

Original article

Influence of food processing on the allergenicity of celery: DBPCFC with celery spice and cooked celery in patients with celery allergy

Background: Celery root is often consumed in a processed form as a cooked vegetable or as a spice. So far, however, there has been no information about the allergenicity of processed celery in celery-allergic patients.

Methods: In 12 patients with a history of allergic reactions to raw or raw and cooked celery, double-blind placebo-controlled food challenges (DBPCFCs) with raw celery ($n=10$), cooked celery ($110^{\circ}\text{C}/15\text{ min}$; $n=11$), and celery spice ($n=5$) were performed. Nine patients underwent an open mucosal challenge with four samples of canned celery retorted at Co-values (cooking effect) of 7.45–76.07 (corresponding to the time periods in minutes at a thermal influence of 100°C). IgE immunoblot analysis of celery extract was performed with sera of all challenged patients. The thermal stability of celery allergen was investigated by enzyme allergosorbent test (EAST) inhibition. Furthermore, intraperitoneal immunization of mice followed by a rat basophil leukemia (RBL) cell mediator release assay was used as a biological *in vitro* model to assess the allergenicity of processed celery.

Results: Six out of 11 patients showed a positive DBPCFC to cooked celery and five out of five patients to celery spice. Allergenicity of celery was preserved in four patients with a positive DBPCFC to cooked celery even if celery was treated at a Co-value of 76.07. Patients with positive DBPCFC to cooked celery reacted to known celery allergens (Api g 1, Api g 4, cross-reactive carbohydrate determinants CCD). EAST inhibition showed that heat resistance of celery allergens decreases in the following order: $\text{CCD} > \text{Api g 4} > \text{Api g 1}$.

Accordingly, five of six patients with a positive DBPCFC to cooked celery were sensitized to profilin and/or CCD. The murine model reflected the reactivity of patients sensitized to the major allergen Api g 1.

Conclusions: 1) In a subset of patients with a positive DBPCFC to cooked celery, celery remains allergenic even after extended thermal treatment ($76.07\text{ min}/100^{\circ}\text{C}$). 2) Celery spice is allergenic for patients with an allergy to raw celery. 3) RBL cells sensitized with mouse IgE to raw celery may serve as a useful tool for screening the potential allergenicity of heat-processed products containing celery.

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Key words: celery allergy; DBPCFC; double-blind placebo-controlled food challenge; food allergy; immunoblotting; processed celery; spice; thermal treatment.

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Celery (*Apium graveolens*) which belongs to the Apiaceae family is a frequent cause of pollen-related food allergy, particularly in European countries (1–6). Celery allergy is highly associated with birch pollen and mugwort pollen sensitization referred to as birch–mugwort–celery syndrome (6, 7). Recently allergy to raw celery root was, for the first time, confirmed (8) by double-blind placebo-controlled food challenge (DBPCFC). Sera of DBPCFC-positive patients recognized known

cross-reactive allergenic structures: The Bet v 1-related major celery allergen, Api g 1, was detected by IgE from 59% of patients, 55% were sensitized to cross-reactive carbohydrate determinants (CCD) and 23% to the celery profilin, Api g 4 (9).

Celery root is not only consumed raw as fresh salad but also as a cooked vegetable and as a constituent of sauces and soups. Furthermore, dried and powdered celery root is widely used as an ingredient of spice mixtures. Jankiewicz and coworkers have recently investigated *in vitro* the immunochemical stability of the three known allergenic structures of celery when processed by microwaving, drying, γ -irradiation, ultra

Abbreviations: CCD: cross-reactive carbohydrate determinants; DBPCFC: double-blind placebo-controlled food challenge; OAS: oral allergy syndrome; SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SPT: skin prick test.

high pressure treatment and high voltage impulse treatment. The heat stability of the known celery allergens decreased in the following order: carbohydrate epitopes > profilin > Api g 1 (10). However, so far the allergenicity of processed celery root *in vivo* is largely unknown. The purpose of this study was:

1) to assess the allergenicity of processed celery by performing DBPCFCs with cooked celery and celery spice in subjects with a positive DBPCFC with raw celery;

2) to determine the optimal time period of heat treatment to reduce allergenicity of celery in allergic subjects;

3) to compare the allergens recognized by IgE from patients sensitized to raw and cooked celery with those from patients sensitized to raw but not to cooked celery;

4) and to establish a biological *in vitro* model to assess the potential allergenicity of processed celery.

Material and methods

Patients

Twelve patients with a history of allergic reactions to raw or raw and cooked celery were recruited at the Allergy Unit of University Hospital Zürich from January 2000 to February 2001. Exclusion criteria were: pregnancy, history of a severe life-threatening anaphylactic reaction after celery consumption, significant concurrent disease, medication with glucocorticosteroids, H₁-receptor antagonists, angiotensin-converting-enzyme inhibitors, or beta-blocking agents.

