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Influence of Food Processing on the Immunochemical Stability of Celery Allergens

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Abstract: Celery roots were processed by microwave heating, cooking, drying, γ -irradiation, ultra high pressure treatment and high voltage impulse treatment. The immunochemical stabilities of the three known allergenic structures of celery were tested with sera from patients who were sensitised to celery. In addition, rabbit antisera were used to detect the allergens profilin and Api g 1 on celery immunoblots. The specificity and reactivity of IgE from the patients' sera were investigated by immunoblotting, by an enzyme allergosorbent test (EAST) and by dose-related IgE inhibition experiments. The results of all three methods agreed closely and indicated high antigenic and allergenic activity in native celery which was reduced by thermal processing. The heat-stability of the known celery allergens decreased in the following order: carbohydrate epitopes > profilin > Api g 1. In contrast, the allergenicity was only mildly reduced by non-thermal processing. The results obtained with human IgE were confirmed by an *in vitro* mediator-release assay that is based on rat basophil leukemia cells (RBL cells) which were passively sensitised with celery-specific murine IgE. With sera from mice that had been immunised with native celery, the native sample and non-thermal celery preparations elicited the strongest mediator release, whereas a weak response was obtained with samples from heat-processed celery. These results agreed closely with the data obtained in allergic patients whose IgE antibodies were directed against the major protein allergen Api g 1. Our results may be helpful in risk assessment and in selecting food preparations which can be consumed without symptoms by a subgroup of celery-allergic patients with a known sensitisation pattern.

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INTRODUCTION

Immediate type allergy against food is based on immunological mechanisms and is, in most cases, related to the activity of specific antibodies of the immunoglobulin class E (IgE). Celery roots belong to the most important

allergenic plant foods (Wüthrich 1993). Severe anaphylactic reactions can occur after ingestion of foods which contain technologically processed or heated celery roots (Pauli *et al* 1988; Wüthrich *et al* 1990; Wüthrich, 1993; André *et al* 1994). This observation is particularly important since dried celery powder is widely used as a cheap spicing ingredient. Wüthrich and his colleagues (1990) were the first who demonstrated a partly thermostable allergenic activity of celery roots at the serological level. Recently, we have shown that immuno-reactive celery allergens can be present even

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after 30 min microwave treatment of celery roots at 100°C (Jankiewicz *et al* 1996; Vieths *et al* 1996). For example, 11/20 sera with a positive enzyme allergosorbent test (EAST) to celery roots had a positive test result to celery proteins of the heat-denatured sample. Hypersensitivity to celery is very common in birch pollen- as well as in mugwort pollen-allergic patients and is due to the existence of cross-reactive pollen-specific IgE (Wüthrich *et al* 1990; Vieths *et al* 1992a). The three known allergenic structures of celery are: (i) Api g 1 (allergen 1 from *Apium graveolens*), a 16-kDa protein which shares high sequence identity with the major birch pollen allergen, Bet v 1, and other major allergens in birch pollen related fruits and vegetables (Ebner *et al* 1995; Schöning *et al* 1995; Vieths *et al* 1995a; Breiteneder *et al* 1995). (ii) Celery profilin, belonging to a group of recently identified plant pan-allergens, with homologues being present in almost all pollen species and all kind of plant food (Vallier *et al* 1992; van Ree *et al* 1992; Ebner *et al* 1995; Fähr *et al* 1995; Vieths *et al* 1995a). (iii) Multiple allergen bands in the mass range between 35 kDa and 90 kDa, that are recognised by patients' IgE on celery immunoblots, are suspected to carry ubiquitous cross-reactive carbohydrate determinants (CCD). These determinants can therefore mediate extended cross-reactions between celery and a wide variety of pollen and plant food (van Ree and Aalberse 1993; Vieths *et al* 1994a).

Celery roots are consumed as ingredients of fresh salads, as cooked vegetables, in vegetable juices and soups, and as fermented or pickled preserves. Ready-to-serve meals spiced with celery powder can undergo microwave heating or conventional heating. Apart from

thermal processing, modern technologies for the inactivation of enzymes and microorganisms, the enhancement of juice yield and the modification of texture have been developed (Diehl 1990; Kühne and Knorr 1990; Mertens and Knorr 1992). In spite of the clearly identified immunological activity of heated celery, no conclusive data have been published about the stability and the behaviour of individual celery allergens during these processes. Therefore, the aim of the present investigation was to study, at the serological level, the influence of food technological treatment on the antigenic and allergenic structures of celery roots under carefully controlled conditions.

MATERIALS AND METHODS

Food processing and extract preparation

Most of the treatments were performed on the same lot of native celery roots of the 'Brillant' variety. The vegetables were grown in the Netherlands and obtained from a wholesaler. The thermal and non-thermal processes applied and the objectives of the treatments are summarised in Table 1. All conditions were controlled carefully. In the case of thermal processing, product temperature was monitored with a 3-channel fibre thermometer (Takaoka 1100). Dried celery powder was obtained from a commercial manufacturer (Worlée, Hamburg, Germany), and a preserve of pickled celery was purchased at the local market ('celery salad', Spreewald, Lübben, Germany). Subsequent to processing, the plant material was shock-frosted in liquid nitrogen,

TABLE 1
Processed preparations from celery roots

Extract	Treatment	Conditions	Objective
N	Native	—	—
MWH10	Microwave	750 W, 10 min, product temperature: 100°C	Cooking
MWH30	Microwave	750 W, 30 min, product temperature: 100°C	Cooking
C	Conventional cooking	20 min, product temperature: 100°C	Cooking
CW	Cooking water	20 min, 100°C	—
HVI	High voltage impulse treatment	10 kV, Pulse rate: 50 min ⁻¹	Cell permeation, enhanced juice yield ^a
UHP	Ultra high pressure	600 mPa, 20°C	Preservation, enzyme inactivation ^b
IR	γ -Irradiation	Total dose: 10 kGy	Preservation ^c
CP	Celery powder	Commercially dried	Preservation
PCE	Pickled celery	Commercial preserve containing vinegar and citric acid	Preservation

^a Coster (1965), Mertens and Knorr (1992).

^b Kühne and Knorr (1990), Mertens and Knorr (1992).

