



Impact of thermal processing and the Maillard reaction on the basophil activation of hazelnut allergic patients

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ABSTRACT

Food allergy, an abnormal immunological response due to sensitization to a food component, has become an important health problem, especially in industrialized countries. The aim of this study was to investigate the impact of thermal processing and glycation on the basophil activation by hazelnut proteins using a basophil activation test. Patients with systemic allergic reactions (SR; $n = 6$) to hazelnut as well as patients with an isolated oral allergy syndrome (OAS; $n = 4$) were investigated. Thermal processing of hazelnut proteins either in the presence or absence of wheat proteins did not result in major changes in the stimulatory activity of the basophils for patients with SR or OAS. For the patients with OAS, incubation of hazelnut proteins with glucose led to complete depletion of the stimulatory activity of the basophils. An increase in stimulatory activity of the basophils for two out of six patients with SR was observed. For the other four patients slight or complete abolition of the stimulatory activity was observed. These results indicate that some patients with SR to hazelnut are at risk when exposed to hazelnut proteins, even in processed foods.

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1. Introduction

Food allergy, an abnormal immunological response due to sensitization to a food component, has become an important health problem, especially in industrialized countries. It is estimated that IgE mediated food allergy affects about 2% of the adult population and up to 8% of the children (Monaci et al., 2006). However, the prevalence of sensitization is much higher (patients with specific IgE to certain foods without manifesting clinical allergy) and in Belgium it represents about 6% for hazelnut (Burney et al., 2010). The symptoms manifested upon inadvertent ingestion of hazelnuts can vary from a mild oral allergy syndrome (OAS) (Hirschwehr et al., 1993; Luttkopf et al., 2002) to more severe anaphylactic reactions (Hansen et al., 2009; Schocker et al., 2004). Hazelnuts (*Corylus avellana*) are widely used nowadays in the food industry, especially in confectionary products. The European Commission Directive 2007/68/EC proposed hazelnut containing foods to be labeled regardless of the amount present (European Parliament and Council, 2007). However, foods may be unintentionally contaminated via allergen-containing ingredients or carry-over during processing, posing a serious threat to allergic consumers.

In a previous study, we have shown that modifications of hazelnut proteins through reactions typically occurring during food

processing, lead to significant changes in the detectability of hazelnut proteins by the commercial kits leading to especially false negative results (Cucu et al., 2011). This indicates that food products declared free of hazelnut allergens by the commercial kits could still affect the allergic consumers. However, it is not known yet whether changes in the triggering effect of the effector cells involved in the IgE-mediated allergies should be expected as well. Abundant evidence exists on how thermal processing and Maillard reaction can greatly influence the IgE binding activity of some food components (Gruber et al., 2004, 2005; Nakamura et al., 2005, 2008). Decreases but also increases of the IgE binding activity were observed, without clear trends being established. It was previously shown that roasting of hazelnut can lead to a reduction of the allergenicity nevertheless exceptions in some patients were observed (Muller et al., 2000; Pastorello et al., 2002; Schocker et al., 2000; Wigotzki et al., 2001; Worm et al., 2009). Remarkably, most of the patients considered for the study of the impact of roasting on the allergenicity were patients with OAS. It is reported that patients with OAS are mostly allergic to the labile pollen related hazelnut allergens such as *Cor a 1* and/or *Cor a 2* (Worm et al., 2009). It is therefore not surprising that thermal processing as well as glycation lead to severe or complete reduction of the allergenicity of roasted hazelnuts. For the patients with systemic reactions, known to react to components resistant to heat treatment and digestion (*Cor a 8*, *Cor a 9* and *Cor a 11*) lesser effect were observed (Dooper et al., 2008; Muller et al., 2000; Pastorello et al., 2002).

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Generally, the allergenicity is assessed using IgE-immunoblotting and IgE-inhibition tests, allergenicity being evaluated based on the affinity between the IgEs and the allergens (Chen et al., 2006; Gruber et al., 2004, 2005; Schocker et al., 2000). However, these tests do not reveal information about the functional properties of the allergenic components and IgE detection is not always indicating that symptoms would be manifested. On the contrary, mast cells and basophils play an important role in IgE mediated allergies and the capacity of food allergens to trigger basophils and mast cells can be used in *in vitro* assays to diagnose allergies (Erdmann et al., 2003). In the early 1990s, the discovery of the human basophil activation marker CD63 (Knol et al., 1991) led to the development of a flow cytometric method to analyze allergen-specific *in vitro* basophil activation named basophil activation test (BAT) (Ebo et al., 2004, 2006). Basophils, upon encountering specific allergen recognized by surface-bound IgE, secrete and generate quantifiable bioactive mediators and up-regulate the expression of different markers (e.g. CD63, CD203c). These markers can be detected by multicolor flow cytometry using specific monoclonal antibodies because high amounts of CD63 glycoprotein are generated in the activated basophils after fusion of the granules with the cytoplasmic membrane (Erdmann et al., 2003).

