

Original article

Roasted hazelnuts – allergenic activity evaluated by double-blind, placebo-controlled food challenge

Background: Allergy to hazelnuts is a common example of birch pollen related food allergy. Symptoms upon ingestion are often confined to the mouth and throat, but severe systemic reactions have been described in some patients. The aim of the study was to evaluate the reduction in allergenicity by roasting of the nuts.

Methods: Double-blind, placebo-controlled food challenges (DBPCFC) with roasted hazelnuts (140°C, 40 min) were performed in 17 birch pollen allergic patients with DBPCFC-confirmed food allergy to raw hazelnuts. The effect of roasting was further evaluated by skin prick test (SPT), histamine release (HR), measurement of specific IgE, and IgE-inhibition experiments.

Results: In 5/17 patients the DBPCFC with the roasted nuts were positive. The symptoms were generally mild and included OAS (oral allergy syndrome) in all patients. Roasting of the nuts significantly reduced the allergenic activity evaluated by SPT, HR, specific IgE, and IgE-inhibition. Immunoblotting experiments with recombinant hazelnut allergens showed sensitization against Cor a 1.04 in 16/17 patients and against Cor a 2 in 7/17 patients. None of the patients were sensitized to Cor a 8. Challenge-positive patients did not differ from the rest in IgE-binding pattern.

Conclusions: All the applied methods indicated that roasting of hazelnuts reduces the allergenicity, but since 5/17 birch pollen allergic patients were DBPCFC-positive to the roasted nuts, ingestion of roasted hazelnuts or products containing roasted hazelnuts can not be considered safe for a number of hazelnut allergic consumers. For patients with a history of severe allergic symptoms upon ingestion of hazelnuts, thorough and conscientious food labelling of hazelnuts and hazelnut residues is essential.

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The association between allergy to various plant foods and allergy to birch pollen has been known for many years and several groups have established that food allergy to hazelnuts (HN) is an important clinical example of birch pollen related food allergies (1–4). In a recent study, with participation of the allergy centres in Milan, Zurich and Copenhagen, food allergy to HN was evaluated in 86 patients by double-blind, placebo-controlled food challenges (DBPCFC) (5). Tree nuts and among them HN are common causes of food allergic symptoms and sometimes even food-induced anaphylaxis (6–10).

Abbreviations: AP alkaline phosphatase, DBPCFC double-blind, placebo-controlled food challenge, EAACI European Academy of Allergology and Clinical Immunology, EAST enzyme allergosorbent test, HN hazelnut, HR histamine release, IB incubation buffer, LTP lipid transfer proteins, mAb monoclonal antibody, NC nitrocellulose, OAS oral allergy syndrome, r recombinant, SPT skin prick test.

In Northern Europe, most cases of food allergy to fruits and nuts can be explained by the resemblance between the major birch pollen allergen Bet v 1 and the allergens from various nuts and fruits, but there seems to be regional differences in the sensitization pattern in Europe (3, 11). The connection to birch pollen allergy seems to be less predominant in the southern parts of Europe, and recent results indicate that non-pollen related allergens may play an important role in food allergy to HN in some patients (12–14). The Bet v 1-homologous food allergens are known to be relatively labile to heat treatment and gastric digestion, whereas the non-pollen related food allergens have varying degrees of heat stability (15). The major HN allergen, Cor a 1.04 is homologous to Bet v 1 both at the sequential and the structural level, and also exhibits extensive IgE cross-reactivity with IgE directed against Bet v 1 (3, 16). According to a number of studies, heat treatment substantially reduces the reactivity of this protein

(12, 14, 17). Proteins belonging to a protein family called Non-Specific Lipid Transfer Proteins (LTP) have been shown to play a role in food allergy to HN, at least in Southern Europe, and these proteins seem to be heat resistant (12–14, 18, 19). Other heat-stable allergens may also be involved but the clinical significance is less documented (17).

