of each dilution was used for 2 separate DNA preparations. Duplicates of the prepared DNA were used as template for the PCR assay (Table 2).

The PCR results indicated that the limit of detection (LOD) for the PCR was between 4 and 10 mg/mL celery. To correlate

Table 1. Contents of model products

Content	Worst-case recipes (WCR)	
	Celery (WCR-Ce)	Peanut (WCR-Pn)
Water	50 L	50 L
Celery mush	40 kg	— ^a
Peanut mush	—	30 kg
Maltodextrin	10 kg	10 kg
Starch	—	10 kg
Neutral oil	—	—
Total weight	100 kg	100 kg

^a — = Recipe does not contain this ingredient.

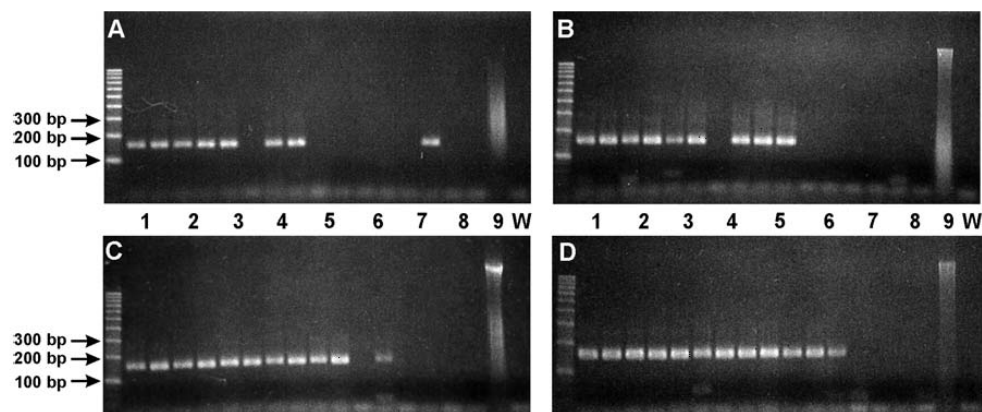


Figure 2. For determination of PCR sensitivity, DNA from celery mush (A), celery powder (B), raw celery (C), and celery seed (D) were isolated and used as template for PCR. PCR reactions were analyzed in a 3% agarose gel stained with ethidium bromide. The following DNA amounts were used: 1000 pg (lane 1); 500 pg (lane 2); 250 pg (lane 3); 125 pg (lane 4); 62.5 pg (lane 5); 31.25 pg (lane 6); 15.6 pg (lane 7); 7.8 pg (lane 8); water-control (lane 9). To estimate DNA quality, 200 ng genomic DNA of each isolate was applied to the agarose gel (lane 9). PCR reactions were run in duplicate.

this to a specific level of total protein, we extracted protein from 1 g mush in 10 mL water for 10 min at room temperature. After centrifugation, the protein concentration of the supernatant was determined. The conditions for protein extraction were adapted to the processing procedure of the WCR-Ce. We found that the celery mush contained approximately 0.36% extractable protein. Comparing the PCR and Bradford results, we concluded that it should be possible to isolate amplifiable DNA from washing water that contains celery protein between 14 and 36 $\mu\text{g/mL}$.

Monitoring of the Cleaning Procedure

For validation of the cleaning procedure, the WCRs with high contents of peanut and celery were processed by the slurry preparation equipment. Samples of the washing water and of the artificial follow-up product were collected from 4 different

production processes. Each sample was analyzed for the presence of peanut or celery by specific methods. Furthermore, the washing water was tested for protein traces by a commercial Bradford assay. The follow-up products could not be analyzed with this assay because of a strong background signal that was due to maltodextrin (data not shown).