Ethical considerations

The study was reviewed and approved by the local ethical committee. All subjects provided written informed consent before enrolment in the study.

Skin tests

Skin prick tests (SPT) were performed on the flexor aspect of the forearm with a standardized prick needle (Stallerpoint, Stallergènes, Antony Cedex, France). Histamine dihydrochloride (10 mg/ml) was used as a positive control and the glycerol-containing diluent of the prick solution (Soluprick®, ALK, Hørsholm, Denmark) as a negative control. Patients were tested with celery extract (Allergopharma, Reinbeck, Germany). Raw native celery was tested using the prick-to-prick-technique (11). Reactions were recorded after 15 min. A wheal with a diameter of at least 3 mm was considered positive (12).

In vitro diagnosis

Specific IgE to celery, birch pollen, recombinant (r)Bet v 1, rBet v 2, rBet v 6 and mugwort pollen were measured by the CAP FEIA system (Pharmacia & Upjohn, Uppsala, Sweden). ImmunoCaps coupled with rBet v 6 were produced and kindly provided by Dr J. Lidholm of Pharmacia & Upjohn, Uppsala, Sweden (13).

Double-blind placebo-controlled food challenge (DBPCFC) with cooked celery and celery spice

DBPCFC was performed in 12 patients with raw celery root (I), in 11 patients with celery cooked at 110°C for 15 min (II) and in five

patients with celery spice, dried and pulverized celery (III), kindly provided by Dr N. Sauerwald (Nestlé, Frankfurt, Germany), using the two-step spit (local mucosal challenge) and swallow procedure as previously described (8). Briefly, two different drinks, identical in colour, texture and taste, were prepared for each challenge. The active drink contained: 20 g of raw celery (I), or 25 g of cooked celery (II), or 4.5 g of celery spice (III); 30 g (I) or 25 g (II) or 50 g (III) of cooked broccoli; 12.5 g of broccoli fond; 5 g of cream; 50 g of yoghurt without flavour; 25 g of water; 1 g of salt; mixed together in a blender. The protein content of celery spice is about 4.5 times higher than the protein content of raw celery. Thus 4.5 g of celery spice has a similar allergen content to 20 g of raw celery. The placebo drink contained the same ingredients but with no celery. Apart from the celery all ingredients were known to be tolerated by each patient.

One ml of active drink contained 0.144 raw celery, 0.18 cooked celery and 0.03 g celery spice.

Open provocation with cooked celery

Open mucosal challenges were performed with four samples of canned celery retorted under controlled conditions with Co-values (cooking effect) of 7.45, 13.12, 23.64, 76.07 (corresponding to the time period in minutes at a temperature of 100°C) at intervals of 15 min or until all symptoms had disappeared. Patients had to chew 25 g of cooked celery samples without having to swallow. Concerning the degree of thermal processing the challenge was performed in a single-blinded way. Challenges were started with celery cooked at Co-value 76.07 and continued with gradually less-processed samples.

Preparation of canned celery

Celery roots purchased on the market were manually peeled, cut into pieces of approximately 10 × 10 × 50 mm, and sliced by a common household machine.

Enamelled tin-plate cans (74 × 104 mm) were filled with 180 g of sliced celery and 220 g of tap water, then mechanically sealed. Four groups of cans were prepared and each group was retorted at 100°C, but for different times, in order to simulate different cooking processes. After thermal treatment the cans were put in running water to be cooled. Before the retorting treatment, a thermocouple was inserted in the centre of one can from each group, in order to record the whole time-temperature profile.

The four heat treatment conditions were normalized, expressing them as the Co value, analogous to Fo value (sterilizing effect), using the following equation (14):

$$Co =$$

where Co represents the equivalent time of treatment (min) at a reference temperature ($T^* = 100^\circ\text{C}$), t is the treatment time, T is the actual temperature and z represents the temperature increase that causes a 10-fold increase of the reaction rate of the chemical reaction taken as reference. We assumed for z the value of 25°C, which represents a mean value for the reactions of thermal damage in foods.

The Co-values calculated for the different thermal treatments were: 7.45, 13.12, 23.69, and 76.07 min.

Protein extracts and recombinant allergens

Celery extract was prepared from raw celery root as previously described (8). Freeze-dried and re-dissolved extracts were kept at -20°C until used. In the same way, extracts were prepared from celery root cooked at 100°C for 30 min and from celery retorted at Co-values of 7.45, 13.12, 23.64 and 76.07. Recombinant major celery allergen rApi g 1 (15) was obtained from Biomay (Linz, Austria).