Since HN contain different allergens with varying susceptibility to heat, it is not clear whether roasting results in an elimination of the allergenic potential or merely a reduction of allergenicity. For the hazelnut-allergic patient these questions are essential, since many processed foods, such as sweets, cookies, etc., contain hazelnuts. If the hazelnut content in the food is low or arises from inadvertent cross-contamination, hazelnut traces may act as “hidden allergens” with the patient unaware of their presence (20).

The aim of this study was to assess the reduction in allergenic potency of HN by roasting, and thereby indirectly evaluate the safety of products containing roasted HN for patients with food allergy to HN.

Material and methods

Design

Two clinical centres participated in this study: the allergy units at the University Hospitals in Copenhagen and Zurich. The serological assays were performed at the Paul-Ehrlich-Institut in Langen and the HR experiments at the Reference Laboratory in Copenhagen. The study took place from 1998 to 2000.

The clinical reactivity to HN was evaluated in birch pollen and HN allergic patients by blinded food challenges with raw and roasted nuts. Further, the impact of roasting on the biological activity of the nuts was evaluated by means of SPT, HR, specific IgE, and IgE-inhibition experiments. Finally, the allergen profiles of the HN allergic patients were determined by immunoblotting using a panel of recombinant allergens. The study was approved by the local ethics committees and all subjects gave written informed consent before entering the study.

Study population

The patients were included on the basis of food allergy to HN, previously confirmed by DBPCFC, except for one patient (no. 17), who had a convincing history and therefore was included directly in this study. A total of 17 patients (11 females and 6 males) were included, 10 in Copenhagen and 7 in Zurich. The median age was 24.5 years (range 14–65). All patients presented an allergic history including oral allergy syndrome (OAS) (2) upon ingestion of HN. In 14 patients the symptoms were confined to the oral cavity, whereas three had also experienced other allergic symptoms such as asthma, rhinitis, or gastrointestinal discomfort. None of the patients had a history of anaphylaxis caused by ingestion of HN.

Medication

Prior to challenges and SPT, medication was discontinued according to the guidelines on skin testing from the European Academy of Allergology and Clinical Immunology (EAACI) (21). None of the

patients received inhaled corticosteroids at the time of the oral challenges.

Allergen sources and extracts

Raw and roasted (140°C, 40 min) HN of the Piemonte variety were used for oral challenges, HR, SPT and as source material for production of allergen extracts. The nuts were supplied by Sorematec, Arlon-Schoppach, Belgium. HN extracts, prepared as previously described (17) were used for SPT, HR, measurement of specific IgE, and inhibition experiments. Further, a commercial birch pollen extract (Soluprick, ALK-Abelló, Hørsholm, Denmark) and a commercial HN extract (Stallergènes SA, Antony Cedex, France) were used for SPT. Additionally, recombinant (r) birch pollen allergens rBet v 1, rBet v 2 (Biomay, Vienna, Austria) and recombinant HN allergens rCor a 1.04 (GenBank Accession No. AF327622) (16), rCor a 2 (profilin) (AF327622) and rCor a 8 (LTP) (AF329829) were included for the IgE measurements and immunoblotting experiments.

DBPCFC

The oral challenges were performed as previously described (5). In summary, the challenge consisted of increasing doses of an HN pudding. The first dose was a test dose (= placebo) in order to evaluate the level of symptoms and possible reactions to the placebo challenge. If symptoms appeared upon a given dose, the following dose was not served until symptoms had disappeared or diminished significantly. The procedure was discontinued when the patients had a convincing and/or repeated reaction to a particular dose. In Copenhagen, the challenge procedure consisted of three challenges (raw nut, roasted nut, and placebo) performed on separate days, whereas in Zurich, the challenges took place on the same day with an interval of at least 1 h. In Zurich, DBPCFC with raw nuts and placebo had been performed 1 year previously, and the same patients were called in again for the challenge with the roasted nuts and placebo. The current activity of the HN allergy to raw HN was assessed by history. A dietician prepared all challenges. In Copenhagen the maximal total dose of HN was 10 g and in Zurich 18.2 g. The challenge order was randomized and the code was not broken until all challenges were served/all challenge days completed and the results entered in an observation sheet. All challenges were performed out of the pollen season.