During the last years, the BAT has proven to be an easily accessible, rapid and reliable diagnostic method, allowing simultaneous testing of different allergens (including foods) using minimal amounts of blood (Ebo et al., 2008). Moreover, BAT proved to be a valuable tool for the diagnosis of hazelnut allergy (Erdmann et al., 2003) and for the functional evaluation of different hazelnut protein extracts (Platteau et al., 2010). In the study we present here, we used the BAT for the assessment of the impact of modification of hazelnut proteins through thermal processing and the Maillard reaction on the basophil activation of hazelnut allergic patients.

2. Materials and methods

2.1. Reagents and standards

Hazelnuts from nine different brands were purchased in Belgian local supermarkets; eight brands were virgin and one was roasted. Soluble wheat proteins “Meripro 711” were kindly provided by Syral Belgium N.V. (Aalst, Belgium). Chemicals and standards of analytical grade were purchased from Sigma–Aldrich (Bornem, Belgium), VWR (Leuven, Belgium) and Acros Organics (Geel, Belgium). Analytical grade solvents and technical grade hexane were obtained from Chem-Lab (Zedelgem, Belgium). Endotoxin-free heparinised tubes were purchased from Vacuette, Greiner Labortechnik GmbH (Kremsmünster, Austria). Mouse monoclonal anti-human IgE (as stimulator) and monoclonal phycoerythrin (PE)-conjugated anti-human CD63 were purchased from BD, Biosciences (Erembodegem, Belgium). Monoclonal anti IgE was purchased from Sigma–Aldrich, Chemtec GmbH (Steinheim, Germany) and labeled in-house with Alexa Fluor 488 (Invitrogen) as described by the manufacturer.

2.2. Selection of patients

Well-defined patients with a compelling history of true hazelnut allergy ($n = 6$) and with an oral allergy *Betulla verucosa* syndrome (OAS) ($n = 4$) during consumption of hazelnuts, were enrolled. In all patients, clinical suspicion was documented with a positive Cor a 1.04-spiked specific IgE for hazelnut (f17, ImmunoCAP FEIA, Uppsala, Sweden). A healthy non-atopic control individual tolerant for hazelnut and with a negative specific IgE for Cor a 1.04-spiked hazelnut (<0.10 kU/L) was also included. All patients and control individuals had responsive basophils (vide infra).

2.3. Total IgE and specific IgE

Quantification of total IgE and sIgE to Cor a 1.04-spiked hazelnut, Cor a 1, Cor a 8 and Cor a 9 and rBet v 1 from birch (*B. verucosa*) pollen was performed with the FEIA ImmunoCAP assay (Phadia, Uppsala, Sweden) with a detection limit of 0.10 kU/L and according to the manufacturers' recommendations.

2.4. Hazelnut protein extraction

A mixture of the hazelnuts (eight unpeeled and one roasted peeled hazelnut) was made by taking equal amounts of each kind and the hazelnut protein extracts were obtained following the protocol described earlier (Cucu et al., 2011). Briefly,

100 g of ground nuts were defatted four times using 1 L technical hexane. Further, 10 g of the defatted powder was mixed with 100 mL of extraction buffer and mixed at 4 °C over night. Protein extraction buffer consisted of 1 M NaCl, 1.47 mM KH_2PO_4 , 2.7 mM KCl and 10 mM Na_2HPO_4 . Sodium azide 0.2 g L^{-1} was added to the extraction buffer to avoid microbial growth. Further, a mixture of protease inhibitors (2 mM ethylenediaminetetraacetic acid (EDTA), 5 mM sodium diethyl-dithiocarbamate (DIECA), 0.5 mM benzamidine hydrochloride (BAHC) and 0.2 mM phenylmethylsulfonyl fluoride (PMSF)) was added to the extraction buffer to increase the stability of the protein isolate during extraction and storage (Bjorksten et al., 1980). The protein mixture was further centrifuged for 1 h at 4 °C and 20,000g to separate the lees. The clear supernatant was dialyzed (Spectra/Por 3 membrane tubing, MWCO 3.5 kDa) using the extraction buffer with the protease inhibitors containing only 0.137 M NaCl, for 48 h at 4 °C and then filtered through a 0.45 μm membrane filter (Puradisc FP 30, Novolab, Belgium). Finally, the protein solution was lyophilized and stored at -20 °C until further use.