Analysis of the Washing Water for the Presence of Peanut

All washing water samples taken during the prewash procedure were positive by ELISA and by the Bradford assay (Table 3). The concentration of peanut protein measured by ELISA varied between 98.5 and 1379 $\mu\text{g/mL}$. These results were in good correlation with the protein concentrations measured by the Bradford assay. The washing water samples collected after the alkaline and acidic wash procedure were all

Table 2. Determination of PCR sensitivity and correlation to protein concentration

Celery mush, mg/mL	Corresponding celery amount, ^a mg	Corresponding protein concentration, $\mu\text{g/mL}$	PCR results, positive/total
100	15	360	4/4
10	1.5	36	2/4
1	0.15	3.6	0/4
Dilution of WCR-Ce celery concn, mg/mL			
1:10 (40)	6	140	4/4
1:100 (4)	0.6	14	2/4
1:1000 (0.4)	0.06	1.4	0/4

^a Absolute amount of celery mush in a dilution volume of 150 μL . This volume was used for DNA isolation.

negative by both ELISA and Bradford. Surprisingly, one follow-up product (FP 2) was contaminated with 1.1 ppm peanut protein. This contamination could be traced back to an application error during the manufacturing process.

Analysis of the Washing Water for the Presence of Celery

As expected, in each of the washing water samples from the prewashing procedure we observed protein contaminations ranging from 2.4 to 12.6 $\mu\text{g/mL}$ (Table 4). When analyzed by PCR, only samples 1B and 1C from test run No. 1 and sample 28C from test run No. 4 were positive. The lower sensitivity of the PCR may result from a decreased detectability of celery mush, because the conditions of celery mush processing may have caused increased DNA degradation or because of the presence of PCR inhibitors in the template DNA. In all samples taken after the alkaline and acidic cleaning, no protein contaminations were observed. In addition, the follow-up products were tested negative for celery in all cases.

RBL Cell Mediator Release Assay

To compare the allergenic potential of different celery sources, we tested extracts from raw celery, cooked celery, and from the celery mush in an RBL mediator release test. In the case of peanut, we compared extracts from roasted peanuts and from the mush. Three different protein concentrations (0.1, 1, and 10 $\mu\text{g/mL}$) were tested. For passive sensitization of the RBL cells, mouse sera containing IgE antibodies raised against alum-adsorbed cooked celery and roasted peanuts were used. Figure 3A shows that the mediator release induced by the extract of celery mush was clearly below the release induced by extracts of raw and cooked celery. This indicates a lower allergenic potential of celery mush than in native and cooked celery. In contrast, the allergenic potential of the peanut mush was higher than that of the roasted peanuts (Figure 3B).

General Considerations and Conclusions

To evaluate the risk of carryover contaminations and to test the possibility of monitoring a cleaning process with a simple Bradford assay, we examined the washing water of slurry preparation equipment, which is used to produce spray-dried flavorings, for celery and peanut contamination. The results of this study indicate that the applied cleaning process was effective and that it was possible to monitor the cleaning process with a simple Bradford assay for total protein.

For analyzing the washing water for the presence of peanut traces, we used a sandwich ELISA based on polyclonal sheep and rabbit sera. As expected, we found in all samples taken after the prewash procedure contamination with peanut protein ranging from 98.5 to 1379 $\mu\text{g/mL}$. The concentrations of peanut protein measured by ELISA were in good correlation with those measured by the Bradford assay. All washing water samples analyzed after alkaline and acidic cleaning were negative for the presence of peanut protein by

the sandwich ELISA and by the Bradford assay. In contrast to these results, we found in the follow-up product FP 2 a contamination with peanut protein at a level of 1.1 ppm. This contamination could be traced back to an application error. A funnel was used to fill the slurry preparation equipment with the ingredients of the WCR. The funnel was then cleaned with hot water. This cleaning procedure was done insufficiently, resulting in residues of peanut mush contaminating the follow-up recipe. However, we cannot fully exclude the possibility that this contamination was caused by peanut residues in the slurry preparation equipment in spite of the fact that no peanut residues were found in the rinse water after alkaline and acidic cleaning.