^c Delincée (1983), Diehl (1990).

homogenised in a commercial blender, extracted with 0.01 M phosphate buffered saline, pH 7.4 (PBS), dialysed against distilled water overnight at 4°C, freeze-dried and stored at -20°C until used. The amount of extracted protein was estimated by a commercial dye-binding assay (Pierce, Rockford, USA).

Polyclonal antisera

Api g 1 was immuno-detected by means of a rabbit antiserum (aBv) which had been raised against Bet v 1 (Allergopharma, Reinbek, Germany). Due to the structural similarities of the two allergens, this serum strongly cross-reacted with Api g 1 (Schöning *et al* 1995; Jankiewicz *et al* 1996; Vieths *et al* 1996). Rabbit antisera against celery profilin (aCp) and ragweed pollen profilin (aRp) (kindly supplied by Dr P Deviller, Lyon, France) were used to detect antigenic profilin in celery extract and the processed preparations (Vallier *et al* 1992; Vieths *et al* 1995a; Jankiewicz *et al* 1996). The reactivity of serum aRp to celery profilin was again based on cross-reactivity.

Patients' sera

All sera were taken from birch pollen allergic patients at the Department of Dermatology and Allergology, Borkum Riff Hospital, Borkum, Germany (Head physician: Dr H Aulepp). Most of the patients were also sensitive to grass pollen and/or weed pollen (for further data see Jankiewicz *et al* 1996). Sera with increased levels of IgE specific for celery (EAST class ≥ 2 , see below) were used as probes to characterise celery allergens. No selection was made on the basis of clinical history. Additional pooled human sera with EAST scores ≥ 2 to celery were purchased from Allergopharma (Reinbek, Germany): Serum BiP ($n = 7$), serum BmP ($n = 12$) and serum WeP ($n = 7$). The EAST scores of these were: BiP: native celery, 2.2; heated celery, 0.6; birch pollen, 4.0; mugwort pollen, 2.5; grass pollen (timothy), 4.0. BmP: native celery, 2.5; heated celery, 2.2; birch pollen, 3.8; mugwort pollen, 0.5; grass pollen (timothy), 4.0. WeP: native celery, 3.4; heated celery, 3.0; birch pollen, 3.2; mugwort pollen, 3.2; grass pollen (timothy), 3.0. Serum from a nonatopic individual was used as a negative control.

Determination of specific IgE

Extracts of native celery and the technologically treated preparations were adjusted to a total protein concentration of 12 $\mu\text{g ml}^{-1}$ and were coupled to cyanogen bromide activated filter paper disks as described for apples and extracts from other plant foods (Vieths *et al* 1992b, 1994a, 1995b). These allergen disks were incu-

bated with 50 μl of human serum overnight at room temperature. After washing, IgE specifically bound to the immobilised allergens was semi-quantitatively determined by a commercial EAST (Allergopharma, Reinbek, Germany) using the calibration system of the manufacturer. In this indirect, non-competitive ELISA, IgE bound to allergens is immuno-detected by a mouse monoclonal anti-IgE antibody labelled with alkaline phosphatase. The enzymatic activity is monitored by hydrolysis of *p*-nitrophenylphosphate. After reading the absorbances at 405 nm, the results are expressed as EAST classes (class 0, no specific IgE; class 4, very high content of specific IgE corresponding to 17.5 kU litre $^{-1}$). To distinguish more precisely, EAST classes were expressed to one decimal place (Vieths *et al* 1994b, 1995b).

IgE inhibition assays

A quantitative estimation of the IgE binding potency of the celery preparations was derived from a competitive ELISA inhibition assay. Patient sera were diluted to an optical density of approximately 70% of the EAST signal (usually 1 : 2–1 : 10) in 0.05 M Tris buffer, pH 7.4, 0.15 M NaCl, 0.3 g litre $^{-1}$ bovine serum albumin (BSA) and 1 ml litre $^{-1}$ Tween 20 (TBS/BSA/Tween). A 10-fold dilution series of the inhibitor extracts was prepared in five steps in TBS/BSA/Tween. Volumes of 50 μl diluted serum and 50 μl inhibitor solution were incubated with an allergen disk overnight at room temperature. Subsequently, the EAST was proceeded according to the standard procedure. The results were expressed as % inhibition. Extract potencies were quantitatively compared by estimating the protein concentration responsible for a 50% inhibition of the IgE binding to the solid phase (C_{50}) from the inhibition graphs. Control inhibitions were performed with ovalbumin and an extract from low-fat milk at a single protein concentration of 250 $\mu\text{g ml}^{-1}$. Generally, all determinations were run in duplicate.

Electrophoresis and immunoblotting

Electrophoresis and immunoblotting were carried out as previously described (Vieths *et al* 1992c, 1995a). Briefly, the extracts were separated by discontinuous SDS-PAGE according to the method of Lämmler (1970), using 50 g litre $^{-1}$ stacking gels (1.0 mm, T = 5.0%, C = 2.6%) and 130 g litre $^{-1}$ resolving gels (T = 12.8%, C = 2.7%). The protein load on the gels was 18 $\mu\text{g cm}^{-1}$ in 5.5 cm preparative slots. Thereafter, the extracts were transferred onto nitrocellulose (NC) membranes by semi-dry blotting (Khyse-Andersen 1984). Cut NC-strips (0.4 mm) were either stained with Indian ink or used for immuno-detections. The latter were achieved by incubating one NC-strip in 1.0 ml of

diluted serum. The sera were diluted as follows: human sera, 1 : 6·7; rabbit aBv, 1 : 2000; aCp, aRp, 1 : 30 000. Immuno-detections were carried out by an amplified system that is based on a biotinylated tertiary antibody and a subsequent streptavidin horseradish peroxidase conjugate incubation (Vieths *et al* 1992c, 1994b). Bound enzymatic activity was stained with 3,3', 5,5'-tetramethylbenzidine.

