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REVIEW ARTICLE

A systematic review of the effect of thermal processing on the allergenicity of tree nuts

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Abstract

Background: Allergenicity of foods can be influenced by processing. Tree nuts are an important source of nutrition and increasingly consumed; however, processing methods are quite variable and data are currently lacking on the effects of processing on allergenicity.

Objective: To perform a systematic literature review on the effects of food processing on the allergenicity of tree nuts.

Methods: A systematic literature search of PubMed and Embase databases was performed, with screening of references, related articles and citations. Studies were included if they assessed the allergenicity or immunogenicity of processed nuts.

Results: The search resulted in 32 articles suitable for analysis. Clinical studies indicate that roasting reduces the allergenicity of hazelnut in individuals with a birch pollen allergy and reactivity to raw hazelnut. Thermal processing may reduce the allergenicity of the PR-10 protein in hazelnut and almond *in vitro*. The majority of the *in vitro* studies investigating the allergenicity of nonspecific lipid transfer proteins (nsLTPs) and seed storage proteins in hazelnut, almond, cashew nut, Brazil nut, walnut, pecan nut and pistachio nut show heat stability towards different thermal processing methods.

Conclusion: Thermal processing may reduce allergenicity of PR-10 proteins in hazelnut and almond, in contrast to nsLTPs and seed storage proteins. This has important implications for source materials used for IgE testing and food challenges and diet advice.

Tree nuts (e.g. hazelnut, walnut) are an important source of nutrients. A diet rich in tree nuts has been shown to improve cardiovascular risk markers (1). On the other hand, tree nuts are a major cause of food allergy (2). Tree nut allergy can result from cross-reactivity after primary sensitization to birch pollen (BP). The major allergen in these foods appeared structurally related to the major allergen in BP, Bet v 1, and belongs to the pathogenesis-related protein 10 (PR-10) family. Of BP allergic individuals, 73% reported a BP-related food allergy (3) involving Rosacea fruits and nuts and in addition vegetables, legumes and seeds (almond) (4). Hazelnut allergy is one of the most frequently reported BP-related food allergies and leads often to mild oral allergy symptoms (3, 5). Allergy for almond and walnut often accompanies a hazelnut allergy in our allergy clinic, suggesting a relation with BP. The BP-related allergens Cor a 1 (hazelnut) (6) and Pru du 1 (almond) are PR-10 proteins, and Cor a 2 and Pru du 4 (7) are profilins (panallergens that are present in most pollens and fruits). Non-BP-related allergens such as nonspecific lipid transfer proteins (nsLTPs) and seed storage proteins might be associated with a severe allergy (8–10) For hazelnut, Cor a 8, 9, 11, 12, 13 and 14 (11–14) have been described. For almond, Pru du 2S, 3, 5 and 6 (15, 16) were identified. Table 1 shows the major allergens from tree nuts, including their structural relationship.

Table 1 Major allergens and structural relation for different tree nuts, according to allergome.org. Estimated molecular weight (kD) of each allergen is indicated in brackets

	PR-10 protein MW (kD)	Profilin MW (kD)	Ribosomal protein MW (kD)	Nonspecific lipid transfer protein LTP MW (kD)	11S globulin MW (kD)	7S globulin MW (kD)	Oleosin MW (kD)	2S albumin MW (kD)
Hazelnut	Cor a 1 (17)	Cor a 2 (14)		Cor a 8 (9)	Cor a 9 (30–40)	Cor a 11 (48)	Cor a 12, 13 (14–17)	Cor a 14 (12–14, 17)
Almond	Pru du 1	Pru du 4 (14)	Pru du 5 (10)	Pru du 3 (9)	Pru du 6 (41)			Pru du 2S (12)
Cashew					Ana o 2 (52)	Ana o 1 (50)		Ana o 3 (14)
Pecan nut					Car i 4 (55)			Car i 1 (16)
Pistachio					Pis v 2 (53) Pis v 5 (36)	Pis v 3 (55)		Pis v 1 (17)
Walnut		Jug r 5		Jug r 3 (9)	Jug r 4 (58)	Jug r 2 (44)		Jug r 1 (14)
Brazil nut					Ber e 2 (52)			Ber e 1 (9)

Tree nut consumption shows geographical differences in Europe, with highest consumption in Mediterranean countries. Walnut is the most popular nut, followed by almond and hazelnuts, respectively (17). The ingestion of tree nuts increased over the last decades, and raw nuts are increasingly available and consumed (17, 18). This might contribute to the severity of allergic reactions in tree nut allergic patients, because raw nuts might be more allergenic than processed nuts. Processing, such as heating, might lead to denaturation of food allergens and disruption of conformational IgE epitopes (19), while linear T-cell epitopes may preserve. For peanut, a decreased allergenicity was observed after boiling, while roasting increased the allergenicity (20).