2.5. Maillard reaction in the presence or absence of soluble wheat proteins

The model systems were prepared in 0.1 M phosphate buffer pH 7.4 by mixing 1% (w/v) hazelnut proteins with 6% (w/v) glucose. Two types of model systems containing wheat proteins were also considered. One model system was obtained by mixing 0.25% (w/v) of hazelnut proteins with 0.75% (w/v) of wheat proteins and 6% (w/v) of glucose. Another model system was obtained by mixing 0.5% (w/v) hazelnut proteins with 0.5% (w/v) wheat proteins and 6% (w/v) glucose. Respective model systems without glucose were prepared as control. The model systems in closed falcon tubes were incubated in a water bath at 70 °C for up to 48 h (Cucu et al., 2011). The modified hazelnut protein solutions were used immediately for the characterisation of the modification degree and the rest was lyophilized and stored at -20 °C until further use.

2.6. Basophil activation test (BAT)

BAT was carried out as described previously (Ebo et al., 2006; Platteau et al., 2010). Within 3 h after sampling, aliquots of 100 μL endotoxin-free heparinised whole blood were pre-incubated for 15 min at 37 °C with stimulation buffer (Hanks balanced salt solution containing 1 mol/L HEPES and 7.5% NaHCO_3). Pre-warmed blood samples were stimulated for 20 min with the different hazelnut extracts (0.01 and 1 $\mu\text{g/mL}$), with 100 μL mouse monoclonal anti-human IgE (10 $\mu\text{g/mL}$) as a positive control or with 100 μL buffer as a negative control (to measure spontaneous CD63 expression) at 37 °C in a water bath. The optimal stimulation time and temperature were determined in preliminary experiments. The reaction was stopped on ice. To estimate the number of activated basophils, cells were stained in the dark for 20 min on ice with 20 μL monoclonal Alexa Fluor 488-coupled anti IgE and 10 μL monoclonal phycoerythrin (PE)-conjugated anti-human CD63. Red blood cells were lysed and white blood cells were fixed by adding 2 mL FACS Lysing solution for 10 min at room temperature. After centrifugation (5 min, 250g, 4 °C), 200 μL of washing solution was added to the cell pellets. Flow cytometric analysis of basophil activation was performed on a FACSCalibur flow cytometer (BD Immunocytometry Systems). Green fluorescence (FL1 channel) and side scatter were employed to gate on at least 500 basophils that expressed high density of surface IgE. Subsequently, within this gate the percentage of activated basophils, i.e. co-expressing CD63 (FL2 channel) and IgE, was measured. For this purpose, the marker (M1) was set on fluorescence minus one (Herzenberg et al., 2006). Percentages of activated basophils were corrected by subtracting spontaneous CD63-expression (negative control stimulation) from the value obtained with allergen stimulation. The coefficient of variation of the method was at all times below 10%.

3. Results

A total of six patients with systemic allergic reactions (Table 1) and four patients with OAS (Table 2) were enrolled. Hazelnut protein extracts non-treated or incubated with or without glucose at 70 °C were tested using BAT. By inducing Maillard reaction in a buffered system it was possible to make abstraction of possible consequences of protein insolubility and eventual extractability as a result of the induced modifications.

Severe modifications of hazelnut proteins due to incubation with glucose either in the presence or absence of wheat proteins were observed as evidenced by the electrophoretic pattern (Fig. 1). Nevertheless, the acid and basic subunits (≈ 35 and 25 kDa, respectively) of the Cor a 9 allergen were the most stable upon incubation with or without glucose. On the contrary, the Cor a 11 allergen with a molecular mass of about 48 kDa, was fairly unstable already upon incubation in the absence of glucose as evidenced by the loss of the band intensity. Moreover, aggregate for-

Table 1

Characterization of the patients with systemic allergic reactions. Total and specific IgE (kU/L).