Table 1. Symptoms and dose (g) of celery products producing symptoms during double-blind placebo-controlled food challenge with raw celery, cooked celery and celery spice

Patient number*	Celery raw			Celery cooked		Celery spice	
	Symptom history	Symptoms DBPCFC	Dose	Symptoms DBPCFC	Dose	Symptoms DBPCFC	Dose
1	OAS	OAS	0.7	OAS	0.9	OAS	0.16
2	U,AE,R,C,D	—	—	OAS	1.8	—	—
3	OAS	OAS	0.7	OAS	0.9	—	—
4	OAS	OAS	0.7	OAS	1.8	OAS,R,F	0.16
5	OAS	—	—	OAS	0.9	—	—
6	R,C,D,GIT	OAS,D,R,C	0.7	F,P,C,D	34.5	—	—
7	OAS,U	F,V	28.5	neg	—	—	—
8	F,GIT	AE	28.5	neg	—	F,AE,GIT	5.85
9	OAS	OAS	0.7	neg	—	—	—
10	OAS	OAS	0.7	neg	—	OAS,R,C,AE	0.32
11	U,GIT	OAS	0.7	neg	—	—	—
12	OAS	AE	28.5	—	—	OAS	0.16

*Number of patient corresponds to immunoblot results (lane) in Figures 1A and 1B.

OAS: oral allergy syndrome; U: urticaria; AE: angioedema; R: rhinitis; C: conjunctivitis; D: dyspnea; GIT: gastrointestinal symptoms; F: flush; V: vertigo; P: pruritus; neg: no symptoms.

Electrophoresis

Celery extract was separated by Tricine-SDS-PAGE according to Schägger and von Jagow using a Bio-Rad (Munich, Germany) Mini Protean cell (16). The 14% T, 2.6% C separating gel was overlaid by a 4% T, 2.6% C stacking gel. Proteins were reduced by 1,4-dithiothreitol (DTT) (Sigma-Aldrich, Deisenhofen, Germany) and loaded onto the gel at a concentration of 25 µg celery protein/cm.

Immunoblotting

The proteins were transferred onto nitrocellulose (NC) membranes by semidry blotting (17) and blocked twice in 50 mM Tris(hydroxymethyl)aminomethane/HCl buffer (pH 7.4) containing 0.15 M sodium chloride and 0.3% Tween 20 (TBST). The NC strips were incubated overnight with 80 µl of patients sera and control serum of a nonallergic donor in 520 µl TBST containing 0.1% bovine serum albumin (BSA). IgE antibody detection was performed with alkaline phosphatase-conjugated mouse antihuman IgE (1:1000, 4 h) (PharMingen, San Diego).

Enzyme allergosorbent test (EAST) inhibition

Extract from raw celery was coupled to cyanogen bromide-activated filter paper disks (Hycor, Kassel, Germany) at a protein concentration of 5 µg per disk (30 µg/ml in the coupling solution) according to the method originally described by Ceska and Lundkvist (18). The enzyme allergosorbent test (EAST) was performed with 'Allergopharma Spez. IgE ELISA' according to the instructions of the manufacturer (Allergopharma, Reinbek, Germany). Dose-related EAST inhibition studies were performed as described (10, 19). Concentrations of inhibitors and dilutions of sera are given in the legends of corresponding figures.

Rat basophil leukemia cell mediator release assay (RBL assay)

The RBL-cell mediator release assay has been developed as a murine model that mimics an essential event of the Type I allergic reaction (20, 21). Briefly, specific IgE was raised against extract from raw or cooked celery tuber (30 min, 100°C) in BALB/c mice by repeated low-dose intraperitoneal injections. BALB/c mice were injected 4–5

Table 2. Specific IgE to birch and mugwort pollen, rBet v 1, rBet v 2, rBet v 6 as determined by CAP and results of IgE reactivity to celery proteins detected on immunoblot in patients with positive and negative DBPCFC to cooked celery

Patient number*	CAP (kU/l)						Blot celery extract		
	celery	birch	rBet v 1	rBet v 2	rBet v 6	mugwort	14 kDa	17 kDa	CCD
History + ve/DBPCFC + ve									
1	9.05	26.3	26.3	neg	neg	0.71	—	+	+
2	1.13	6.57	3.08	4.26	neg	22.1	+	—	—
3	15.8	71.6	56.2	7.38	neg	2.42	+	+	+
4	5.42	>100	>100	4.47	neg	2.24	+	+	+
5	0.76	2.56	0.53	1.85	neg	0.44	—	—	—
6	4.92	38.4	27.7	neg	neg	neg	—	+	—
History + ve/DBPCFC – ve									
7	neg	4.15	5.59	neg	neg	0.89	—	—	—
8	3.38	65.3	55.4	7.55	neg	10.1	+	(+)	+
History – ve/DBPCFC – ve									
9	6.53	37.0	36.8	neg	neg	neg	—	+	—
10	neg	41.8	28.6	neg	neg	neg	—	—	—
11	19.7	40.9	37.7	neg	neg	1.51	—	+	+
History – ve/DBPCFC n.d.**									
12	>100	>100	>100	0.84	n.d.	16.8	+	+	+

*Patient number corresponds to immunoblot results (lanes) in Figures 1A and 1B.