The influence of processing on the allergenicity of tree nuts is largely unknown. Different heating methods are used when processing various tree nuts, of which an overview is shown in Table 2. Information about the effect of processing on the allergenicity of tree nuts is vital in the diagnosis and treatment advice provided to tree nut allergic patients. Therefore, we performed a systematic literature search to evaluate the current knowledge on the influence of processing on the allergenicity and immunoreactivity of tree nuts.

Methods

Search strategy

A systematic search of the PubMed and Embase databases was performed by two reviewers using the terms 'processing' and 'nuts' and synonyms, with screening of references, related articles and citations (Web of Science and SCOPUS) (Fig. 1). From the major list of tree nuts according to the FDA official list, seven (hazelnut, almond, cashew nut, Brazil nut, pecan nut, walnut and pistachio nut) are described in this study, because the others have hardly been studied or have no established allergenicity.

Study selection

Studies were included if they assessed the allergenicity or immunogenicity of processed nuts. Included studies were published in peer-reviewed journals and written in English. Reviews and case reports were excluded along with studies, of which full-text articles were not available (Fig. 1). Screening for eligibility was performed independently by two reviewers.

Data collection

Data on patient characteristics (age, hazelnut and BP sensitization, skin prick test, food challenge), source and type of antibodies, tree nut variety, temperature of process, duration and way of processing and *in vitro* techniques to assess allergenicity or immunogenicity were collected independently by two reviewers. Data were discussed and interpreted by both reviewers. Disagreements were discussed to reach consensus, if needed a third reviewer was consulted. Clinical studies with food challenges were given highest strength of evidence score, followed by *in vitro* studies measuring IgE reactivity (allergenicity), and the lowest score was given to studies measuring *in vitro* IgG reactivity (immunogenicity).

Results

The systematic search of the literature resulted in 846 articles. Of these 846 articles, 825 articles did not meet our inclusion criteria and were excluded (Fig. 1). After thoroughly screening related articles of the 21 articles that initially met our inclusion criteria, an additional 11 articles about allergenicity or immunogenicity of processed nuts were found and included in our review. This resulted in 32 articles for our final analysis (Fig. 1). A summary of the results from clinical studies and measured IgE reactivity is shown in Table 3. A detailed summary of the effects of processing on the allergenicity of each tree nut is provided in the following sections.

Decreased allergenicity of hazelnut by roasting in individuals with a hazelnut and BP allergy

Two clinical studies investigated the influence of roasting on the allergenicity of hazelnut by double-blind placebo-controlled food challenges (DBPCFCs). Both studies reported a decreased allergenicity.

Table 2 Usual processing methods in the food industry

Tree nut	Usual processing methods	Temperature
Hazelnut	Raw	
	Blanched*	100°C†
	Dry roasted	Quickly roasted to remove skin at 100°C for 4–5 min‡ Further roasted until 160°C†
	Fried§	Fried at 150–160°C for 1–4 min¶
Almond	Raw	
	Pasteurized (not entirely raw)	Superficial: till 70°C for 30 min, quick: high temperatures for short duration (e.g. 135°C for 2 s)**
	Blanched*	100°C†
	Dry roasted	Roasted until 160°C† or 120°C for 20–25 min‡
	Fried§	Soaked in water, blanched and dried in heated cabinet at 70°C gradually increasing to 115°C for 25 min†† or 150–160°C for 1–3 min¶
Cashew	Raw	First heated till 150°C for 20–35 min to remove the shell†
	Dry roasted (US)	Roasted until 160°C† or 120°C for 20 min‡
	Fried§ (the Netherlands)	Slowly fried: 93°C gradually increasing to 135°C in 35–40 min†† or 150–160°C for 1–3 min¶
Brazil	Raw	First heated till 150°C for 20–35 min to remove the shell†
Walnut	Raw	No prior heating to break open the shell¶
Pecan nut	Raw	
	Dry roasted	Roasted until 160°C† or quickly, because of delicacy of the nut: 120°C for 10 min‡
	Fried§	Fried at 80°C gradually increasing to 115°C in 15–18 min†† or 150–160°C for 1–3 min¶
Pistachio	Raw	
	Dry roasted	Roasted until 160°C† With shell: 140–150°C for 20–30 min‡ Peeled: 120°C for 15 min‡
	Fried§	Fried at 150–160°C for 1–3 min¶