Patient no.	Sex/year birth	Total IgE	IgE hazelnut	IgE Bet v 1	IgE Cor a 1.04	IgE Cor a 8	IgE Cor a 9
1	F/1981	324	14.2	5.3	3.2	1.4	n/d
2	M/1988	421	20.7	31.5	18.9	7.2	n/d
3	M/2002	410	4.3	0.8	n/d	n/d	n/d
4	M/1996	272	3.8	n/d	n/d	n/d	n/d
5	M/2008	287	4.6	n/d	n/d	n/d	0.3
6	F/1984	454	16.7	55.7	n/d	n/d	n/d

Table 2

Characterization of the patients with an OAS. Total and specific IgE (kU/L).

Patient no.	Sex/year birth	Total IgE	IgE hazelnut	IgE Bet v 1	IgE Cor a 1.04	IgE Cor a 8	IgE Cor a 9
7	M/1967	26.0	2.03	3.1	2.5	n/d	n/d
8	F/1969	47.0	7.6	12.3	9.0	n/d	n/d
9	F/1955	88.0	12.7	19.6	16.7	n/d	n/d
10	F/1970	62.0	1.4	1.8	1.6	n/d	n/d

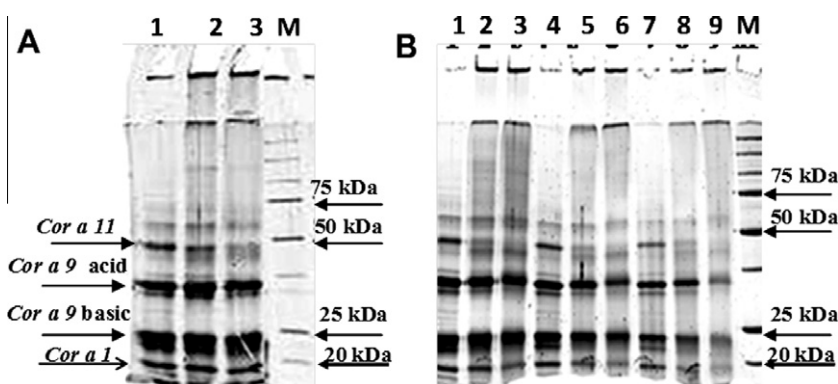


Fig. 1. Changes in SDS–PAGE pattern of the hazelnut proteins upon progressive Maillard reaction. (A) SDS–PAGE pattern of hazelnut proteins incubated at 70 °C in the absence of glucose; lane 1 – non-treated, lane 2–3 incubated for 24 and 48 h, respectively, M – molecular weight marker. (B) SDS–PAGE pattern of hazelnut proteins incubated with glucose at 70 °C; lane 1–3 – 1% hazelnut protein native, 24 and 48 h incubated; lane 4–6 – 0.5% hazelnut and 0.5% wheat proteins native, 24 and 48 h incubated; lane 7–9 – 0.25% hazelnut and 0.75% wheat proteins native, 24 and 48 h incubated.

mation was observed, which was manifested by their presence at the boundary of the resolving and stacking gel. Interestingly, the protein band with the mass of approximately 18 kDa, representing most probably the *Cor a 1* allergen, was fairly stable upon incubation in the absence of glucose, while upon incubation with glucose a time dependent decrease in the intensity was observed.

No basophil activation by hazelnut proteins was observed in the control individual (data not shown). The basophils of patients with systemic allergic reactions to hazelnut could be clearly stimulated with hazelnut extracts although with high heterogeneity between the patients (Fig. 2). Indeed, the basophils of patient number 1 seemed to be the least reactive, whereas basophils of patients 2 were the most reactive with native hazelnut proteins. For the later one, 48 h incubation of the hazelnut protein extract at 70 °C did not have a great impact on the basophil activating capacity. Increases in the percentage of CD63 positive cells for patients 1, 3, 4 and 6 were observed. Only for the patient 5 the activating capacity decreased slightly. A 48 h incubation of hazelnut proteins with glucose led to a decrease of the basophil activation activity for patients 1, 2 and 6. For patients 3 and 4, on the other hand, an increase in the basophil activation was observed while for patient number 5 still no changes were observed compared to the non-treated hazelnut proteins.

The basophils of patients with OAS could be stimulated as well with some heterogeneity (Fig. 3). The basophils of patient 7 could be activated the least compared to the other patients. Heating in the absence of glucose for 48 h did not lead to significant changes

in the stimulation of the basophils for patients 8, 9 and 10 while for patient 7 a slight increase could be observed. However, 48 h incubation with glucose led to an almost complete abolishment of the basophil activation capacity for all the investigated patients.