**In patient 12 DBPCFC performed with celery spice but not with cooked celery.

DBPCFC: double-blind placebo-controlled food challenge; +ve: positive; –ve: negative; n.d.: not done.

times with allergen extract containing 1 µg of protein adsorbed to 5 mg Al(OH)₃ in 0.5 ml phosphate buffered saline at intervals of two weeks. Final bleeding was performed 10 days after the last immunization. Animals were graded as responders when a mediator release of at least 20% was obtained in the RBL cell assay with 1 : 50 diluted serum used for passive sensitization and allergen stimulation at 1 µg/ml. Sera from responder mice were pooled. Permanently growing RBL-2H3 cells were harvested in the stationary phase, transferred at 10⁵ cells per well to 96-well cell-culture plates (Nunc, Wiesbaden, Germany) and cultivated for 18 h at 37°C in Eagle's minimal essential medium (MEM) containing 5% of fetal calf serum (FCS). The attached cells were passively sensitized for 1 h with pooled murine reagent antiserum, diluted 1 : 50 in Eagle's MEM, 5% FCS. After sensitization, the cells were washed three times with Tyrode's buffer. Subsequently, the RBL-cells were incubated with 100 µl of serial allergen extract dilutions (as indicated in the results section) in Tyrode's buffer (130 mM NaCl, 5 mM KCl, 1.4 mM CaCl₂, 1 mM MgCl₂, 5.6 mM glucose, 10 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid, and 0.1% BSA, pH 7.4) in a humidified atmosphere at 37°C for 1 h. The specific release was determined by measurement of β-hexosaminidase activity. After transfer of 30 µl of the supernatant to a second microtiter plate, the released enzymatic activity was detected by hydrolysis of p-nitrophenyl-N-acetyl β-D-glucosaminide for 1 h. Absorbance was measured at 405 nm. The total release was quantified by treating the cells with the buffer containing 1% Triton X-100. Controls were run with Tyrode's buffer without allergen to measure spontaneous release, and by stimulation of cells with antiserum of divergent specificity (e.g., anti-grass pollen IgE). Results were expressed as percentage of the total release after subtraction of the spontaneous release (20, 21).

Results

Patients

Twelve patients (seven females and five males) entered the study. The mean age of the study population was 27.9 ± 7.3 years (range 21–42 years). Case histories with respect to celery allergy are summarized in Table 1.

Skin tests

All 11 patients undergoing DBPCFC with cooked celery were positive for the prick-to-prick test with raw native celery and 10 patients for SPT with celery extract.

In vitro diagnosis

All six patients with a positive DBPCFC to cooked celery and three out of five patients with a negative DBPCFC had a positive CAP test for celery (>0.35 kU/l). Table 2 shows the CAP test results for celery, rBet v 1, rBet v 2 and rBet v 6, birch and mugwort pollen of all 12 patients.

DBPCFCs with raw and processed celery

Symptoms occurring during DBPCFC with raw celery root, cooked celery root and celery spice are summarized in Table 1.

Eleven patients underwent DBPCFC with cooked celery; eight patients (numbers 1–8) had a positive history of allergic reaction after consumption of cooked

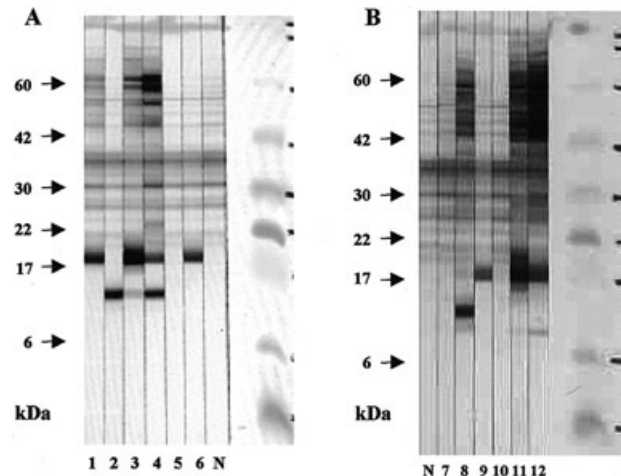


Figure 1. Immunoblot of celery extract tested with patients' sera. A. Sera no. 1–6: Patients with positive histories of allergic reaction to cooked celery and positive DBPCFC to cooked celery. In addition patients 1 and 4 had a positive DBPCFC with celery spice. No IgE-binding was observed with a nonallergic control: (N). B. Sera no. 7–8: Patients with positive histories of allergic reaction to cooked celery but negative DBPCFC. Sera 9–11: Patients with negative histories of allergic reaction to cooked celery and negative DBPCFC. Sera 8, 9–10, 12: Patients with positive DBPCFC with celery spice.