*Blanched by means of steam or quickly roasted in an oven to remove the skin.
†Delinuts, Ede, the Netherlands, H. Budding, personal communication, 18 April and 11 May 2011.
‡Hazel Noten and Zuidvruchten, Amsterdam, the Netherlands, personal communication 25 October 2011.
§Duration of frying is dependent on the size of the oven used.
¶De NotenBeurs bv, Zevenhuizen, the Netherlands, personal communication 25 October 2011.
**http://www.naturalnews.com/021776.html.
††Blumenthal S. Food manufacturing: a compendium of food information, with practical factory-tested commercial formulae for the food manufacturer, chemist, technologist, in the canning, flavouring, beverage, confectionery, essence, condiment, dairy products, meat and fish and allied industries. Chemical Publishing Company, Inc. Brooklyn, New York, USA; 1942, p. 279–281.

Hansen et al. performed a DBPCFC with roasted hazelnut (140°C, 40 min) in 17 patients with a BP allergy and a DBPCFC-confirmed food allergy to raw hazelnut. Serum IgE from 94% (16/17) of patients in the study recognized rCor a 1, 41% (7/17) rCor a 2, and none recognized rCor a 8 on immunoblot. All 17 patients experienced oral symptoms, and three of them reported additional symptoms such as asthma, rhinitis and gastrointestinal discomfort after consumption of raw hazelnut. Five patients (29%) experienced oral symptoms with roasted hazelnut consumption; one of them also experienced rhinoconjunctivitis. Eliciting doses were elevated after roasting (median eliciting doses were at least doubled). More than 50% of patients lost reactivity to prick-to-prick and specific IgE (sIgE) with roasted hazelnut compared with raw hazelnut. In addition, histamine release test (HRT) reactivity was significantly reduced as well as enzyme allergosorbent test (EAST) inhibition. Together, these results indicate decreased allergenicity of hazelnut after roasting; however, clinical symptoms were not completely alleviated in all patients (21). Worm et al. performed a DBPCFC with roasted hazelnut (144°C, duration unknown) in 20 patients (with BP allergy) who were previously challenged with raw hazelnut. Seventeen patients (85%) developed oral symptoms during the challenge with roasted hazelnut. Eliciting doses were elevated compared with the eliciting dose of raw hazelnut in the majority of patients (median eliciting doses were doubled). Skin prick test (SPT) and basophil reactivity was decreased to roasted hazelnut (22). A thorough component resolved evaluation of the patients against other hazelnut allergens was not conducted. It is not described whether patients who experienced clinical symptoms upon consumption of roasted hazelnut may have had some reactivity to hazelnut allergens that remain stable during heat processing.

PR-10 proteins and profilins in hazelnut

Two *in vitro* studies (23, 24) found decreased allergenicity after roasting (140°C, 40 min) by EAST inhibition using sera from

Database	Search[a]
Pubmed	#1:Roast OR roasting OR heat OR heating OR cook OR cooking OR boil OR boiling OR frying OR fry OR bake OR baked OR microwave heating OR microwave OR roasted OR heated OR cooked OR boiled OR baking OR autoclaving OR autoclave OR blanch OR blanching OR blanched OR dry OR drying OR thermal processing
	#2: Nut OR nuts OR tree nut OR tree nuts OR hazelnut OR hazelnuts OR filbert OR filbert OR Corylus OR walnut OR walnuts OR Juglan OR Juglans OR pecan OR pecans OR hickory OR hickory nuts OR Carya OR Carya illinoensis OR almond OR almonds OR Prunus dulcis OR Prunus amygdalus OR cashew OR cashews OR Anacardium OR Anacardium occidentale OR pistachio OR pistachios OR Pistacia OR Pistacia vera OR macadamia OR macadamias OR Brazil nut OR Bertholletia excelsa
	#3: #1 AND #2 Field: Title/Abstract
Embase	As in Pubmed Field: ti,ab; AND [embase]/lim NOT [medline]/lim
[a] Unfound terms are not shown	