The presence of other proteins during thermal treatment and glycation was also evaluated. In this study wheat proteins were used as a model. The addition of wheat soluble proteins did not affect the stimulatory effect of the basophils of patients with systemic allergic reactions 1, 2, 5 and 6 (Fig. 4). However, an increased stimulatory effect was observed for patients 3 and 4 where addition of soluble wheat proteins led to almost a doubling compared to hazelnut proteins alone. This effect was not due to cross-reactivity to the wheat proteins because the stimulatory effect to wheat proteins was below 5% for all six patients analyzed. Thermal treatment of hazelnut proteins at 70 °C in the presence of wheat proteins did not lead to considerable changes in the stimulatory activity of the basophils from patients 2, 3, 5 and 6 while for patients 1 and 4 a slight decrease was observed. Upon 48 h incubation with glucose the stimulatory activity of the basophils decreased for patient 1, 4 and 6, increased for patient 3 and remained fairly stable for patient 2 and 5. For patient 2, incubation with glucose for 24 h showed a decreased stimulatory activity of the basophils. The observed decrease was however due to an inhibition effect on the basophils by the supra-optimal stimulation with allergen. This was confirmed by the results obtained upon stimulation with 0.01 µg protein, where the activity was around 70%, similar as for the non-treated proteins (data not shown).

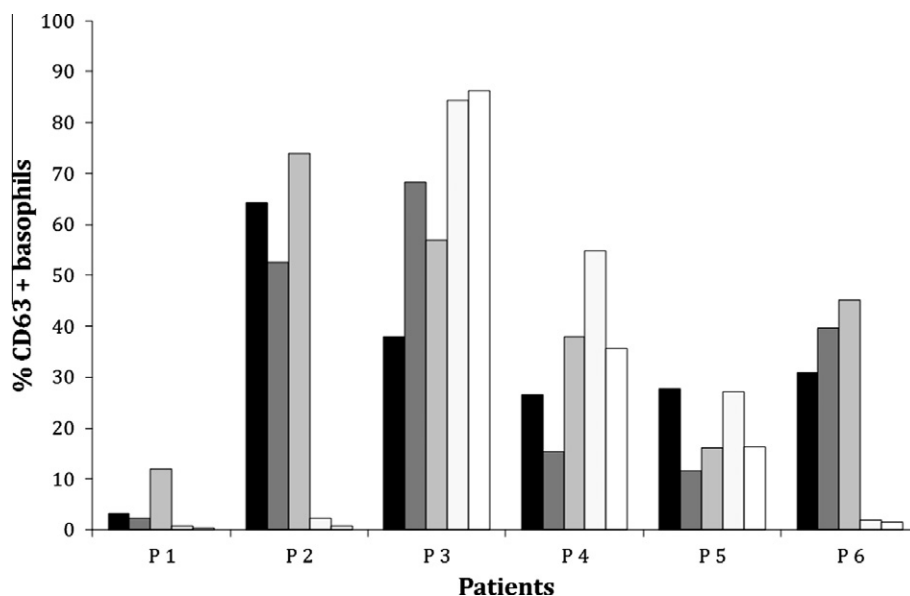


Fig. 2. Basophil activation (expressed as percentages of CD63 positive cells) upon stimulation with 1 μ g hazelnut protein/mL in patients with systemic allergic reactions: ■ – native hazelnut proteins, ■ – hazelnut proteins incubated at 70 °C for 24 h, ■ – hazelnut proteins incubated at 70 °C for 48 h, □ – hazelnut proteins incubated with glucose at 70 °C for 24 h, □ – hazelnut proteins incubated with glucose at 70 °C for 48 h.