For analyzing washing water samples with a celery-specific method and because of a lack of commercially available or published immunological assays, we established a real-time PCR. The sensitivity of the PCR for the material used in this study was evaluated by diluting celery mush and the processed WCR-Ce. The LOD was determined to be 0.6–1.5 mg celery mush, corresponding to approximately 10–30 $\mu\text{g/mL}$ protein in the washing water. However, this estimate is based on the assumption that protein solubility parallels solubility and extractability of DNA in a dilution series. For approaches in which a higher sensitivity is required, it should be possible to enhance the sensitivity by modifying the DNA extraction procedure, e.g., by increasing the amount of sample.

Because of cross-reactivities with some spices (data not shown), we do not recommend use of this assay for analyzing food products with complex recipes. In our study, contaminations with celery were identified only after the prewash procedure. The protein content ranged between 2.4–12.6 $\mu\text{g/mL}$, which was much lower than the results of the peanut study. A reason for this observation could be the high protein content of the peanut mush compared with the celery mush.

All washing water samples that were collected after the equipment were cleaned with alkaline or acidic solution, and all follow-up products were negative for celery and protein traces. In the case of the celery WCR, it is difficult to evaluate the efficiency of the whole cleaning procedure with the applied methods because, although a large amount of celery was used in the WCR, the level of extractable celery protein was relatively low.

This study demonstrates the possibility of monitoring a wet cleaning process by a fast and cost-effective protein determination. In the case of celery, the Bradford assay was even more sensitive than the real-time PCR used for analysis. For processing the celery mush, celery roots were treated with pectinases followed by evaporation. This procedure presumably leads to DNA degradation and consequently to reduced sensitivity of the PCR. Nevertheless, the PCR was sensitive enough to amplify celery DNA when the protein content of the washing water was within 10–30 ppm. This protein concentration corresponds to our suggested threshold value for contamination of the washing water. The rationale for this threshold is based on the assumption that the protein

Table 3. Analysis of washing water samples and follow-up products for the presence of peanut

Procedure	Sample	ELISA, µg/mL	ELISA, %CV	Bradford, µg/mL	Bradford, %CV	Result
Test run 1						
Prewash	4A	204.1	8.5	116.8	6.5	Positive
	4B	167.6	15.5	103.7	6.0	Positive
	4C	153.7	4.4	117.7	6.8	Positive
After alkaline cleaning	5A	0	/ ^a	0	/	Negative
	5B	0	/	0	/	Negative
	5C	0	/	0	/	Negative
After acidic cleaning	6A	0	/	0	/	Negative
	6B	0	/	0	/	Negative
	6C	0	/	0	/	Negative
Follow-up product	FP 1	0	/	ND ^b	— ^c	Negative
Test run 2						
Prewash	13A	120.6	10.0	114.1	4.3	Positive
	13B	1379.0	2.7	917.6	7.4	Positive
	13C	127.2	7.5	119.1	7.1	Positive
After alkaline cleaning	14A	0	/	0	/	Negative
	14B	0	/	0	/	Negative
	14C	0	/	0	/	Negative
After acidic cleaning	15A	0	/	0	/	Negative
	15B	0	/	0	/	Negative
	15C	0	/	0	/	Negative
Follow-up product	FP 2	1.1	6.2	ND	—	Positive
Test run 3						
Prewash	22A	150.1	7.5	140.9	5.3	Positive
	22B	99.5	17.1	136.0	3.7	Positive
	22C	202.4	13.9	138.5	3.4	Positive
After alkaline cleaning	23A	0	/	0	/	Negative
	23B	0	/	0	/	Negative
	23C	0	/	0	/	Negative
After acidic cleaning	24A	0	/	0	/	Negative
	24B	0	/	0	/	Negative
	24C	0	/	0	/	Negative
Follow-up product	FP 3	0	—	ND	—	Negative
Test run 4						
Prewash	31A	105.1	9.7	85.9	0.9	Positive
	31B	98.5	11.8	95.1	6.1	Positive
	31C	163.8	16.4	116.9	8.9	Positive
After alkaline cleaning	32A	0	/	0	/	Negative
	32B	0	/	0	/	Negative
	32C	0	/	0	/	Negative
After acidic cleaning	33A	0	/	0	/	Negative
	33B	0	/	0	/	Negative
	33C	0	/	0	/	Negative
Follow-up product	FP 4	0	/	ND	—	Negative