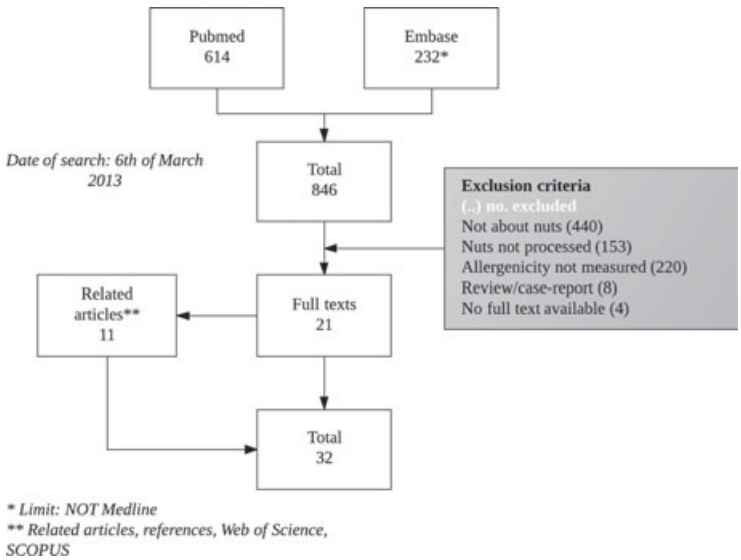

Figure 1 Overview of systemic search method.

patients with a hazelnut and BP allergy. Furthermore, recognition of Cor a 1 was completely lost after roasting (23, 25) at 140°C for 20–40 min. Wigotzki et al. (26) demonstrated that Cor a 1 was heat resistant to treatment at 100°C for up to 90 min, using immunoblots and an EAST inhibition assay with sera from 19 patients, whereas at 185°C, Cor a 1 (18 kD) and Cor a 2 (14 kD) detection was lost. Decreased allergenicity of hazelnut by EAST inhibition assay was reported after processing into hazelnut chocolates, nougat products, hazelnut cake, hazelnut cookies and hazelnut croquants. Furthermore, detection of Cor a 1 and Cor a 2 on immunoblot was reduced (27). One study reported decreased rat basophilic leukaemia cell (RBL) activity after roasting (25).

Together, data from clinical and *in vitro* studies indicate that roasting reduces the allergenicity of PR-10 proteins and profilins in hazelnut.

Nonspecific lipid transfer proteins and seed storage proteins in hazelnut

No DBPCFC studies in patients with hazelnut allergy recognizing nsLTPs or seed storage proteins in hazelnut were found. Seven *in vitro* studies were published. Patients with a hazelnut allergy without birch pollinosis showed similar reactivity to raw and roasted hazelnut (140°C, 40 min) by EAST inhibition (23, 24), while BP extract showed no significant inhibition, indicating involvement of heat-resistant nsLTPs or seed storage proteins. In a hazelnut allergy with recognition of PR-10 proteins combined with nsLTPs or seed storage proteins, roasting resulted in a decreased allergenicity but this decrease was less pronounced than in a hazelnut allergy with PR-10 protein recognition only (24). The decreased IgE binding by IgE blotting and EAST inhibition may be caused by a decreased solubility.

IgE binding to roasted and unroasted hazelnut was comparable in two different studies: two patients with severe hazelnut allergy [180°C, 15 min, by ELISA (enzyme-linked immunosorbent assay)] (28) and five patients sensitized to Cor a 9 (±170°C, 10 min, using ELISA and immunoblot). Intact Cor a 9 was detected in roasted hazelnut paste (29). Another study showed that Cor a 9 and Cor a 11 and an allergen <14 kD (Cor a 14) were stable after roasting at 185°C for 15 min (26). Muller et al. (25) showed heat stability (140°C, 20–40 min) of a 12–14-kD allergen (Cor a 14)

Table 3 (A and B) Summary of the results after often used processing methods. (A) Decreased allergenicity after processing, (B) conflicting data about effect of processing or limited effect shown after processing. The strength of evidence is based on the source data: from clinical studies (+++), measured IgE reactivity (++) in more than two patients, IgE reactivity measured in only one or two patients (+), IgG data are not depicted in this table

Decreased allergenicity after processing

Tree nut	Allergens involved	Processing method	Conditions	Magnitude of effect after processing compared with raw or native tree nut*	Strength of evidence
(A)					
Hazelnut	Cor a 1	Roasting	140°C–40 min (21) 144°C–duration unknown (22)	15–71% of patients reacted to roasted hazelnuts in DBPCFC, median eliciting dose were doubled (21, 22)	+++
			140°C–40 min (21, 23–25) 144°C–duration unknown (22) 170–185°C–15 min (26)	<50% of patients reacted to sIgE, SPT or prick-to-prick with roasted hazelnut (21, 22) 50% reduction histamine release after roasting (21) IC50 increased 10–100 times (21, 24, 26) AC50 increased 50 times (22) IC 50 not reached (23) B-hexosaminidase release 50% reduction after roasting in RBL cell assay (25)	++
	Cor a 1 Cor a 2	Processing into commercial hazelnut products	Unknown, commercially processed (27)	IC50 increased 5–20 times (27)	++
Almond	Pru du 1	Blanching Roasting	Unknown (35, 36) Unknown (35, 36)	Complete loss of recognition on Western blot of Pru du 1 after blanching and roasting (31, 32)	++