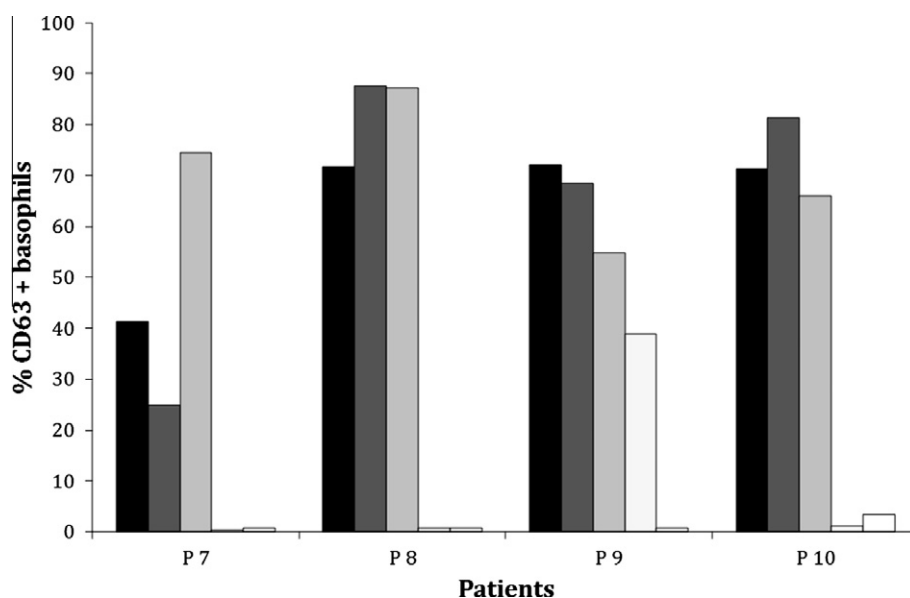


Fig. 3. Basophil activation (expressed as percentages of CD63 positive cells) upon stimulation with 1 μ g hazelnut protein/mL in patients with OAS: ■ – native hazelnut proteins, ■ – hazelnut proteins incubated at 70 °C for 24 h, ■ – hazelnut proteins incubated at 70 °C for 48 h, □ – hazelnut proteins incubated with glucose at 70 °C for 24 h, □ – hazelnut proteins incubated with glucose at 70 °C for 48 h.

For patients with OAS to hazelnut, the stimulatory effect of the hazelnut protein in the presence of soluble wheat proteins was similar as for the hazelnut proteins alone (Fig. 5). For patient 8 only, the stimulatory effect was decreased compared to the hazelnut proteins alone, which was observed upon stimulation with either 1 or 0.01 μ g protein (data not shown). Incubation with glucose completely depleted the basophil activation similar with the model systems containing no wheat proteins.

4. Discussions

Symptoms of hazelnut allergy can vary between an OAS and potentially dangerous generalized anaphylactic reactions. It is therefore mandatory to predict the impact of thermal processing

and glycation on the basophil activation for both groups of patients. Although Belgium is a birch endemic region, implying that many of the hazelnut sensitizations observed are mainly due to cross-reactivity with birch pollen, a study has shown that systemic allergic reactions are frequent as well, particularly in young children (De Knop et al., 2011).

In this study, the BAT was used for the assessment of the activatory potential of hazelnut extracts after modification of constituent proteins/allergens through thermal processing and the Maillard reaction.

From the SDS–PAGE patterns of the native and treated proteins it was seen that severe modifications of hazelnut proteins were taking place. Especially the *Cor a 11* was affected by heat treatment even in the absence of glucose while *Cor a 1* seemed to be affected