Skin prick test

SPT was performed according to EAACI guidelines (21). SPT with raw and roasted nuts was performed by the prick–prick technique (22, 23). The protein content of the HN extracts was 1 mg/ml (in Copenhagen the biological potency was determined by SPT with a serial five-fold dilution of a stock of 2 mg/ml). All skin tests were performed in duplicate or quadruplicate, and a skin prick test was considered positive when the wheal area was $\geq 7 \text{ mm}^2$ (diameter $\geq 3 \text{ mm}$). For quantitative determination of the biological potency, the skin wheal areas to the extract of raw and roasted nut were determined by computer scanning (24) in nine patients.

Specific IgE

Specific IgE against HN and pollen allergens including rBet v 1 and rBet v 2 was measured by the CAP-system (Pharmacia Diagnostics, Uppsala, Sweden), according to the instructions of the manufacturer. IgE against extracts of raw HN and roasted HN was

determined by an enzyme allergosorbent test (EAST) (Allergopharma, Reinbek, Germany) (50 µg of extract of raw or heated HN per allergen disk) according to (16) as described in (25). IgE to recombinant allergens was detected by IgE immunoblotting.

Histamine release

Biological potency was compared by histamine release tests performed by the glass-fibre method (26). The extracts of raw and roasted nuts (protein content of stock solutions 100 µg/ml) and fresh preparations of raw and roasted whole HN were applied for HR. The whole nuts (5 g of HN in 5 ml buffer) were prepared as described for apples (27). The extracts and fresh preparations were added to the microtitre plates in 12 3.5-fold dilutions. Histamine release ≥ 14 ng/ml was considered as significant. Control experiments were performed with blood from a healthy, non-atopic individual and all dilutions of the allergen extracts produced a release < 10 ng/ml. The HR was calculated as the mean value of the maximal release (ng/ml) and as the number of 3.5-fold dilution of the extract at which the release was 50% of the maximal release ($1/2$ max HR), e.g. the higher the number, the greater the allergenic potency of the extract.

Tris-Tricine-PAGE and immunoblotting

The recombinant HN allergens Cor a 1.0401 and Cor a 2 (0.5 µg/cm each) were separated by Tris-Tricine-PAGE according to Schägger and von Jagow (28) with a 16.5% T (T = total concentration [wt/wt] of acrylamide plus bis-acrylamide), 6% C (C = percentage [wt/wt] of the cross-linker relative to T) separating gel overlaid by a 4% T, 3% C stacking gel under reducing conditions in a Mini Protean II Cell (Bio-Rad, Munich, Germany), transferred onto nitrocellulose (NC) membranes (Protran 0.2 µm, Schleicher & Schuell, Dassel, Germany) by semi-dry blotting according to (29). NC-strips (3 mm $\times$ 7 cm) were incubated with 120 µl patient serum and 480 µl incubation buffer (IB: 50 mM Tris/HCl, 150 mM NaCl, pH 7.4, 0.05% Tween 20, 0.1% bovine serum albumin) overnight. Bound IgE was detected by an alkaline phosphatase (AP) labelled mAb mouse anti-human IgE-AP (Pharmingen, Hamburg, Germany), diluted 1 : 750 in IB (1 ml per NC-strip), and AP Conjugate-Substrate Kit (Bio-Rad, Munich, Germany). To achieve a suitable sensitivity of the immunodetection, Tris-Trichine-PAGE of LTP rCor a 8 (0.5 µg/cm) had to be performed under non-reducing conditions, and this allergen was therefore blotted separately. Sera from two Spanish patients with HN allergy and IgE against HN LTP were applied as positive controls (Schocker et al., submitted).