Conflicting data about effect of processing

Tree nut	Allergens involved	Processing method	Conditions	Strength of evidence
(B)				
Hazelnut	Cor a 1 Cor a 9 Cor a 11	Maillard	145°C–20 min (31) 70°C–48 h (32–34)	++

Limited effect shown after processing

Allergen	Allergens involved	Processing method	Conditions	Strength of evidence
Hazelnut	Cor a 8 Cor a 9 Cor a 11 Cor a 14	Roasting	140°C–20–40 min (23–25) 170–185°C–10–15 min (26, 28, 29)	++
	Cor a 9	Processing into commercial hazelnut products	Unknown, commercial hazelnut paste (29)	++
	Not shown Cor a 9	Storage	19 weeks (26) Six months (29)	++
Almond	Pru du 6	Blanching Roasting	Unknown (35–37) 180°C–15 min (28) Unknown (35–37)	++
	Pru du 1 Pru du 6	Processing into almond butter	Unknown (35)	++

Table 3 (continued)

Limited effect shown after processing				
Allergen	Allergens involved	Processing method	Conditions	Strength of evidence
Cashew nut	Not shown	Roasting	180°C–15 min (28)	+
Brazil nut	Not shown	Roasting	180°C–15 min (28)	+
Pecan nut	Car i 1	Blanching	100°C–10 min	++
	Car i 4	Roasting	148°C–30 min 172°C–12 min (49)	
Pistachio	Unknown	Roasting	37–150°C–8 h (52)	++

DBPCFC: Double-blind placebo-controlled food challenge, sIgE: specific IgE, SPT: skin prick test, IC50: concentration of inhibitor to reach 50% inhibition of native protein extract by EAST inhibition, AC50: concentration of inhibitor to reach 50% inhibition of native protein extract by basophil activation test, RBL cell assay: rat basophilic leukaemia cell assay.

and Cor a 11. Heat stability of Cor a 8 (140°C, 40 min) was shown by EAST inhibition (23). One study investigated the effect of autoclaving and showed decreased IgE binding on Western blot and protein bands on SDS-PAGE after autoclaving (138°C, 15–30 min), most likely due to decreased solubility (30).

Together, these *in vitro* studies indicate that roasting does not affect the allergenicity of nsLTPs or seed storage proteins in hazelnut.

Maillard reaction of hazelnut

The effect of Maillard reaction or caramelization, a chemical reaction between an amino acid and a reducing sugar, usually caused by heat, is not unequivocal.

A decreased immunoreactivity of Cor a 11 was shown after glycation (heated at 145°C in the presence of glucose) by SDS-PAGE, immuno-dot blot and IgG on ELISA. However, RBL activity was increased. Such a discrepancy might be caused by precipitation of glycosylated Cor a 11 in the RBL assay (31). This was confirmed by Cucu et al. who showed a decrease in intensity of the 49-kD band (Cor a 11) on a SDS-PAGE gel after glycation of hazelnut at 70°C, which caused precipitation. In addition, Cor a 9 was unaffected and appeared stable, while a Cor a 1 showed only some decrease (32, 33). Cucu et al. (34) recently showed in six patients with systemic reactions to hazelnut that glycation of hazelnut enhanced (2/6) or decreased (3/6) the allergenic property of hazelnut in the basophil activation test.

Effect of storage on the allergenicity of hazelnut

Storage of hazelnuts for 1–19 weeks at room temperature had no effect on the protein pattern of hazelnut as investigated by SDS-PAGE and immunoblot. The EAST inhibition assay showed very little difference in the C50 values over the 19-week storage period (26). Dooper et al. (29) found a decrease in detection of a Cor a 9 using ELISA and immunoblot after storage of more than 6 months, likely due to loss of solubility of the protein than a true decrease in allergenicity.

Almond

Clinical studies of patients with almond allergy have not been published. *In vitro* studies reported that heat reduces the allergenicity of a 15–17-kD protein, which may be the Bet v 1 homologue, Pru du 1 after blanching and roasting (35, 36). Immunoblot recognition was similar for almond butter and raw almond, suggesting that processing into almond butter (no extreme heat required) did not influence allergenicity (35).

de Leon et al. (28) found no difference in IgE binding to roasted and unroasted almond (180°C, 15 min), by ELISA in one patient with a almond allergy.