^a / = No % CV calculated, as sample was tested negative.^b ND = Not determined by the Bradford assay.^c — = No % CV calculated, as sample was not tested.

Table 4. Analysis of washing water samples and follow-up products for the presence of celery

Procedure	Sample	Real-time PCR, positive/negative	Final PCR result	Bradford, µg/mL	Bradford, %CV	Final Bradford result
Test run 1						
Prewash	1A	0/4	Negative	2.8	3.8	Positive
	1B	4/0	Positive	12.6	4.3	Positive
	1C	3/1	Positive	3.3	4.8	Positive
After alkaline cleaning	2A	0/4	Negative	0	/ ^a	Negative
	2B	0/4	Negative	0	/	Negative
	2C	0/4	Negative	0	/	Negative
After acidic cleaning	3A	0/4	Negative	0	/	Negative
	3B	0/4	Negative	0	/	Negative
	3C	0/4	Negative	0	/	Negative
Follow-up product	FP1	0/4	Negative	ND ^b	— ^c	Negative
Test run 2						
Prewash	10A	0/4	Negative	3.3	4.1	Positive
	10B	0/4	Negative	3.2	4.4	Positive
	10C	0/4	Negative	3.1	2.9	Positive
After alkaline cleaning	11A	0/4	Negative	0	/	Negative
	11B	0/4	Negative	0	/	Negative
	11C	0/4	Negative	0	/	Negative
After acidic cleaning	12A	0/4	Negative	0	/	Negative
	12B	0/4	Negative	0	/	Negative
	12C	0/4	Negative	0	/	Negative
Follow-up product	FP2	0/4	Negative	ND	—	Negative
Test run 3						
Prewash	19A	0/4	Negative	2.4	5.5	Positive
	19B	0/4	Negative	2.5	7.1	Positive
	19C	1/3	Negative	2.6	8.1	Positive
After alkaline cleaning	20A	0/4	Negative	0	/	Negative
	20B	0/4	Negative	0	/	Negative
	20C	0/4	Negative	0	/	Negative
After acidic cleaning	21A	0/4	Negative	0	/	Negative
	21B	0/4	Negative	0	/	Negative
	21C	0/4	Negative	0	/	Negative
Follow-up product	FP3	0/4	Negative	ND	—	Negative
Test run 4						
Prewash	28A	1/3	Negative	2.5	7.8	Positive
	28B	0/4	Negative	2.7	3.7	Positive
	28C	4/0	Positive	2.9	4.7	Positive
After alkaline cleaning	29A	0/4	Negative	0	/	Negative
	29B	0/4	Negative	0	/	Negative
	29C	0/4	Negative	0	/	Negative
After acidic cleaning	30A	0/4	Negative	0	/	Negative
	30B	0/4	Negative	0	/	Negative
	30C	0/4	Negative	0	/	Negative
Follow-up product	FP4	0/4	Negative	ND		Negative

^a / = No % CV calculated, as sample was tested negative.^b ND = Not determined by the Bradford assay.^c — = No % CV calculated, as sample was not tested.