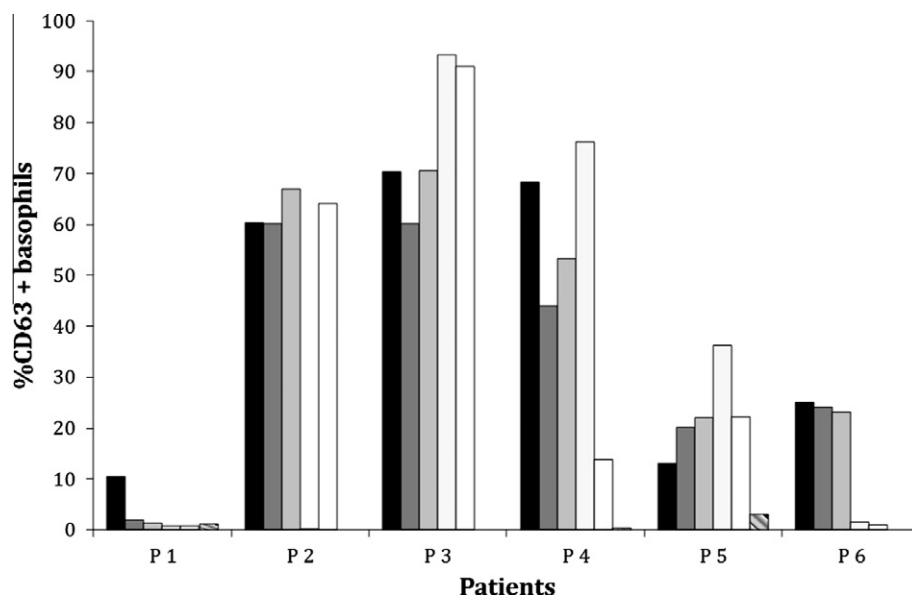


Fig. 4. Basophil activation (expressed as percentages of CD63 positive cells) upon stimulation with 1 μ g hazelnut protein/mL in patients with systemic allergic reactions: ■ – 0.25% hazelnut proteins and 0.75% wheat proteins nontreated, ■ – 0.25% hazelnut proteins and 0.75% wheat proteins incubated at 70 °C for 24 h, ■ – 0.25% hazelnut proteins and 0.75% wheat proteins incubated at 70 °C for 48 h, □ – 0.25% hazelnut proteins and 0.75% wheat proteins incubated with glucose at 70 °C for 24 h, □ – 0.25% hazelnut proteins and 0.75% wheat proteins incubated with glucose at 70 °C for 48 h, ▨ – wheat.

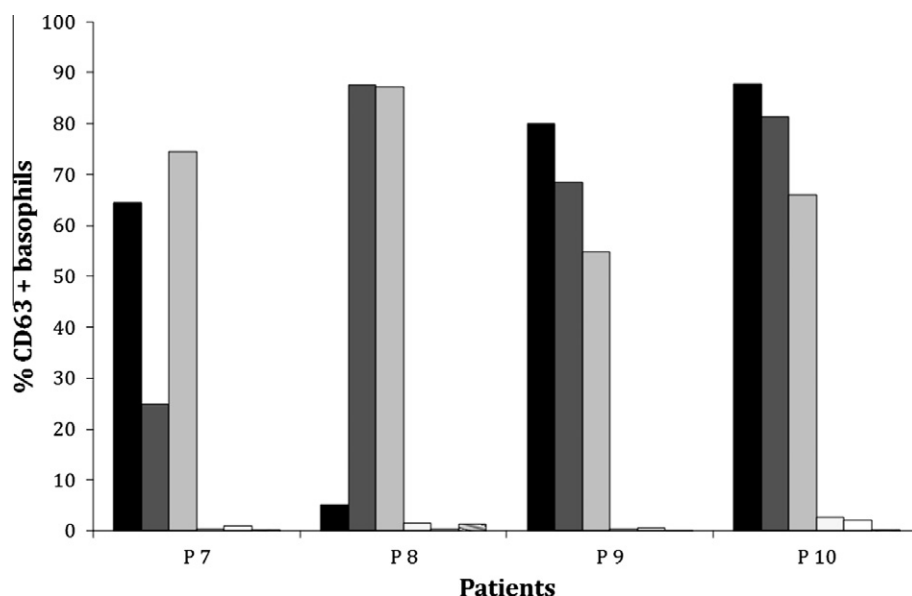


Fig. 5. Basophil activation (expressed as percentages of CD63 positive cells) upon stimulation with 1 μ g hazelnut protein/mL in patients with OAS: ■ – 0.25% hazelnut proteins and 0.75% wheat proteins nontreated, ■ – 0.25% hazelnut proteins and 0.75% wheat proteins incubated at 70 °C for 24 h, ■ – 0.25% hazelnut proteins and 0.75% wheat proteins incubated at 70 °C for 48 h, □ – 0.25% hazelnut proteins and 0.75% wheat proteins incubated with glucose at 70 °C for 24 h, □ – 0.25% hazelnut proteins and 0.75% wheat proteins incubated with glucose at 70 °C for 48 h, ▨ – wheat.

only upon incubation with glucose. Only *Cor a 9* allergen seemed to be the most resistant to glycation. These changed indicated that an impact on the basophil activation could be expected as well.

4.1. Patients with systemic allergic reactions

In the case of the patients with systemic reactions to hazelnut thermal treatment alone did not greatly decrease the stimulatory activity of the basophils. In the case of thermal treatment in the absence of wheat proteins for 1 out of 6 patients and in the presence of wheat proteins for 2 out of the 6 patients investigated a decrease was observed. The decrease observed upon thermal treatment in

the presence of wheat proteins was probably due to protein–protein interactions taking place between hazelnut and wheat proteins which masked the IgE binding epitopes in hazelnut proteins. This indicated that the presence of matrix proteins might have an impact on allergenicity as well. Hazelnut proteins are rarely present alone in foods, with other food proteins representing the bulk, or if present as contamination, hazelnut proteins are even a minor component. As a consequence, during processing the traces of hazelnut proteins will primarily cross-link with the matrix proteins instead of traces of other hazelnut proteins present. This might imply that the hazelnut proteins surface may become covered with matrix proteins which is leading to the observed effect

on basophil activation. Interestingly, for 4 out of 6 patients investigated, thermal treatment of hazelnut proteins alone led to an increase in the basophil activation. This might indicate that the thermally induced changes on the three dimensional structure of the proteins might have led to the exposure of some buried epitopes, therefore facilitating the upregulation of the CD63 marker.