The effect of different heating methods on the allergenicity of 11S globulin, Pru du 6 (also known as amandin, or almond major protein, 37–66-kD protein bands on Western blot) (16), was investigated in six *in vitro* studies. Most bands were very stable towards blanching and roasting (28, 35–37), except for two bands between 55 and 65 kD (35–37). This thermostability of amandin was also illustrated in three studies with polyclonal IgG antibodies (36–38). No major changes in secondary structure were found with circular dichroism spectroscopy after heating amandin from 13 to 77°C (39). However, fluorescence spectroscopy revealed significant changes in secondary structure of amandin, after heating to 100°C for 10 min, whereas immunoreactivity was not effected on dot blot (40). Acosta et al. (41) showed conflicting data with a decrease (up to 87%) in immunoreactivity after blanching, moist heat >100°C, roasting and processing into almond paste with competitive ELISA, not confirmed by SDS-PAGE and Western blot. In agreement with the 15–17-kD almond allergen, Bargman et al. (35) showed similar IgE-binding patterns of almond butter compared with raw almond on electro- and immunoblot with sera obtained from eight almond allergic patients. Summarizing, most *in vitro* studies indicate that Pru du 1 but not Pru du 6 is affected by blanching and roasting.

Cashew nut

Only one study investigated the effect of roasting of cashew nut using patient sera (one allergic and one only sensitized).

No significant effect on IgE binding was found after roasting at 180°C for 15 min (28). Several *in vitro* studies investigated binding of monoclonal and polyclonal antibodies (IgG) to cashew major protein (CMP or Ana o 2), Ana o 1 and Ana o 3 after different heating methods. Roasting resulted in a slight decreased immunoreactivity of Ana o 1 and Ana o 3. Immunoreactivity of Ana o 2 was not affected by heating, although more extreme roasting conditions (160°C for 30 min or 200°C for 15 min) resulted in a decrease (38, 42). The effect of blanching was limited and primary due to leakage of proteins in the blanching water. Cashew frying (at 191°C for 1 min) showed no significant effect on Ana o 2 (38). Microwave heating and autoclaving resulted in conflicting data (38, 42). The available data showed a limited effect of roasting on the allergenicity of cashew nut with Ana o 2 seemingly more heat stable than Ana o 1 and Ana o 3.

Brazil nut

de Leon et al. (28) found no significant effect of roasting at 180°C for 15 min on the IgE binding of Brazil nut in two patients (one allergic, one only sensitized). Brazil nut consists of two major allergens, Ber a 1 (30%) and Ber e 2 (60%). IC50 determinations suggested that Ber e 1 is less immunogenic than Ber e 2 (43). It was shown that an irreversible denaturation of Ber e 1 starts at temperatures above 110°C (44–48). Denaturing conditions for Ber e 2 have not been published yet.

One *in vitro* study confirmed the limited effect of different heating methods on the immunoreactivity. The measured effect was not consistent with the different methods (ELISA, dot blot and Western blot). No effect or only a slight decrease (3–36%) was reported after blanching for 3 and 10 min, roasting, autoclaving for 30 min and frying. In contrast to this, the ELISA showed an increased immunoreactivity of 32% after microwave heating at 500 watt for 3 min (43).

Overall, *in vitro* data (human IgE and rabbit IgG) showed only limited effect of different heating methods on the allergenicity and immunoreactivity of Brazil nut.

Pecan nut

A limited effect of heating on pecan nut was detected by Western blot using pooled patient sera. Most protein bands of Car i 1 and Car i 4 seemed very stable or showed some decrease after blanching for 10 min, roasting at 148°C, 30 min or 172°C, 12 min and autoclaving for 5 min. Some subunits of Car i 4 almost disappeared after blanching, roasting and autoclaving, likely due to irreversible loss of protein solubility rather than protein epitope destruction. Polyclonal antibodies showed also stability towards blanching and roasting, with a significant decrease after roasting at 160°C, 20 and 30 min and autoclaving (49) or microwave heating for 15 min (50). These processing conditions resulted in a dark unappealing external appearance, so it is unlikely that these extreme conditions are used in commercial pecan processing and thus would not be representative of the type of pecan that allergic consumers may be exposed to (49). The decrease in immunogenicity due to extreme conditions could be due to the loss of protein solubility.