celery, and three patients (numbers 9–11) had a negative history to the cooked vegetable but reported adverse reactions to raw celery. Six patients reacted to the drink containing cooked celery but not to the placebo drink (numbers 1–6) and were regarded to be responders. Five patients complained about symptoms strictly localized to the oral cavity (OAS) during the local mucosal challenge ('spit' phase) at a mean provocation dose of 1.26 ± 0.44 g cooked celery. In one patient, symptoms were not restricted to the oral cavity and consisted of flush, cough and dyspnea. These symptoms occurred at a provocation dose of 34.5 g cooked celery when the patient was ingesting the active meal. Five patients, two with a positive history and three with a negative history, neither responded to the drink containing cooked celery nor to the placebo drink (nonresponders).

Five patients underwent DBPCFC with celery spice. All five patients reacted to the active drink, but not to the placebo drink and were regarded to be responders. Two patients complained about symptoms strictly localized to the oral cavity (OAS), both at a provocation dose of 0.16 g celery spice during the local mucosal challenge. In three patients symptoms were not restricted to the oral cavity and consisted of rhinitis, conjunctivitis, flush, angioedema and gastrointestinal pain. These symptoms occurred in two patients during the local mucosal challenge at 0.16 g and 0.32 g, and in one patient when he was ingesting 5.85 g of celery spice.

DBPCFC with raw celery root had been performed in all but two of the 12 patients (Table 1). Both patients responded to the provocation with cooked celery and denied another provocation with raw celery.

Table 3. Symptoms during open mucosal challenge with canned celery retorted under standardized conditions at Co-values from 7.45 to 76.07

Patient*	Co-values of retorted celery samples			
	7.45	13.12	23.64	76.07
1	OAS	OAS	neg	OAS
2	OAS	OAS	OAS	OAS
3	OAS	OAS	neg	neg
4	OAS	OAS	OAS	OAS
5	OAS	OAS	OAS	OAS
7	OAS	neg	neg	neg
9	neg	neg	neg	neg
10	neg	neg	neg	neg
11	neg	neg	neg	neg

*Number of patient corresponds to immunoblot results (lane) in Figures 1A and 1B.

Co: cooking effect: equivalent time (min) at 100°C of the four thermal treatments applied; OAS: oral allergy syndrome.

No reactions to placebo were observed in our patients either during the DBPCFC with raw celery, cooked celery or with celery spice.

Open provocation with canned celery

Five patients (numbers 1, 3–6) with a positive DBPCFC to cooked celery underwent an open provocation with canned and retorted celery. Clinical reactivity disappeared in one patient after heat treatment of celery at a Co-value of 23.64 and was preserved in four patients even if celery was treated at a Co-value of 76.07. In three patients (numbers 9–11) with a negative DBPCFC to cooked celery all four samples of retorted celery did not provoke any allergic symptoms (Table 3). One patient with a negative DBPCFC to cooked celery but positive case history reacted with OAS to the sample treated at a Co-value of 7.45 but not to celery treated at higher Co-values (Table 3).

Immunoblot analysis

IgE immunoblot analysis of celery extract was performed with sera of all patients undergoing DBPCFC to cooked celery or celery spice (Fig. 1; Table 2). In addition, identification of the allergens was done by immunoblot inhibition assays with recombinant celery allergens and purified N-glycans as described (9).

Five out of six patients with positive DBPCFC to cooked celery displayed strong IgE-antibody reactivities to one or more proteins from celery extract whereas in one serum (number 5) no IgE antibody binding was detected. Four patients' sera (66.6%) recognized a protein with a molecular mass of 17 kDa, corresponding to the Bet v 1-related celery allergen, Api g 1 (9, 15). All of these patients were sensitized to Bet v 1 (Table 2). Three sera (50%) reacted with a 14-kDa protein, i.e. the Bet v 2 related celery profilin, Api g 4 (22, 23). All were CAP-positive for Bet v 2. IgE to multiple bands in the range of 34–70 kDa were detected