Confirmation of the allergen specificity of human sera

In addition to comparative immunoblot testing with the rabbit antibodies, allergen specificity of human IgE was confirmed by spot-checking selected sera as follows: (i) pre-incubation of sera with 10–15 µg of purified Bet v 1 prior to immunoblotting deleted the binding of IgE to Api g 1, but not to other celery allergens (Vieths *et al* 1995a). (ii) Immunoblotting with profilin that was purified by poly-L-proline affinity chromatography (Vallier *et al* 1992). (iii) IgE binding to carbohydrate determinants on multiple allergen bands was destroyed by incubating blot strips in 0·1 M NaIO₄, pH 5·0 for 2 h as described (Vieths *et al* 1994a, 1995a) and/or by a glycan ELISA which was kindly performed by Dr F Altmann, Vienna, Austria (Tretter *et al* 1993; Petersen *et al* 1996).

Rat basophil leukemia cell mediator release assay

Allergic symptoms are not directly linked to serum IgE, but to specific crosslinking of IgE bound to the surface of mast cells and basophils. A murine *in vitro* model of this essential event of the type I allergic reaction which utilizes permanently growing rat basophil leukemia cells (subline RBL-2H3) as a mast cell equivalent has recently been applied to estimate the biological activity of allergen extracts (Hoffmann *et al* 1997a,b). In short, the assay was performed as follows: Specific IgE was raised against extract from native celery in BALB/c mice as described for birch pollen extract (Hoffmann *et al* 1997a). RBL cells were harvested in the stationary phase and attached to the surface of 96 well Nunc cell culture plates at a density of 10⁵ cells per well for 18 h at 37°C and were passively sensitised with pooled serum from 5 mice in Tyrodes' buffer (37°C, 2 h) at a serum dilution of 1 : 65. For crosslinking and degranulation, sensitised RBL cells were incubated with 100 µl of serial allergen extract dilutions in a humidified atmosphere at 37°C for 1 h. The protein concentration of the extract dilutions ranged from 0·001 µg ml⁻¹ to 10 µg ml⁻¹. The specific release was assayed by measurement of β-hexosaminidase activity, which is present in the same granula as histamine (Schwartz *et al* 1981). Enzymatic activity in the supernatant was detected by hydrolysis of *p*-nitrophenyl-*N*-acetyl-β-D-glucosamine for 1 h. Absorbance was measured at 405 nm. Controls were run with Tyrode's buffer without allergen to measure

spontaneous release, and with the buffer containing 1% Triton X-100 to quantify the total release. The results were expressed in % of the total release after subtraction of spontaneous release (Hoffmann *et al* 1997a,b).

RESULTS

Immunoblot reactivity of the technologically processed preparations of celery roots

Rabbit antisera

Electrophoretically separated extracts from native celery and all technologically processed preparations were transferred to NC membranes and tested for their antigenicity with three rabbit polyclonal antisera: Api g 1 was detected by the anti-Bet v 1 serum aBv and profilin by anti-celery profilin aCp and anti-ragweed pollen profilin aRp. As previously described, Api g 1 appeared as a single band with a mass of approximately 16 kDa and profilin as a double band at 15–16 kDa (Vieths *et al* 1995a; Jankiewicz *et al* 1996). Cooked celery and the cooking water were omitted from blotting, but were used for specific IgE determinations as described below. As shown in Fig 1 microwave heating of celery for 30 min at 100°C completely destroyed the antibody binding of all three sera, an effect which most probably indicated the destruction conformational epitopes. In contrast, all other processed preparations and commercial celery powder contained antigenic Api g 1 and celery profilin. With a standardised amount of protein, however, increased antibody binding occurred with most of these preparations.

Patients' sera

Similar experiments as above were performed with three patients' sera containing IgE specific for the known celery allergens: Serum S 69 (Api g 1), Serum 131 (profilin) and serum 153 (multiple bands, probably carrying CCD). With the latter serum, IgE reactivity to carbohydrates was confirmed by a glycan ELISA and by periodate oxidation of blot strips with celery allergens and subsequent immuno-detection as described (results not shown) (Jankiewicz *et al* 1996; Petersen *et al* 1996). As shown in Fig 2(a), 30 min heating also deleted the binding of patient's IgE to celery allergens on immunoblots except serum 153 which was highly reactive and recognised as many bands as before. Microwaving for 10 min deleted IgE binding to Api g 1, but not to celery profilin and the multiple bands at higher molecular masses. Figure 2b illustrates that all other preparations retained strong IgE reactivity. Api g 1, however, was only weakly detected after UHP treatment and in commercial celery powder. In γ-irradiated celery roots, the celery-positive human sera recognised a strong new band at 18–19 kDa which was not detected by the non-allergic control (the latter not shown). The