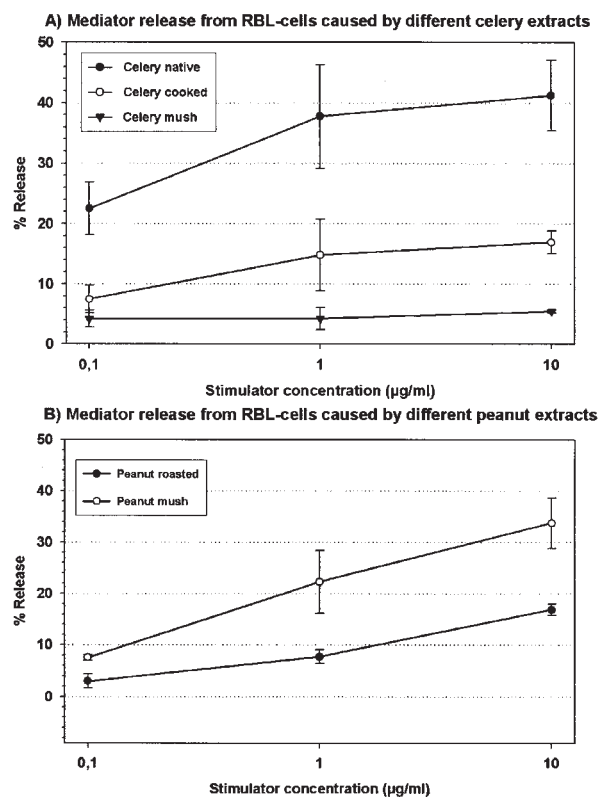


Figure 3. For assessing the allergenic potential, protein extracts of peanut and celery mush were analyzed by the RBL cell mediator release assay and compared with extracts from raw and cooked celery (A) and with an extract of roasted peanuts (B). Different concentrations of protein (0.1, 1, and 10 µg/mL) were applied to the sensitized RBL cells, and the mediator release was measured.

residuals are diluted at least 10-fold when a follow-up product is processed on the equipment. This reduction would result in a hypothetical contamination of a follow-up product at approximately 1 ppm protein. As suggested by Taylor and Nordlee (40) this level of contamination is acceptable for allergic individuals. Similar suggestions were made by the German Society of Allergy and Clinical Immunology in a position paper (41).

Another approach to assess the allergenic risk for the consumer is to evaluate the allergenic potential of the processed food. In this study, we compared an extract of the celery and peanut mush with extracts of raw and cooked celery and with an extract of roasted peanuts by an RBL mediator release assay. The results showed that the mediator release induced by celery mush was clearly decreased in comparison to raw and cooked celery. We therefore assume that the allergenic potential of the celery mush was drastically reduced. Similar results indicating a decreased allergenicity of heat-treated celery were obtained in studies by Hoffmann et al. (37) and Jankiewicz et al. (42). Nevertheless, we cannot fully exclude the possibility that this celery material is sufficient to trigger allergic reactions because, in a clinical

study by Ballmer-Weber et al. (43), some patients presented allergic reactions against celery that was heat-treated for 76 min at 100°C. In contrast, the mediator release induced by the protein extract of peanut mush was even higher than that induced by a protein extract of roasted peanuts, indicating a similar or higher allergenic potential of the commercial food ingredient.

We have shown that the risk of carryover contamination can be evaluated by a simple unspecific protein determination of the washing water. Moreover, carryover contamination is unlikely to occur with the described cleaning procedure. Such an evaluation of the cleaning effectiveness can increase food safety because precautionary labeling, such as “may contain...”, can be omitted in cases where cross-contamination of food products is at low risk. In our opinion, this would be a great benefit for the allergic consumer, as food can be labeled in a precise and transparent fashion.

Future studies should examine other production processes, for example, of confectionery products, because a high percentage of such foods are contaminated with peanut and hazelnut. Furthermore, rapid tests such as dipstick ELISAs or lateral flow devices for detecting trace amounts of the “big eight” allergens in processed foods should be developed to monitor the implementation of new labeling guidelines and to make fast and easy in-process controls possible for food manufacturers.

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