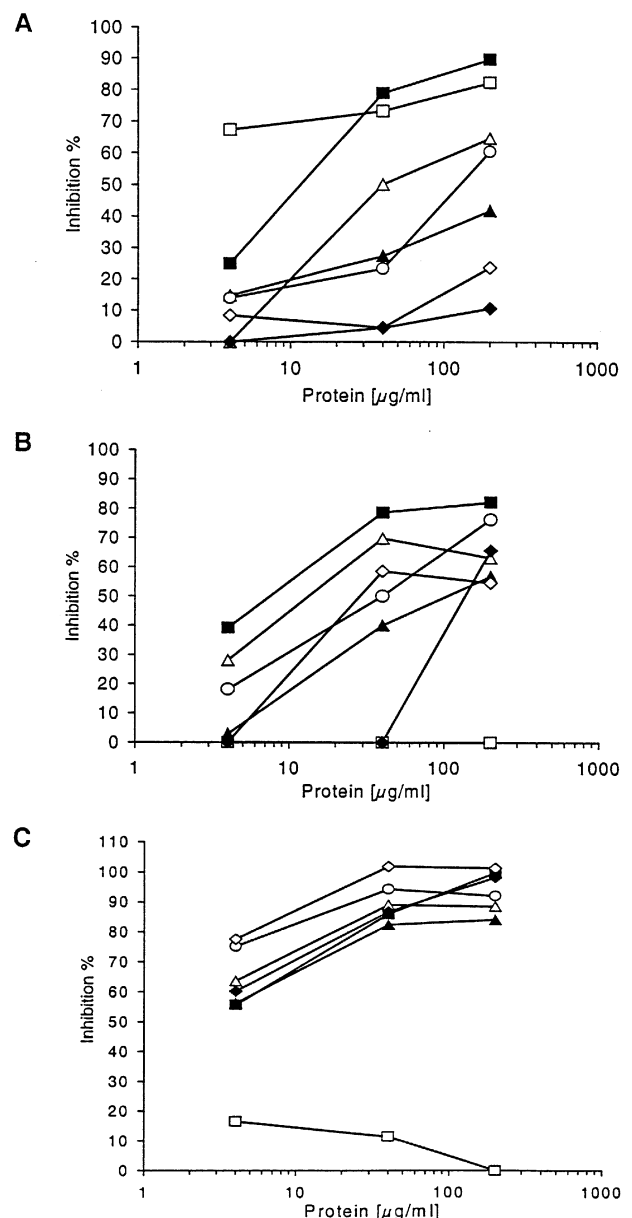


Figure 2. EAST inhibition experiments. Solid phase antigen: raw celery. A. Serum from a patient with IgE specific for Api g 1, diluted 1 : 2.5. B. Serum from a patient with IgE specific for profilin, diluted 1 : 10. C. Serum from a patient with IgE specific for cross-reactive carbohydrate epitopes, diluted 1 : 2.5. Inhibitors: raw celery (■), rApi g 1 (□), canned celery retorted at Co 7.45 (△), at Co 13.12 (▲), at Co 23.64 (○), at Co 76.07 (◇), celery cooked for 30 min at 100°C (◆).

with three sera (50%). Among the five patients with a negative DBPCFC to cooked celery, three patients showed IgE-reactivity to celery proteins. All three sera showed IgE-binding to the 17 kDa protein, one serum to the 14 kDa protein and two sera to multiple bands in the range of 34–70 kDa. Patient number 12, who was only challenged with raw celery and celery spice, showed IgE reactivity to all three allergenic structures in celery. No IgE-binding bands were detected by the

nonallergic control. Two out of five patients with a negative DBPCFC to cooked celery were negative in the immunoblot, one patient had IgE to celery profilin, two patients to Api g 1, and two to CCD.

Investigation of the thermal stability of celery allergens by EAST inhibition

To determine the heat stability of individual celery allergens, sera from patients with confirmed allergy to celery and a known sensitization pattern were used (9). Extract from raw celery was coupled to paper disks and extracts from processed celery were used as inhibitors. In a patient monosensitized to the major celery allergen, Api g 1, almost complete inhibition was obtained with extract from raw celery and the major celery allergen, rApi g 1 (Fig. 2A). The shortest heating period (Co-value 7.45) resulted in about a 10-fold decrease of the inhibition potency of celery. A further reduction of IgE reactivity was observed after prolonged heating times with maximal inhibitions reduced to 24% at a Co value of 76.07, and to 11% after conventional cooking of celery root for 30 min. In a patient with IgE to celery profilin, all retorted samples and extract from celery cooked for 30 min caused more than 50% inhibition at the maximal inhibitor concentration of 200 µg/ml, indicating considerable residual IgE-binding activity of celery profilin in all samples (Fig. 2B). When serum from a celery-allergic patient with IgE to CCD was used in a similar experiment, all samples except the non glycosylated rApi g 1 had a very similar inhibitory activity (Fig. 2C).