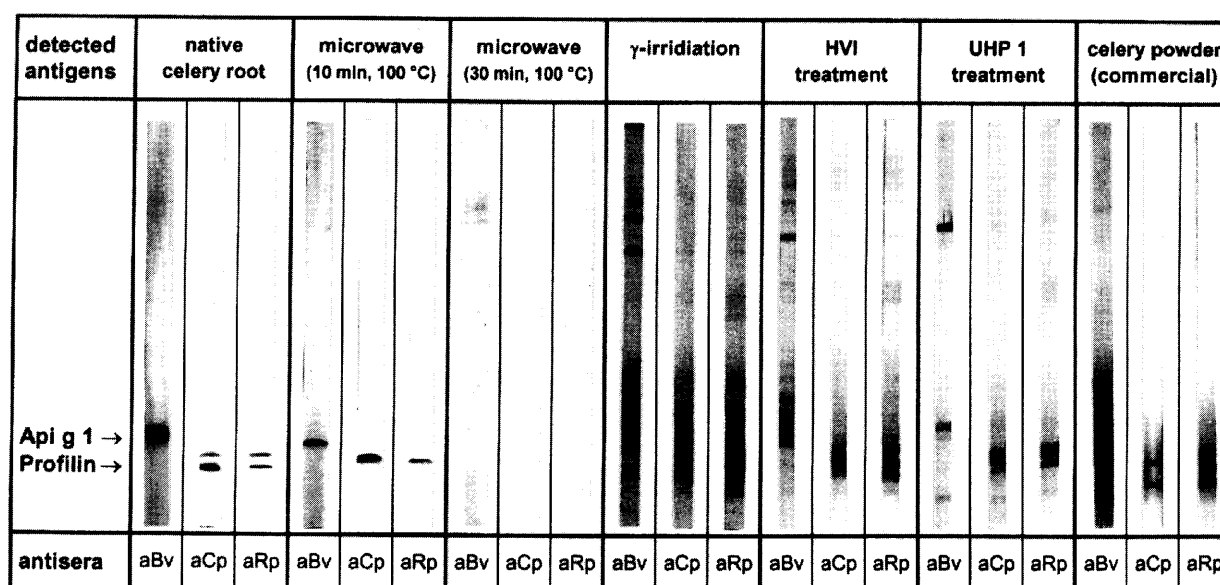


Fig 1. Immunoblot analyses of extracts from processed celery preparations. Extracts were separated by SDS-PAGE and transferred to nitrocellulose. Antibody binding to Api g 1 at approximately 16 kDa and to the profilin double band at 15–16 kDa. aBv: rabbit antiserum raised against Bet v 1, diluted 1 : 2000. aCp: rabbit antiserum raised against celery profilin, diluted 1 : 30 000. aRp: rabbit antiserum raised against ragweed pollen profilin, diluted 1 : 30 000. B: buffer control. Celery preparations as indicated in Table 1.

IgE binding to this preparation could be almost completely inhibited with extract from native celery (results not shown). Currently, we have no further data about the epitopes of this protein. In the case of extract from pickled celery, only serum Bo 153 revealed a multiple reaction pattern as above. In contrast, the sera with IgE against Api g 1 and profilin produced a faint staining below 20 kDa that could not be assigned to specific allergen bands (results not shown).

Extended immunoblot testing was performed with 25 sera from birch pollen allergic patients and a positive EAST result (EAST class of at least 2) to celery as well as with three pooled sera from pollen-allergic patients who were serologically positive to birch pollen, mugwort pollen and celery. Immunoblot reactivity was determined with extract from native celery, after short-time microwaving (extract MW 10, see Table 1), and with extract from completely heat-denatured celery (either MW 30 or C, see Table 1). The results summarised in Table 2 indicate that the observed heat-stabilities of the allergenic structures underlie some variation for individual patients, but nevertheless seem to represent a general trend.

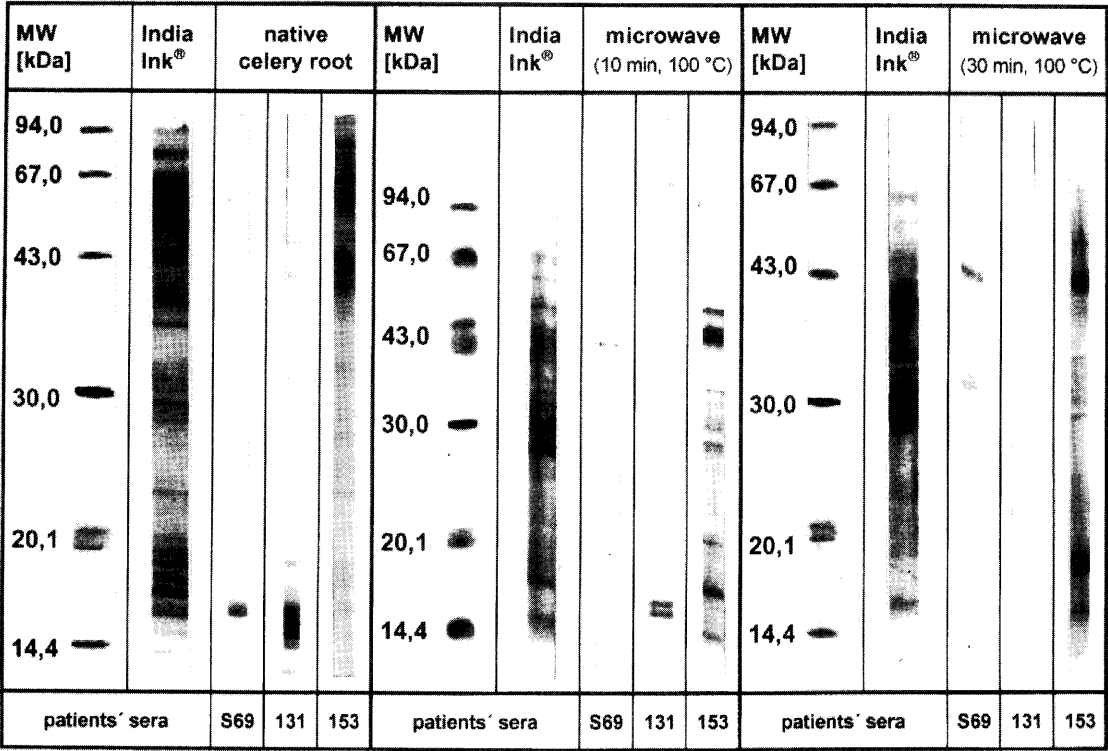
Determination of IgE specific for the processed preparations by EAST

The three patients' sera used for the above immunoblot analyses were also used to determine specific IgE to all technologically treated preparations and moreover, to celery roots that had been cooked for 20 min and the cooking water. The results summarised in Table 3