EAST inhibition

Dose-related EAST inhibition experiments with extract of raw HN (50 µg/disk) or rCor a 1.0401 (0.1 µg/disk) immobilized on disks were performed with serum pools A and B as previously described (12). Patients in pool A (nos. 2 and 9) had a positive DBPCFC to raw HN but a negative DBPCFC to roasted HN, whereas patients in pool B (nos. 3 and 8) were DBPCFC-positive to both raw and roasted HN. Serial dilutions of inhibitors were prepared from extracts of raw and roasted HN, respectively. Inhibitor concentrations are given in the abscissa of the corresponding figures. Non-allergic control sera did not show any unspecific reactivities to the allergen disks.

Statistics

The differences between raw and roasted nuts evaluated by SPT, specific IgE, and HR were compared by a nonparametric method

(Wilcoxon Signed Rank Test). DBPCFC-positive and negative patients were compared by the Chi-squared test and Mann-Whitney Rank Sum Test. The applied statistical programs were SigmaStat 1.0 (Jandel Corporation, San Rafael, CA, USA) and STATISTICA 4.5 (StatSoft Inc., Tulsa, OK, USA).

Results

DBPCFC, skin prick test and specific IgE

Table 1 summarizes the results of the food challenges, SPT, and specific IgE measurements for all 17 patients. Five patients (29%), four in Copenhagen and one in Zurich, reacted to the oral challenges with the roasted nuts. The median doses that gave a positive response were 7 g of roasted HN (0.2–10 g) and 2 g of raw HN (0.1–10 g) in Copenhagen. In Zurich, the single patient with a positive DBPCFC to roasted HN reacted to a dose of 5.2 g, whereas the dose of raw HN that gave a positive response was 2.6 g in all the included patients in Zurich. The symptoms were generally mild. All five patients experienced OAS and no objective signs were registered during the challenges. Because of the differences in challenge procedures between the two centres, e.g. time elapsed between challenge with raw and roasted HN in Zurich, and the low number of responders, the results of the DBPCFC to raw and roasted nut, respectively, were not compared qualitatively. However, in the four DBPCFC-positive patients from Copenhagen, it was noted that larger amounts of the pudding with roasted compared to raw HN were necessary in order to provoke the same level of symptoms (data not shown). One patient in Copenhagen developed a mild skin rash the day after the placebo challenge, but the symptoms was later diagnosed as an virus infection. The patient did not react to the roasted nuts.

Regarding SPT, all patients were positive to the commercial birch and commercial HN extracts (data not shown). All but one patient had a positive SPT by prick-prick with fresh HN (94%) and the same pattern was seen for SPT with the native nut extract. Four out of 10 patients (40%) tested reacted to prick-prick with the roasted nuts, whereas only 1/16 (6%) reacted to the extract of the roasted nuts. None of the nine Copenhagen patients had a skin wheal area ≥ 7 mm² to the extract of the roasted nuts. Calculated from the results of the SPT with the serial dilution of the native nut extract, roasting of the nuts resulted in a decrease of the biological potency of at least 125 times (data not shown).

Eleven out of 17 patients (65%) showed specific IgE to the native HN measured by EAST and only 3/17 (18%) had demonstrable IgE to the roasted HN. Interestingly, neither SPT to roasted HN (prick-prick or extracts) nor measurement of specific IgE predicted clinical reactivity as only one out of five challenge-positive patients demonstrated a positive skin reaction to prick-prick with the roasted nut and IgE to the roasted nut. Sixteen