Immunization of mice and RBL cell mediator release assay

BALB/C mice were sensitized by repeated intraperitoneal injection of extracts from raw or heated celery adsorbed to aluminium hydroxide. The obtained IgE-containing mouse sera were used for passive sensitization of RBL cells. Subsequent to allergen stimulation the mediator release was measured. The rate of sensitization in BALB/c mice was calculated as the ratio of responders (release > 20%) to total number of immunized animals as described in the methods section. With extracts from raw celery root, a high sensitizing rate of 80% (16/20) was obtained. Mouse IgE raised against raw celery reacted with Api g 1, the major celery allergen (Fig. 3A). In contrast, extract from heated celery tuber reduced the IgE induction rate to 15% (3/20) when extract from heated food was used for stimulation. Murine IgE to heated celery did not react with rApi g 1 (Fig. 3B). Pooled sera from responders were used in further RBL cell degranulation studies to assess the residual allergenicity of celery tuber after thermal processing. As shown in Fig. 3A the retorted sample with a Co-value of 7.45 had a slightly reduced allergenic activity in the model system, whereas a more than 10-fold reduction was measured for the sample

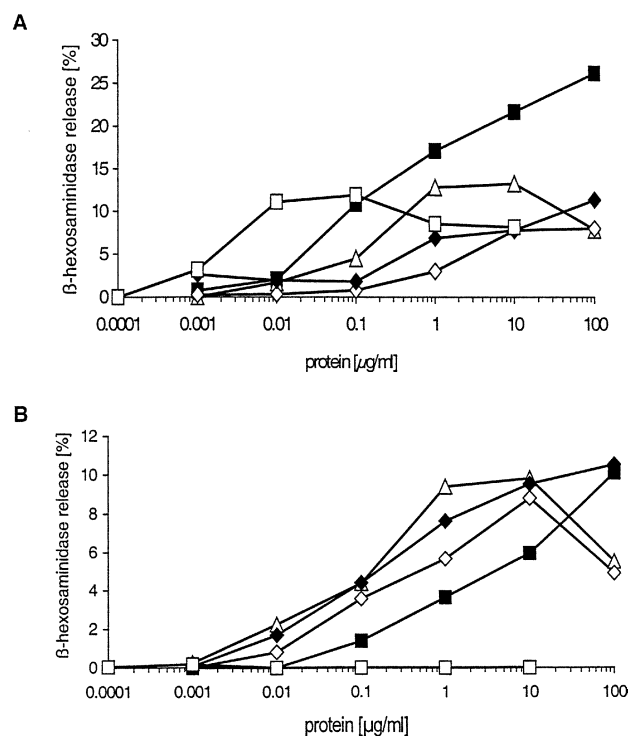


Figure 3. RBL cell mediator release assay. A. RBL 2H3 cells were sensitized by pooled mouse sera with IgE against raw celery (1 : 50) and stimulated with the following allergens: celery raw (■), canned celery retorted at Co 7.45 (△), at Co 76.07 (◇), celery cooked for 30 min at 100°C (◆), rApi g 1 (□). B. RBL 2H3 cells were sensitized by pooled mouse sera with IgE against cooked celery (1 : 50) and stimulated with the following allergens: raw celery (■), canned celery retorted at Co 7.45 (△), at Co 76.07 (◇), celery cooked for 30 min at 100°C (◆), rApi g 1 (□).

with a Co-value of 76.07, and celery cooked for 30 min. When IgE to cooked celery was used for sensitization of the RBL cells, all heated samples had very similar mediator release activity, whereas extract from the raw vegetable had about a 10-fold lower allergenic activity (Fig. 3B).

Discussion

Thermal processing is usually carried out to enhance texture, flavour, digestibility or microbiological safety (24). Heating or cooking of food, however, leads to a modification of protein structures and may influence its antigenicity. It is a well-known phenomenon that cooking substantially reduces allergenicity of fresh fruit, such as apples, and that cooked fruits can be consumed by many allergic subjects without any symptoms. Allergenicity of other foods such as peanuts, however, is hardly influenced by heat (25).

Celery is widely used in the food industry, in particular in Central Europe, because of its aromatic flavour. Consumer product diversification has led to an increase in recipes containing celery as a spice or in

cooked form, e.g. as an ingredient of soups and sauces. To date, there has been no information about the tolerability of cooked celery in celery-allergic patients.

This study confirms for the first time the allergenicity of cooked celery by DBPCFC. In six out of eight patients with a positive case history, allergy (26) to cooked celery was confirmed by our DBPCFC procedure that was originally developed for raw celery (8). It is difficult to assess whether the two nonresponders were indeed not allergic to cooked celery, or if dilution of the celery in the challenge mixture caused the negative results. The fact that patient 7 developed OAS during open challenge with celery retorted at a Co-value of 7.45 may indicate that the two nonresponders react to mildly heated, but not to cooked, celery. The three patients with negative case histories did not show any reactions during DBPCFC with cooked celery.