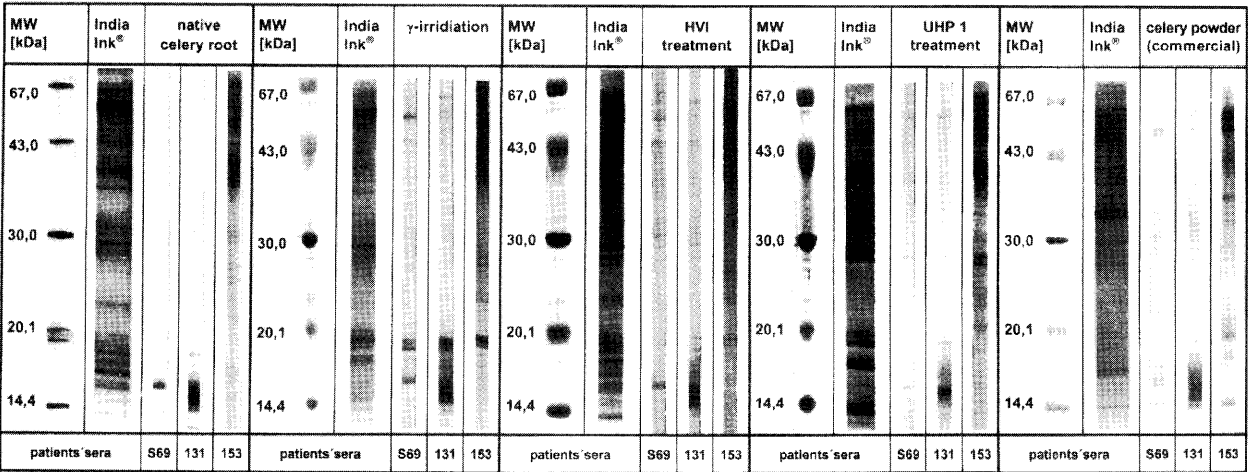
agreed closely with immunoblotting in most of the cases: immuno-reactivity to Api g 1 was destroyed even after short microwave treatment, to profilin by heating for 30 min; and IgE reactivity to CCD survived in all cases. In the case of UHP-processing and celery powder, immunoblot results were doubtful for Api g 1, but the EAST with serum S 69 clearly indicated IgE binding capacity of these preparations. This observation may be due to different detection limits of the two methods. Twenty minutes of conventional cooking destroyed the reactivity to Api g 1. In contrast, the 2 sera with IgE against profilin and CCD, respectively, revealed a positive EAST even with extract of cooked celery and the cooking water. Due to the limited amount of sera the immuno-reactivity of Api g 1 and profilin in pickled celery was tested with the sera of patients Bo 193 and A21, respectively. Binding to CCD was again analysed with serum Bo 153. The following EAST classes were obtained: Bo 193: native: 3·1, PCE: 1·8; A21 native: 3·6, PCE: 1·8; Bo 153: native: 2·5, PCE: 2·0. Due to these results, the EAST with pickled celery indicated a remarkable reduction of the IgE reactivity to Api g 1 and profilin and again a weaker influence on carbohydrate epitopes. Controls with serum of a non-atopic individual were all negative (class 0).

IgE inhibition experiments

In order to compare the IgE-binding potency of celery roots before and after thermal processing, dose-related EAST inhibitions were carried out using native celery on the solid phase and three patients' sera of known



(a)



(b)

Fig 2. Immunoblot analyses of extracts from (a) native and microwave-heated celery and (b) processed celery preparations. Extracts were separated by SDS-PAGE transferred to nitrocellulose and tested with sera of celery-sensitised patients, diluted 1 : 6. Antibody binding to Api g 1 at approximately 16 kDa (serum S69), the celery profilin double band at approximately 15–16 kDa (serum 131) and multiple bands probably carrying CCD (serum 153). Celery preparations as indicated in Table 1. A total protein stain was performed with Indian ink.

allergen specificity. Extract of native celery, 30 min microwaved celery and selected processed preparations were used as the inhibitors. The resulting C₅₀-concentrations of celery protein responsible for a 50% inhibition of the IgE binding to the solid phase, which reflect the extracts' potency, are listed in the legend of Fig 3. Figure 3(a) presents inhibition graphs with serum of patient S69 whose IgE antibodies exclusively reacted

with Api g 1 on celery immunoblots. Figures 3(b) and (c) show the results of similar experiments performed with sera of patients exclusively sensitised to profilin (patient A21) and to carbohydrate epitopes (patient Bo 153), respectively. In the case of IgE binding to Api g 1 (Fig 3(a)) a strong specific inhibition resulted when extract from native celery was used as the inhibitor. In contrast, the inhibition did not exceed 50% for any of

TABLE 2
Immunoblot binding of IgE from human sera to celery allergens extracted from native and heat-treated celery roots^a

IgE binding to	Serum	Extract		
		Native	Short time microwaving (10 min at 100°C)	Thermal denaturation (20 or 30 min at 100°C)
Api g 1	Bo 91	++	nt	—
	Bo 155	+++	—	—
	S 69	+++	—	—
	Bo 163	++	nt	—
	Bra	+++	(+)	nt
	Bo 10	+++	(+)	nt
	Bo 5	+++	(+)	nt
	Bo 2	++	—	nt
	S 64	++	—	nt
	ILC 216	+++	(+)	nt
	Bo 193	++	nt	—
	Bo 196	++	nt	—
	Bip-pool (<i>n</i> = 7)	+	—	—
Profilin	Bo 131	+++	+++	—
	Bo 13	+++	+++	nt
	S 71	+++	+++	—
	A 94	+++	++	—
	A 21	+++	+++	—
	Sec	+++	—	—
	H 01	++	nt	—
	Wep-pool (<i>n</i> = 7)	+++	+++	—
	Bmp-pool (<i>n</i> = 12)	+++	++	—
Multiple bands $\geq$ 30 kDa	Bo 153	+++	+++	+++
	Bo 164	+++	nt	+++
	Bo 13	+++	+++	nt
	Wka	+++	++	++
	Wke	+++	++	++
	Bia	++	++	nt
	Wep-pool (<i>n</i> = 7)	+++	+++	++

^a Intensity of IgE binding: +++ = very strong; (+) = weak/doubtful; —: no binding; nt: not tested.

the thermally processed preparations even at the highest protein concentration of $670 \mu\text{g ml}^{-1}$. Comparing the protein levels causing a 30% inhibition of the IgE binding revealed at least a 20-fold reduction of the inhibition potency after 10 min of microwave treatment. The same procedure had no influence on the IgE reactivity of profilin (Fig 3(b)), but the inhibition potency

clearly decreased at least 15-fold after 30 min microwaving. With the profilin-specific patient serum, the extracts of cooked celery and the cooking water showed a weaker reduction of the inhibition capacity when compared to 30 min microwave heating (Fig 3(b)).