Table 1. DBPCFC, Specific IgE and Skin Prick Test

	patient no	DBPCFC				IgE				SPT				
		hazelnuts		East, class hazelnuts		CAP, class				prick-prick hazelnuts		extracts hazelnuts		
		native	roasted	native	roasted	hazelnut	birch	Bet v 1	Bet v 2	native	roasted	native	roasted	birch
Zurich	1	OAS/RC	/	2	0	2	3	3	0	/	nd	+	/	+
	2	OAS	/	2	0	2	5	6	0	+	nd	+	/	+
	3	OAS	OAS	2	0	1	4	4	0	+	nd	+	/	+
	4	OAS	/	3	1	2	nd	4	0	+	nd	+	/	+
	5	OAS	/	2	1	2	4	4	2	+	nd	+	+	+
	6	OAS	/	2	0	3	5	5	2	+	nd	+	/	+
	7	OAS	/	0	0	0	3	3	0	+	nd	+	/	+
Copenhagen	8	OAS	OAS	3	0	3	4	5	0	+	/	+	/	+
	9	OAS	/	2	0	2	4	4	0	+	+	+	/	+
	10	OAS	/	2	0	2	4	5	3	+	/	+	/	+
	11	OAS	OAS	0	0	0	4	3	0	+	/	+	/	+
	12	OAS	/	1	0	2	4	4	0	+	/	+	/	+
	13	OAS	/	0	0	0	3	0	0	+	+	+	/	+
	14	OAS	/	0	0	0	3	3	0	+	+	+	/	+
	15	OAS	/	0	0	0	4	3	0	+	/	+	/	+
	16	OAS	OAS	0	0	0	3	3	0	+	/	¹	/	+
	17	OAS/RC	OAS	3	3	3	2	1	2	+	+	nd	nd	+

+ = positive; / = negative.

¹ SPT area >7mm² to the concentration 400 microgram/ml, area = 6 mm² to concentrate 2 mg/ml.**Patient no. 3, 8, 11, 16 and 17 DBPCFC-positive to both raw and roasted hazelnuts.**

patients (94%) had specific IgE against Bet v 1, whereas only 4/17 (24%) had measurable IgE to Bet v 2. The Bet v 1-negative patient had previously been tested positive. All five DBPCFC-positive patients were sensitized to Bet v 1 and one was also positive to Bet v 2. There were no significant differences between DBPCFC-positive and negative patients regarding level of IgE to HN, birch, Bet v 1 or 2.

Histamine release

Figure 1 shows the results of the HR (Copenhagen patients), illustrated by the differences in 1/2 max HR between native and roasted HN. Evaluated by use of both whole nuts ($P = 0.01$) and the extracts ($P = 0.02$), roasting resulted in a significantly reduced release, but 9/10 (90%) and 7/10 (70%) showed significant release to the fresh preparation of the roasted nuts and the extract of roasted nuts, respectively. The decrease in release capacity by heating seemed to be more pronounced for the whole nuts than for the extracts evaluated by the slope of the curves. This observation is most likely due to different extraction procedures: a 4-h extraction in phosphate buffered saline was used for preparation of the extracts (17), whereas the whole nuts were ground and suspended in release buffer very shortly before performing the release assay, which may result in an incomplete extraction, but more likely resembles the process which occurs during elicitation of OAS. The maximal HR in ng/ml did not show any significant differences for either

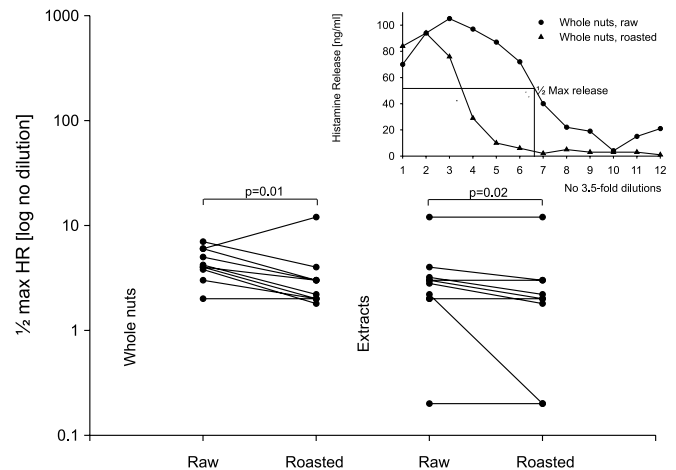


Figure 1. Histamine release (no. 3.5-fold dilution corresponding to 1/2 max HR). Parallel data from 10 patients on raw and roasted whole HN (left) and extracts of raw and roasted HN (right), respectively. Individual release curves (HR in ng/ml) for whole nuts from patient no. 9 are inserted at the upper right corner.