Walnut

The effect of heating on the allergenicity of walnut was studied by circular dichroism spectra and polyclonal IgG antibodies. Sordet et al. (51) showed that the protein structure of nJug r 1 exhibited good resistance to heating at 90°C. One *in vitro* study showed that blanching for 5–10 min did not show a significant effect on immunoreactivity of walnut glutenin (WG), the major storage glutenin fraction in walnut (Jug r 4 and Jug r 2). Roasting at different conditions, frying (191°C) and microwave heating also showed no significant effect on immunoreactivity of walnut. Autoclaving did not effect immunoreactivity tested in ELISA; however, Western blot showed a decreased recognition of 42–45-kD proteins (Jug r 2) and 45–66-kD bands (Jug r 4), not shown after blanching and roasting (38). Concluding, the two studies found showed a limited effect of heating on the immunoreactivity of walnut allergens; however, human studies should be performed to show the clinical relevance of these findings.

Pistachio nut

One study showed the effect of processing in pistachio nut allergy. A limited effect of roasting (dry) was shown on IgE binding in two human serum pools with SDS-PAGE, Western blot and ELISA inhibitions; however, steam roasting strongly reduced the IgE binding in these assays. Steam-roast processing resulted in protein aggregation which contributed to the decrease in IgE binding but it is unknown whether this form of processing decreases the allergenicity of pistachio nut (52).

Discussion

The aim of this study was to review the influence of different heating methods on the allergenicity and immunoreactivity of tree nuts to improve the diagnosis and treatment (diet advices) of tree nut allergic patients. A key factor in evaluating the effect of processing on the allergenicity of tree nuts is the consideration of the solubility of the tree nut allergens after processing. *In vitro* analysis using sera IgE in conjunction with immunoblotting or inhibition ELISAs should be followed up with clinical oral challenge trials to confirm a decrease or removal of the allergenicity before it is determined that processing results in hypo-allergenic tree nuts. The two available clinical studies have shown a decreased allergenicity of hazelnut after roasting in patients with a BP allergy and reactivity to raw hazelnut (21, 22). This was confirmed by *in vitro* studies, illustrating a decrease in Cor a 1 reactivity (23–27). A similar phenomenon was reported for almond Pru du 1 (35, 36). In contrast, nsLTPs and seed storage proteins in hazelnut and almond appeared very stable (23–26, 28, 29, 35–40). Studies examining the effect of thermal processing on the allergenicity of cashew nut, Brazil nut,

pecan nut, walnut and pistachio were scarce and limited in their scope. They all show stability of these foods to different heating methods (28, 38, 40, 42–49, 51).

PR-10 proteins are the most important BP-related allergens and share homology in their tertiary structures (conformational epitopes). PR-10 proteins are generally heat labile, as described for hazelnut (21), celery (53, 54), apple (55), carrot (56) and peanut (57). Heating might lead to unfolding and disruption of conformational epitopes (19). Bohle et al. showed unfolding of PR-10 proteins in BP (Bet v 1), celery (Api g 1), carrot (Dau c 1) and apple (Mal d 1) upon cooking between 50 and 80°C. The structure of Mal d 1 and Dau c 1 remained unfolded upon cooling, whereas the unfolding of Bet v 1 and Api g 1 seemed partly reversible (56). Such a mechanism might explain the retained reactivity to roasted hazelnut in 29–85% of the hazelnut allergic patients (21, 22), although for Cor a 1 (hazelnut) folding experiments have not been published. Recognition of nsLTPs or seed storage proteins in addition to Cor a 1 and Cor a 2 might also lead to a remained reactivity in some patients. Another explanation might be that the hazelnut core was not heated sufficiently as reported for baking of crumps, in which the temperature did not exceed 100°C during baking at 180–230°C, due to the water content inside (48). However, the presence of water is required for denaturation. Dry heat treatment like roasting makes proteins more thermostable than moist heat treatments like blanching, cooking or steam roasting (52, 58). Understanding how heat influences allergenicity and whether this is reversible could lead to strategies to reduce allergenicity in food production.

A 15–17-kD protein in almond (Pru du 1) was also found to be heat labile. The clinical relevance of this finding has not been confirmed yet. Further insight might broaden the product choice of almond allergic patients or reveal processing methods that might eliminate or reduce the allergenicity, like the eliminated allergenicity of Mal d 1 in apple after microwave heating.