With serum of patient Bo 153 which contained IgE against carbohydrate epitopes of celery glycoproteins,

TABLE 3
Determination of specific IgE to processed celery preparations by EAST

Serum	Allergen specificity	EAST scores to allergen disk from								
		Native	MWH10	MWH30	C	CW	HVI	UHP	IR	CP
S69	Api g 1	2.4	0.0	0.1	0.0	0.0	2.9	2.4	2.7	3.2
Bo 131	Profilin	3.3	3.5	0.3	2.8	3.0	3.6	3.7	3.8	3.5
Bo 153	CCD	2.5	2.4	2.2	2.6	1.9	1.8	2.3	2.9	2.5

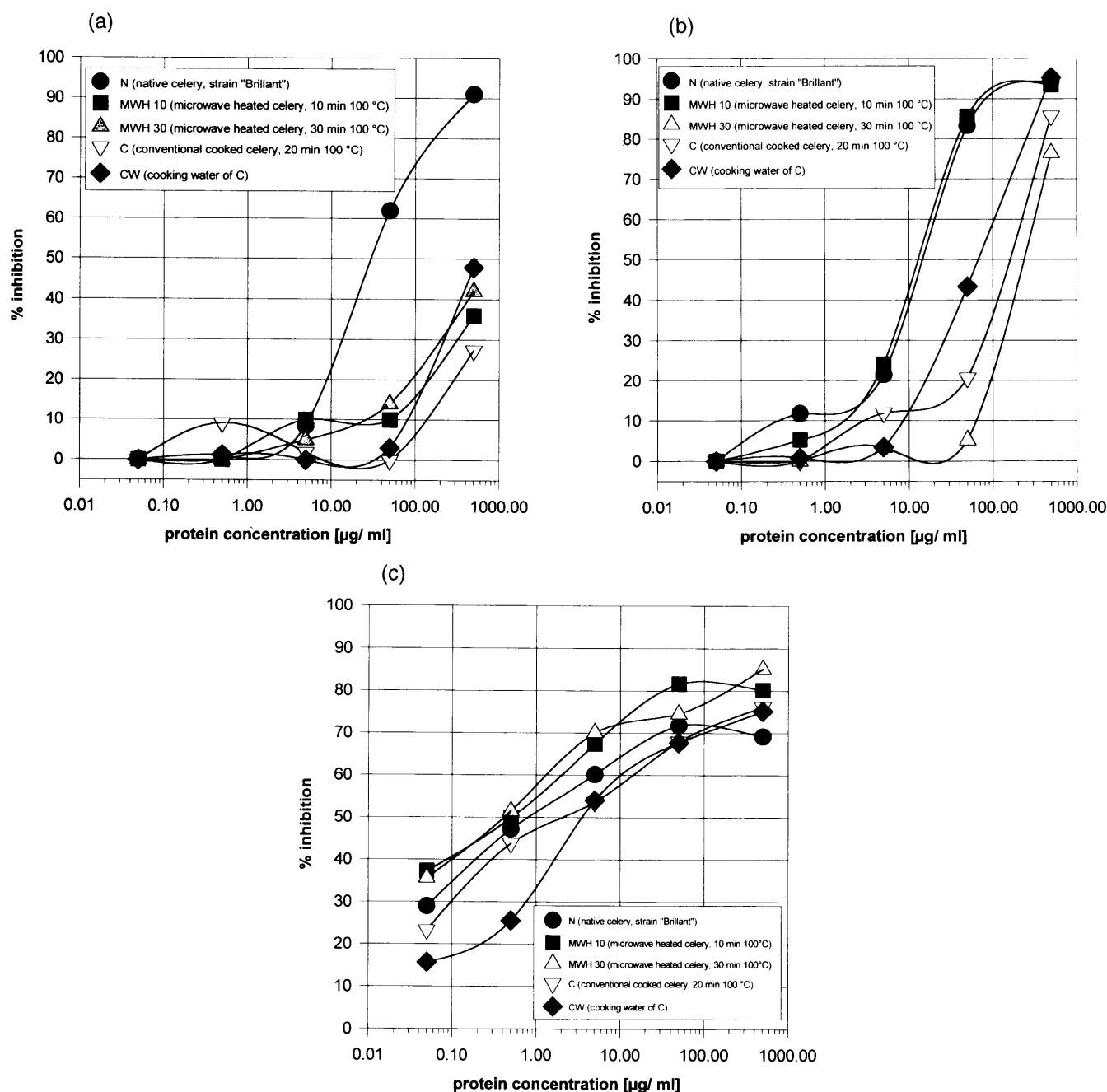


Fig 3. Results of dose-related inhibition of IgE binding to allergen disks with native celery allergens. (a) Serum of patient S69 whose IgE antibodies were directed against Api g 1 was diluted 1 : 2.5. Extracts from native celery roots and heated celery roots were used as the inhibitors as indicated in the figure. Results are expressed as % inhibition. With native celery the C_{50} -concentration was $30 \mu\text{g ml}^{-1}$. In addition, the following C_{30} -concentrations were obtained: native celery: $14 \mu\text{g ml}^{-1}$, microwave heating (10 min): $340 \mu\text{g ml}^{-1}$, microwave heating (30 min): $200 \mu\text{g ml}^{-1}$, cooked celery (20 min): —, cooking water: $230 \mu\text{g ml}^{-1}$. Ovalbumin ($250 \mu\text{g ml}^{-1}$) inhibited 2.8% and $250 \mu\text{g ml}^{-1}$ of protein from low-fat milk to a degree of 1.4%. (b) Serum of patient A21 whose IgE antibodies were directed against celery profilin was diluted 1 : 2.5. Extracts from native celery roots and heated celery roots were used as the inhibitors as indicated in the figure. Results are expressed as % inhibition. The following C_{50} -concentrations were obtained: native celery: $15 \mu\text{g ml}^{-1}$, microwave heating (10 min): $13 \mu\text{g ml}^{-1}$, microwave heating (30 min): $240 \mu\text{g ml}^{-1}$, cooked celery (20 min): $160 \mu\text{g ml}^{-1}$, cooking water: $70 \mu\text{g ml}^{-1}$. Ovalbumin ($250 \mu\text{g ml}^{-1}$) inhibited 0.0% and $250 \mu\text{g ml}^{-1}$ of protein from low-fat milk to a degree of 1.2%. (c) Serum of patient Bo 153, whose IgE antibodies were directed against carbohydrate epitopes of celery glycoproteins, was diluted 1 : 2.5. Extracts from native celery roots and heated celery roots were used as the inhibitors as indicated in the figure. Results are expressed as % inhibition. The following C_{50} -concentrations were obtained: native celery: $0.8 \mu\text{g ml}^{-1}$, microwave heating (10 min): $0.5 \mu\text{g ml}^{-1}$, microwave heating (30 min): $0.4 \mu\text{g ml}^{-1}$, cooked celery (20 min): $2.0 \mu\text{g ml}^{-1}$, cooking water: $3.5 \mu\text{g ml}^{-1}$. Ovalbumin ($250 \mu\text{g ml}^{-1}$) inhibited 35% and $250 \mu\text{g ml}^{-1}$ of protein from low-fat milk to a degree of 26%.