In order to determine the extent of heat treatment necessary to lower the allergenicity of celery, we challenged the patients with four samples of canned celery retorted under controlled conditions with Co-values of 7.45, 13.12, 23.64, 76.07. The three patients with negative DBPCFC lost their clinical reactivity if celery was retorted with a Co-value of 7.45, i.e. heat treatment at a temperature of 100°C for 7.45 min was sufficient to destroy allergenicity of the responsible celery proteins in these patients. In four out of five patients with a positive DBPCFC to cooked celery, however, allergenicity of celery was sustained even after extensive thermal treatment (76.07 min at 100°C) suggesting high heat resistance of a subgroup of celery allergens.

Furthermore, all patients undergoing DBPCFC with celery spice (dried and powdered celery) presented reactions comparable to symptoms observed during DBPCFC with raw celery. Thus, our data clearly show that commercially used celery powder is not safe for patients who are allergic to raw celery. The high *in vivo* allergenicity of commercial celery powder corresponds to the finding of Jankiewicz et al. (10) that all three important celery allergens, Api g 1, Api g 4, and CCD, can be detected by specific IgE antibodies in extracts from this material.

We were unable to identify patients complaining of allergy to cooked celery who were not allergic to the raw vegetable. This finding may indicate that no neoallergens are created by the heating process, and that residual activity of native celery allergens is responsible for the allergenic activity of the cooked vegetable. This view is supported by our mouse immunization studies. We found a high sensitizing rate of 80% to the raw vegetable. Only 15% of the animals, however, developed an IgE response to extract from cooked celery, and the sera of those animals had weak biological activities in the RBL cell mediator release assay (Fig. 3B).

Which are the allergens responsible for the persistent allergenic activity of heat-processed celery? Our EAST

inhibition data are in accordance with the study of Jankiewicz et al. (10), showing that Api g 1 is the most labile allergen in celery, that celery profilin Api g 4 is more stable under thermal processing, and that the IgE reactivity of CCD is not affected by heating (Fig. 2).

In IgE immunoblotting, five out of six patients with a positive DBPCFC to cooked celery were IgE positive. The sixth patient had a CAP class 2 to celery, which we find is often too low to obtain clearly positive immunoblotting results. A positive CAP to Bet v 2 indicated that this patient was sensitized to profilin (Table 1). Four of the five remaining patients had IgE either to Api g 4 or to CCD, which have been reported to be the partially and fully heat-resistant allergens in celery (10). One patient (Table 1, patient 6), however, was exclusively sensitized to Api g 1, the Bet v 1-related major celery allergen, which is the least heat-resistant celery allergen. Remarkably, patient 6 reacted to a much higher dose (34.5 g) of cooked celery than the other positive patients in DBPCFC (0.9–1.8 g; Table 2). Hence, we think that it is possible that an accumulated dose of low amounts of Api g 1 not deactivated in the cooked celery sample was responsible for the allergic reaction in this patient. Most importantly, the comparison of our clinical and *in vitro* data shows that the risk of clinical reactivity of a patient to cooked celery cannot be deduced from the sensitization pattern to individual celery allergens.

Our biological *in vitro* model reflected several aspects of celery allergy: Firstly, an IgE response to the major allergen, Api g 1, was obtained when raw celery was used as the immunogen (Fig. 3A). Secondly, with mouse IgE against raw celery, 'allergenic' activities of processed samples measured by the RBL cell mediator release assay corresponded to the IgE inhibition with sera from patients sensitized to Api g 1: raw celery had the highest mediator release capacity, which was reduced by about five-fold when celery was retorted at a Co-value of 7.45 and 50–100-fold at a Co-value of 76.07 or after cooking for 30 min (Figs 2A and 3A). Thirdly, no IgE response to Api g 1 was obtained when cooked celery was used as the immunogen, indicating that this allergen is not immunogenic in the cooked vegetable (Fig. 3B). Interestingly, raw celery had the weakest mediator release capacity when IgE to heated celery was used for sensitization of the RBL cells, which may indicate that a part of the polyclonal IgE response in the animals was directed against neoallergens, an effect that has so far not been found in celery-allergic subjects.

Taken together, we conclude that RBL cells sensitized with mouse IgE to raw celery may serve as a useful tool for screening the potential allergenicity of heat-processed products containing celery. Even extensive heat treatment of celery did not abrogate clinical reactivity in a subset of celery-allergic subjects. Therefore, we conclude that thermal processing is not

suitable to deplete allergenicity of celery completely. Celery-allergic patients should be instructed to strictly avoid any kind of spice mixtures or prepacked food possibly containing celery spice, since allergenicity of celery powder is comparable to that of raw celery.

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