whole nuts or extracts when the results from all 10 patients were compared, but when the dilution numbers corresponding to the maximal release were compared, roasting significantly reduced the maximal release (whole nuts and extracts, $P = 0.03$) (data not shown). Again, the DBPCFC-positive patients did not differ from the rest of the patients. Significant HR to the

fresh preparation of the whole roasted HN was seen in all four challenge-positive patients in the Copenhagen group and three of the four patients were positive to the extract also. The release curves for whole nuts from a selected patient (no. 9) are inserted in the figure. The curve representing the result for the roasted nuts reached a lower peak value and was shifted to the left compared to the curve for the raw nuts, corresponding to a higher stimulator concentration needed. Evaluated by the difference in 1/2 max release for all 10 patients, the roasting resulted in a reduction of the potency for both the whole nuts and the extracts of about 50% (data not shown).

Immunoblot

Immunoblotting results are shown in Fig. 2. In order to increase the sensitivity, the allergen patterns of the patients were evaluated by immunoblotting with the recombinant HN allergens rCor a 1.0401, rCor a 2, and rCor a 8 instead of native HN extracts. Sensitization against the Bet v 1-related major HN allergen rCor a 1.0401 was detected in 16/17 (94%) patients and against profilin rCor a 2 in 7/17 (41%) patients. None of the patient sera in this study showed IgE-binding to HN LTP rCor a 8 in contrast to positive control sera of Spanish patients with HN allergy (Schocker et al., submitted). Regarding the five patients with positive DBPCFC to roasted HN, one patient showed specific IgE against both rCor a 1.0401 and rCor a 2, in three patients exclusively IgE against rCor a 1.0401 was detected, and only one patient presented IgE against rCor a 2 without concomitant IgE against rCor a 1.0401. Thus, sensitization to Cor a 2 was obtained in 2/5 (40%) patients DBPCFC-positive to both raw and roasted HN and in 5/12 (42%) patients reacting only to the raw nuts.

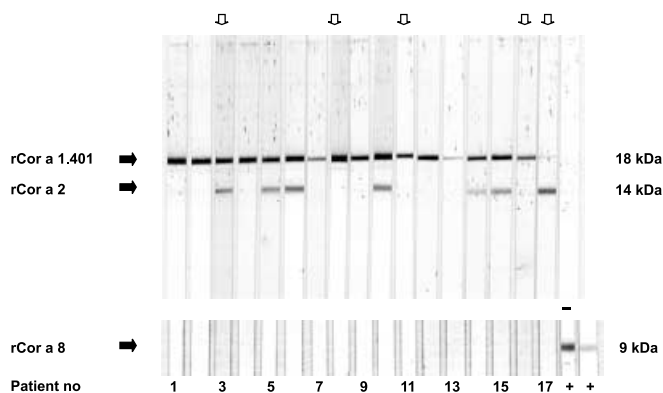


Figure 2. Immunoblots with rCor a 1.0401 and rCor a 2 (upper part of the figure) and rCor a 8 (bottom). 1–17: patient sera; – : non allergic control; + : LTP-positive control sera. The molecular weights of significant bands are indicated to the right. Patient no. 3, 8, 11, 16 and 17 were DBPCFC-positive to both raw and roasted HN (white arrows).