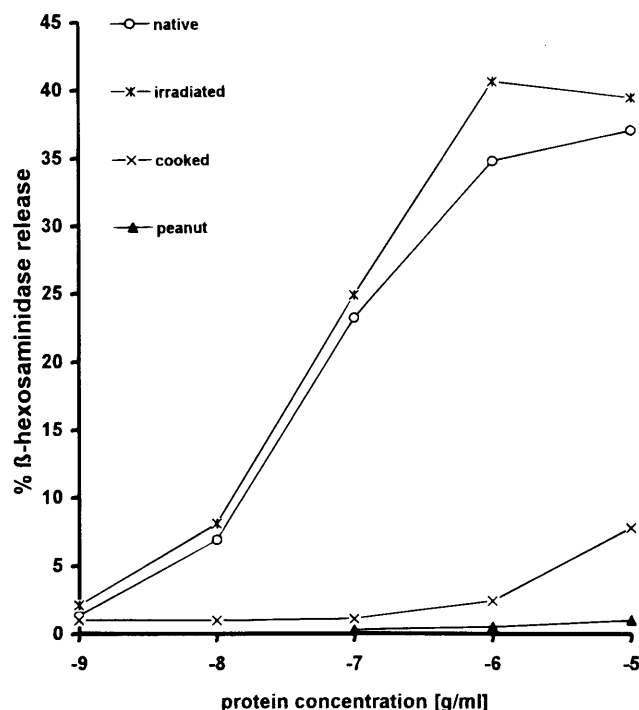


Fig 4. Dose-related mediator-release of RBL cells sensitised with a murine reagenic antiserum against extract from native celery roots. Prior to sensitisation, the serum was diluted 1:65. Stimulator extracts as indicated in the figure. The results are expressed as % of the total release after subtraction of spontaneous release.

all five extracts strongly inhibited the antibody reactivity to the allergens from native celery (Fig 3(c)). Controls with $250 \mu\text{g ml}^{-1}$ of ovalbumin and protein from low-fat milk led to very low inhibitions below 5% for the sera S69 and A21. In the case of serum Bo 153, ovalbumin caused 35% inhibition and low-fat milk 26% inhibition of the IgE binding to celery allergens. Since this serum contained carbohydrate-specific IgE, these

results are most probably due to cross-reacting carbohydrate moieties in the control solutions.

Mediator-release assay

Crosslinking of allergen-specific IgE on the surface of mast cells and basophils and the resulting mediator release are essential events of the type I allergic reaction. We have recently developed a dose-related RBL cell mediator release assay as a murine model to determine the biological activity of commercial allergen extracts (Hoffmann *et al* 1997b). For studying the mediator release caused by celery allergens before and after technological treatment, RBL 2H3 cells were passively sensitised with a reactive murine serum pool containing IgE against native celery. Thereafter the cells were stimulated with serial dilutions of extracts from all technologically processed preparations. An extract from roasted peanuts was used as a negative control.

As an example, typical dose-related release graphs of the RBL cell mediator release assay are depicted in Fig 4. The sensitised RBL cells were strongly stimulated by extract from native celery and from γ -irradiated celery. In contrast, cooked celery had a very low allergenic activity in this model system. No release was elicited by peanut extract.

The allergenic potential of celery extracts in the cell system is well reflected by the total mediator release at a stimulator protein concentration of $1 \mu\text{g ml}^{-1}$. The released enzyme activities caused by all processed preparations at this protein concentration in cells sensitised against native celery are shown in Fig 5. As can clearly be recognised, a relatively high residual allergenic activity was also present in all other non-thermal preparations (CP, UHP, HVI), whereas a reduced allergenicity was measured after application of heat (either cooking or microwaving). No activity was

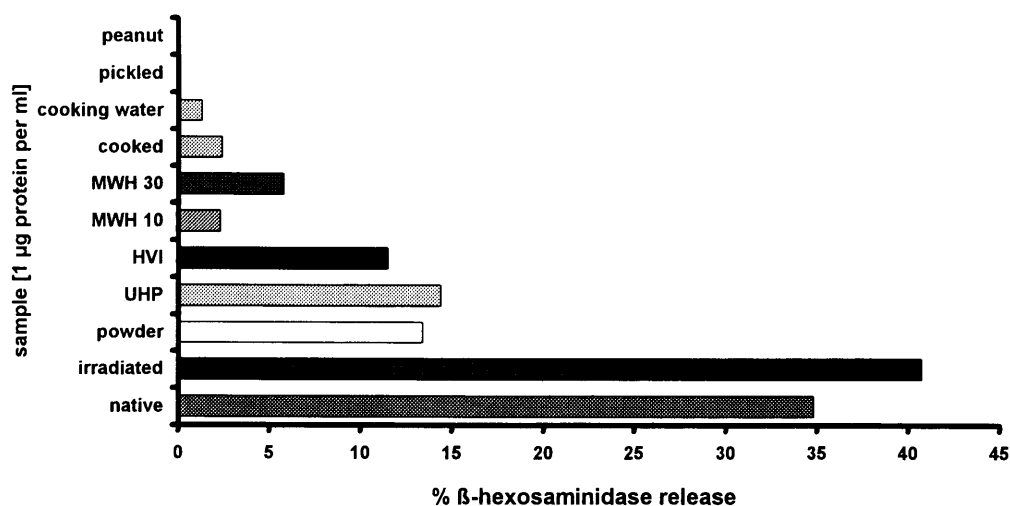


Fig 5. β -Hexosaminidase release of RBL cells sensitised with a murine reagenic antiserum against extract from native celery roots and triggered with extracts from various celery preparations at a protein concentration of $1 \mu\text{g ml}^{-1}$. The results are expressed as % of the total release after subtraction of spontaneous release.

detected in the heat-sterilised preserve of commercially pickled celery (PCE).