Upon incubation in the presence of glucose, a heterogeneous impact on the basophil activation was observed as well. For 2 out of 6 patients a decrease in the basophil activation was observed regardless of the presence or absence of wheat proteins upon incubation with glucose. This might be due to the fact that these patients were also reactive to the *Bet v 1* homologue *Cor a 1* (Table 1). Given that the *Bet v 1* related allergens are known to be labile during glycation as also evidenced by the SDS–PAGE pattern (Fig. 1B), the decrease in the basophil activation for these particular patients was not unusual. On the contrary, for other 2 out of the 6 patients with systemic allergic reactions (Figs. 2 and 4) an increase in the basophil activation was observed. Increases in allergenicity of proteins as result of the Maillard reaction are not unusual, and have been previously reported for peanut and scallop proteins (Gruber et al., 2005; Maleki et al., 2000; Nakamura et al., 2005). Considering that most of the commercial kits failed to detect these thermally treated proteins (Cucu et al., 2011), these results indicate therefore, that the patients with systemic allergic reactions to hazelnut are still at risk when they are exposed to hazelnut proteins even in highly processed foods. Moreover, this evidences that the routinely used methods for hazelnut detection in foods are not able to protect the allergic consumers.

4.2. Patients with OAS

Heat treatment alone did not have a great impact on the basophil activation of the patients with OAS (Figs. 3 and 5). It is generally believed that heat treatment leads to decreases in allergenicity of the hazelnut due to the disruption of conformational epitopes of *Cor a 1* and *Cor a 2* allergens through changes in the tertiary structure such as observed upon roasting of hazelnuts (Hansen et al., 2003; Muller et al., 2000). However, during roasting of whole hazelnuts, interaction with carbohydrates and fatty acids might take place, which would additionally affect the allergenicity. The preservation of the stimulatory activity of the treated hazelnut proteins in patients with OAS is in line with the stability of the *Cor a 1* allergen observed from the SDS–PAGE pattern (Fig. 1A). Previously, the allergenicity of semi purified pollen related allergens such as *Api g 1* from celery (*Apium graveolens*), *Pru av 1* from cherry (*Prunus avium*) and *Mal d 1* from apple (*Malus domestica*) were reported not to be affected by thermal processing (Gruber et al., 2004; Vieths et al., 1998). This indicates that as long as other food components are not present, the pollen-related allergens are rather stable upon thermal treatment alone. On the contrary, upon incubation with glucose a complete abolishment of the basophil activation was observed. This indicates that the loss observed is most probably due to the modification of *Cor a 1* and *Cor a 2* allergens due to the reactive carbonyl species formed during thermal treatment in the presence of glucose which led to modification of the nucleophilic amino acids and changes in the tertiary structure of the protein as previously shown (Cucu et al., 2011). This confirms moreover that the earlier observed decreased allergenicity during roasting of hazelnuts (Hansen et al., 2003) was not solely due to thermal treatment but also to interactions between the carbohydrates and lipids during roasting.

In conclusion, our results indicate that the impact of thermal processing and the Maillard reaction is highly dependent on the type of allergic patient investigated. For the patients with an OAS, thermal treatment alone does not lead to significant changes in the basophil activation, while the presence of carbohydrates (i.e.

glucose) led to depletion of the basophil activation. For the patients with systemic allergic reactions, however, the thermal processing as well as the Maillard reaction might lead to increases as well as to decreases in the basophil activation without clear trends being established. Finally, patients with systemic allergic reactions to hazelnut are still at risk even if hazelnuts are highly processed. Although BAT cannot be routinely used due to limited amounts of blood from allergic patients, it would be most valuable to analyse foods containing clinically relevant allergens which could not be demonstrated by routinely applied methods such as enzyme-linked immunosorbent assay (ELISA) [8]. Additionally, these results indicate that a correct selection of patients, for studies dealing with the impact of food processing on allergenicity is mandatory.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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