EAST inhibition

EAST inhibition experiments (Fig. 3) were performed to investigate quantitative differences in IgE-reactivity between patients with positive DBPCFC to both raw and roasted HN (pool B), and patients with a positive challenge to raw HN but a negative challenge to the roasted nuts (pool A). IgE binding to extract of raw HN (Fig. 3a) and to rCor a 1.0401 (Fig. 3b) was inhibited by extract of raw and roasted HN, respectively. Inhibition graphs of the two serum pools were almost identical in each experiment indicating similar IgE-reactivities of the two patient groups. IgE-binding to both sorts of allergen disks was completely quenched by extract of raw HN and inhibited up to 60–80% by extract of roasted HN. Approximately 100-fold higher concentrations of extract of roasted HN compared to raw were applied for obtaining a 50% inhibition (c_{50}).

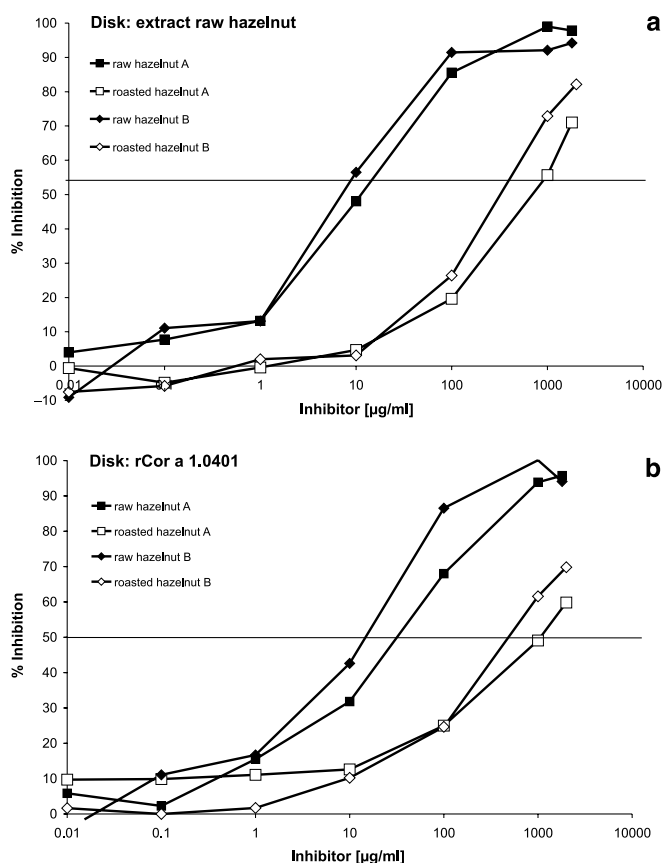


Figure 3. Dose-related EAST inhibition with extract of HN (50 µg/disk) (fig 3a) and rCor a 1.0401 (0.1 µg/disk) (fig 3b) and two serum pools: A (positive DBPCFC to raw HN only, patient no. 2 and 9) and B (positive DBPCFC to both raw and roasted HN, patient no. 3 and 8). Final dilutions of serum pools with HN disks were 1:5 for pool A and 1:10 for pool B, and with rCor a 1.0401 disks 1:8 for both pools.

Discussion

Since allergy to tree nuts is common and in some cases related to food anaphylaxis, and at the same time nuts are widely used as constituents in sweets, cookies, etc., the issue of consumer safety is highly relevant (12, 30).

Case histories of severe reactions to sweets and other food products containing nuts have been reported, but to our knowledge no further controlled studies of the clinical significance of processing of HN have been published (7, 20, 31). The present data confirm, as previously published by our groups, the clinical relevance of food allergy to HN and that HN allergy can be diagnosed by DBPCFC (5, 32). All methods used in this study supported that roasting of the HN leads to significant reduction of allergenic activity, but nevertheless a significant number of patients (5/17) in this study population proved to be clinically reactive to heat-treated nuts evaluated by DBPCFC. The heat treatment was consistent with usual procedures applied in the food industries. The fact that the patients developed very mild symptoms to the roasted nuts, can partly be explained by the set-up: For safety reasons, the challenge procedure was abrogated when the patients developed significant or repeated symptoms and our results therefore do not allow for any evaluation of the maximal level of symptoms. Further, the fact that all patients were sensitized to the Bet v 1-related allergen Cor a 1.04 may have influenced the severity of the symptoms.