DISCUSSION

Changes in the binding of antibodies to food proteins and glycoproteins may be induced by a variety of technological processes and treatments. In principle, epitopes can be modified, eg by conformational changes, covalent linkages, association and thermal as well as hydrolytic fragmentation of proteins. Considering these aspects, a panel of technological processes representing the potential to cause different kinds of structural changes was selected and applied to celery roots. Heat was transferred by traditional cooking and by microwaving for different periods. Despite the absence of cooking water, microwave treatment includes the risk of inhomogeneous heating resulting in the possibility of native allergens being present in specific parts of the food. γ -Irradiation to a maximum of 10 kGy has been recommended for the preservation of spices and is known to induce some specific adulterations such as the formation of o-tyrosine residues in proteins (Delincée 1983; Diehl 1990). Preservation and enzyme inactivation by ultra high pressure is mainly due to denaturation of functional proteins (Mertens and Knorr 1992). High voltage impulse treatment increases the cell permeation and may as a consequence change the pattern of soluble proteins released from plant material. Finally, partial proteolysis which is also the basic process in the production of hypoallergenic formulae can be due to fermentation and acidification and hence most likely cause a reduction of antigenicity. Due to these divergent evidences, different process-specific changes in antigenicity and allergenicity could be expected upon testing the model products with rabbit antisera and IgE from allergic patients. By contrast, the results of the present study indicated that the antibody binding properties are clearly affected by thermal processing of celery but to a relatively weak degree by all non-thermal processes. This may be explained by the view that all non-thermal treatments applied in this investigation maintain a considerable amount of protein in the native state. However, non-thermal fermentation which is likely to reduce allergenicity has not been studied, but an acid preserve of pickled celery had a reduced but remarkable residual capacity of IgE binding in the EAST.

The strategy of using sera from patients who were exclusively sensitised to one specific celery allergen yielded data about diverging allergen stabilities. As indicated by the results of EAST, which were in accordance with immunoblotting in most of the cases, a complete loss of antibody binding to Api g 1 can be achieved by cooking and even by short microwave treatment for at least 10 min (Table 2), whereas inactivation of profilin required 30 min of microwaving but was not observed

after 20 min of conventional cooking. It is remarkable that the latter treatment led to the presence of allergenic profilin even in the cooking water. Taken together these observations may be due to the dominance of sequential epitopes on profilin, whereas the immuno-reactivity of Api g 1 seems to depend on the conformational structure. The detection of IgE-reactive carbohydrate epitopes in all processed preparations can again be explained by the fact that these determinants are not affected by changes of the protein folding. Despite some variations among individual patients, the stability data obtained with sera from single patients were confirmed by immunoblot analyses of sera from 25 celery-sensitised patients and three pooled human sera containing IgE against celery.

According to our own unpublished data of 32 celery-allergic patients, 40% of these were exclusively sensitised to the major allergen Api g 1. For this important subgroup tolerance to foods containing celery may be achieved by a mild thermal treatment.

The RBL cell assay has been designed as a murine *in vitro* model that simulates IgE-mediated reactions. In the case of celery, its results seem to reflect the overall allergenic activity of the products for patients with a predominant sensitisation to Api g 1. This view has been confirmed by immunoblot analyses that indicated the presence of IgE specific for Api g 1 and the absence of considerable amounts of anti-carbohydrate and anti-profilin IgE in the reactive murine serum (results not shown). In the context of human IgE directed against CCD it has to be noticed that, in contrast to the common opinion about Api g 1 and profilin, the clinical significance of these antibodies is doubtful (van Ree and Aalberse 1993; Vieths *et al* 1994a) and requires further investigation.

All patients who participated in the present study were allergic against birch pollen and, in most cases against other kinds of pollen. The immunisation of this particular group of patients is most likely due to the uptake of food-related pollen allergens via the respiratory tract, but not to the ingestion of food (Ortolani *et al* 1988; Ebner *et al* 1991; van Ree and Aalberse 1993; Breiteneder *et al* 1995; Vieths *et al* 1995b). When raised, the pollen-specific IgE cross-reacts with allergens from native plant foods and may therefore be responsible for the symptoms caused by these foods. As a consequence, the symptoms are most likely due to the ingestion of native celery allergens. As demonstrated with IgE of such patients and with sera of mice which were immunised with native celery, these allergens may have residual and often reduced allergenic activity after application of several kinds of technological processing. Therefore, native proteins may indeed represent the immunogens of our patients.

In principle, patients may also become sensitised by the ingestion of heated celery roots. In this case, one would expect a strong antibody response to heat-

denatured celery allergens and a weaker reactivity with native celery. However, we were not able to detect IgE antibodies with such properties neither in this study nor in a group of 46 pollen-allergic patients who were also sensitised against related plant foods (Jankiewicz *et al* 1996). Moreover, the literature lacks data about patients with severe allergies against heated celery who tolerate native celery, and about celery allergy independent of pollinosis. However, the consequences of allergic sensitisation against heated celery were stimulated in initial mediator-release experiments with serum from mice immunised with extract from a microwaved celery preparation. With this serum, the allergenic response of sensitised RBL cells was very intensive even to thermally treated samples such as cooked celery and pickled celery (which weakly bound to IgE from most allergic patients and to murine IgE against native celery) (results not shown). Consequently, one would expect a high risk of allergic reactions to processed celery products in patients with an IgE response against these modified epitopes.

CONCLUSION

We conclude that in pollen-allergic patients who are also sensitised to celery roots the thermal stability of the known celery allergens decreases in the following order: Carbohydrate epitopes > profilin > Api g 1.

Summarising, our model investigation confirms the existence of a partly heat-stable allergenic activity in celery roots. At the molecular level, this stability is primarily related to the pan-allergen profilin and to carbohydrate epitopes. The knowledge about the high lability of the major allergen Api g 1 may be helpful for nutritional recommendations if the specific sensitisation of a celery-allergic patient is known. For the subgroup of patients who are exclusively sensitised to this allergen the elicitation of symptoms upon ingestion of celery may be circumvented by mild thermal food processing. Moreover, the study may assist the optimisation of procedures which probably achieve an additional reduction of the allergenic potency of celery as a constituent of processed foods. Further studies are being undertaken to determine the presence of active celery allergens in commercial food products and to investigate the structural basis of the epitope modifications induced by heat-denaturation of celery roots.

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