The limited effect of heating on the allergenicity of hazelnut, almond (nsLTPs or seed storage proteins), cashew nut, Brazil nut, pecan nut, walnut and pistachio nut might be due to heat-stabile allergens like the seed storage proteins. Heat stability has been illustrated for the 2S albumins: Ber e 1 in Brazil nut until 110°C (44–48) and Jug r 1 in walnut until 90°C (51) and the 7S and 11S globulins in soy: 7S globulins until 70–75°C and 11S globulins until 94°C (59). Heat stability might also be expected for cross-reactive sensitizations to 11S globulins, for example (60). However, some subunits of 11S globulins seem heat labile after denaturing on SDS-PAGE, which has been shown for almond, Brazil nut, pecan nut and walnut. This is likely due to irreversible loss of protein solubility, which does not necessarily indicate a decrease in allergenicity. If allergic consumers eat a tree nut, they would be exposed to both the soluble and insoluble forms of the proteins. Gastric digestion may aid in the resolubilization of some of the allergens; however, little information is currently known about the effect of ingestion and digestion on the resolubilization of allergens in the human body.

The impact of factors like matrix (61) and stability to digestion (62) might further influence the allergenicity of tree nuts and are not discussed in this review. Allergens that are stable to digestion reach the intestinal mucosa intact (nsLTPs in cherry), where absorption and sensitization can occur in contrast to the labile allergens (PR-10 proteins and profilins in cherry) (63, 64). The warranted clinical studies with processed nuts could also provide insight to the contribution of these factors to the allergenicity in tree nut allergy.

In contrast to decreased allergenicity, an increased immunogenicity has been described after microwave heating of nuts (42, 42, 43). For peanut, an enhanced allergenic property has been described for Ara h 1 and Ara h 2 after roasting or browning (Maillard reaction) (20, 65). The effect of Maillard reaction on the allergenicity of hazelnut is not clear yet, because the data found were not consistent. This might be due to precipitation of the proteins. Further investigation to determine the effect of the Maillard reaction is requisite.

Storage of hazelnut up to 19 weeks had little effect on allergenicity (26), storage for more than 6 months resulted in decreased detection of Cor a 9, likely due to a decreased solubility (29). Data on the storage of other tree nuts are lacking.

The decreased allergenicity after processing of tree nuts has important implications for clinical practice. Clinicians should perform a thorough history including reactivity to raw (directly from tree) or processed (blanched or roasted) tree nuts. Reacting to raw or unprocessed tree nuts, without symptoms to heated tree nuts, might result in a modified dietary advice. Reactivity to processed and unprocessed hazelnuts in combination with more severe symptoms makes reactivity to nsLTPs or seed storage proteins more likely, a risk of more severe or anaphylactic reactions (9). These findings further influence the advices concerning consumption of nut products. Raw tree nuts are a risk for all tree nut allergic patients, although processed nuts might be tolerated by patients recognizing only the PR-10 proteins. Unfortunately, it is not possible to discriminate between both groups with current diagnostic *in vivo* or *in vitro* tests. The hazelnut oral challenge with roasted hazelnuts should be the most reliable test; however, the processing conditions of nuts in daily life are not standardized, and therefore, the clinical reaction will be difficult to predict. The component-resolved diagnosis will give more insight into the specific sensitization pattern of the patient and might lead to an individual advice concerning ingestion of processed nuts. If it is possible in the future to totally eliminate the allergenicity of Cor a 1 in hazelnut or Pru du 1 in almond after heating or processing, patients recognizing solely this PR-10 protein could take advantage of this.

Furthermore, the heat lability, storage effect and influence of processing of tree nut allergens warrant us to use fresh, raw nuts for diagnostic food challenges for patients with a PR-10 protein-related tree nut allergy. False-negative outcomes may increase the risk of unexpected allergic reactions during introduction of the nut into the diet.

In conclusion, this study shows that heating and processing may reduce allergenicity of PR-10 proteins in hazelnut and

almond. In the work-up of tree nut allergy, reactivity to raw vs processed nuts should be discussed for diagnosis and dietary advice. In PR-10 protein-related tree nut allergies, raw nuts should be used as source materials for IgE tests and hazelnut challenges. In the future, information on the influence of processing on the allergenicity might lead to the development of hypo-allergenic tree nuts or tree nut products.

Author contributions

L. J. Masthoff and R. Hoff made substantial contributions to conception and design, acquisition, analysis and interpretation of data, drafting article and final approval of version to be published; K. C. M. Verhoeckx and J. L. Baumert made substantial contributions to interpretation of data, revising the article critically for important intellectual content and final approval of version to be published; H. van Os-Medendorp and S. G. Pasmans made substantial contributions to design, revising the article critically for important intellectual content and final approval of version to be published; A. Michelsen-Huisman made substantial contributions to conception, revising the article critically for important intellectual content and final approval of version to be published; and Y. Meijer and A. C. Knulst made substantial contributions to conception and design and interpretation of data, revising the article critically for important intellectual content and final approval of version to be published.

Conflict of interest

None of the authors has any potential conflict of interest to declare.

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