Even though all the applied methods comparing raw and roasted HN showed a reduction in allergenic potency by roasting, there seems to be a lack of consistency between the methods used, since neither the skin tests, HR, nor the IgE assays could discriminate the patients with a positive reaction to the oral challenge with roasted nuts. A similar discrepancy between methods was shown in investigations of allergy to celery root (25, 33, 34). According to the EAST inhibition results, there is no doubt that the Bet v 1-related major HN allergen Cor a 1.04 is affected by heat. Inactivation of Cor a 1.04 by roasting of the nuts at 140°C for 40 min was most intensive, as about 100-fold higher inhibitor concentrations compared to raw HN were necessary to obtain a 50% inhibition. In spite of that, traces of the protein in the roasted nuts may still be able to elicit symptoms in the clinically most sensitive patients, since IgE-binding to Cor a 1.0401 could still be inhibited to more than 60% by extract of roasted HN. Further, factors not addressed by this study such as individual differences in the oral mucosa, clinical threshold, histamine sensitivity, etc. may add to the explanation.

The results confirm the clinical importance of the Bet v 1-homologous allergens in plant food allergy in Central and Northern Europe. All but one serum bound to rCor a 1.04 and seven sera bound to profilin rCor a 2.

No IgE-binding was seen to the LTP rCor a 8, so in these patients HN LTP does not seem to play any significant role. On the other hand, the patients were not pre-selected for severe symptoms and it is possible that the reactivity pattern would have been different in patients with a history of anaphylactic reactions. Recent data from Southern Europe suggest that LTP are major allergens regarding severity of clinical reactions to HN (14), but also other heat-stable HN allergens have been described (12, 17). Interestingly, in three of five patients with positive DBPCFC to roasted HN exclusively IgE against rCor a 1.0401 was detected by immunoblotting with recombinant HN allergens, and one of five patients showed specific IgE against rCor a 1.0401 and rCor a 2. Based on the results of this study, it seems that in our patients from Central and Northern Europe, who are negative to LTP, either trace amounts of un-destroyed Cor a 1.04 or Cor a 2 in roasted nuts, or heat-stable minor allergens different from LTP may trigger the reactions. For reasons of higher analytical sensitivity the allergen pattern of the participants of this study was determined with the three most important allergens in recombinant form and therefore, it is possible that we overlooked some important heat-stable minor allergens. However, sera of all seven patients from Zurich and nine patients from Copenhagen were already analysed by IgE immunoblotting with HN extract (14) without an indication of the presence of other heat-stable HN minor allergens other than LTP. Data recently obtained by Ballmer-Weber et al. (35) support the hypothesis of trace amounts of pollen-related HN allergens causing allergy to roasted HN, since in that study one patient with a positive DBPCFC to cooked celery was exclusively sensitized to the Bet v 1-related major celery allergen Api g 1. Furthermore, profilin in celery is known to be partially heat-resistant (33, 34). Therefore, the HN profilin Cor a 2 may also be involved in triggering the reactions in the patient group with positive DBPCFC to roasted HN. Although, similar to Cor a 1.04, no correlation was obtained between profilin sensitization and allergy to roasted HN, since IgE to rCor a 2 was obtained with almost the same prevalence in patients with positive (40%) and negative (42%) DBPCFC to roasted HN. Furthermore, the observation that the two patient groups, represented by serum pools B and A in the EAST inhibition experiments, did not show any differences in the inhibition potency of raw and roasted HN, underlined that identical allergens were recognized by IgE in both serum pools.

In conclusion, the allergenicity of HN was clearly reduced by roasting, in particular in patients sensitized to the birch pollen-related HN allergens Cor a 1.04 and Cor a 2, but roasted HN are still not safe for a considerable number of HN-allergic patients. The results of this study emphasize the importance of a thorough food product labelling when known allergenic offenders such as tree nuts are involved.

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