

Review

Stability of food allergens and allergenicity of processed foods

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Abstract

The allergenicity of food could be altered by several processing procedures. For various foods of animal and plant origin the available literature on this alteration is described. Investigations on hidden allergens in food products are also dealt with.
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1. Introduction

During the manufacture of food their allergenicity may be altered by various processing parameters. The allergenic activity may be unchanged, decreased or even increased by food processing. The molecular basis of changes in the allergenic activity is the inactivation or destruction of epitope structures or the formation of new epitopes or better access of cryptic epitopes by denaturation of the native allergen.

Table 1 gives an overview of important manufacturing procedures which potentially affect the allergenicity of food allergens. To date, there are only a few studies on alteration of the allergenicity of processed foods. Most studies were done with native foods or allergen extracts of native foods. Some studies were initially started by a single case of adverse reaction to a certain processed food without systematic investigations of processing parameters and without appropriate numbers of patients. There are a few model studies which imitate processing parameters listed in Table 1. Mainly heating (dry

heating, boiling or cooking) and enzymatic digestions were investigated.

In the following, studies available in the literature will be described. It should be noted, that the “allergenicity” of food allergens was assessed in the minority of studies by double-blind placebo-controlled food challenge (DBPCFC). Most investigations were done by determination of the relative “IgE-binding potencies” of food allergens applying radio-allergosorbent test (RAST) or enzyme-allergosorbent test (EAST) and RAST or EAST inhibition tests, respectively.

In addition to alteration of allergenic potencies the problem of allergen contamination is present in processed foods. There are no reliable data on the frequency of contamination of processed foods with undeclared allergens. According to the report on control of foodstuffs by the Standing Committee for Foodstuffs of the European Union [1] 2.3% of 838 “milk-free” samples from six EU-member states contained undeclared milk proteins. Related frequencies for contaminated “egg-free” and “gluten-free” products were 1.3% in Germany ($n=319$) and 5.2%

Table 1

Overview of the main preparation and processing procedures which potentially influence the allergenicity of foods

Manufacturing processes

• Preparation

Washing, Peeling, Removing the husk, Storage

• Breaking up processes

Cutting, Grinding

• Thermal procedures

Drying, Heating (Baking, Blanching, Grilling, Steaming, Kiln-drying, Stewing, Cooking, Roasting, Pasteurization, Ultra High Heating, Sterilization), Cooling, Freezing, Evaporation

• Biochemical procedures

Fermentation (Lactic acid, Acetic acid, Rennin-precipitation, Purification, Tenderizing etc.)

• Isolation and purification

Melting, Pressing, Extraction, Distillation, Raffination, Filtration, Decanting, Centrifugation, Sieving, Polishing, Purification

• Chemical preservation

Alcohol, Pickling, Salt, Sugar, pH, Preservatives

• Other procedures

Mixing, Suspending, Emulsifying, Homogenization, Stabilization, Extrudation, Texturation, Colouring, Bleaching, Deodorizing

in ten EU-member states ($n=1583$), respectively. These data could not be generalised, because the majority of sampling was on the basis of suspicion and analytical methods varied from one participating laboratory to another.

2. Allergens of animal origin

2.1. Milk and milk products

The major allergens from cow's milk are constituted by caseins (Bos d 8, 20–30 kDa) and whey proteins beta-lactoglobulin (Bos d 5, 18 kDa), alpha-lactalbumin (Bos d 4, 14 kDa) and serum albumine (Bos d 6, 67 kDa) [2].

2.1.1. Raw milk and processed milk

Host and Samuelsson [3] investigated the allergenic potential of raw milk, pasteurized milk (75°C, 15 s) and pasteurized and homogenized milk (60°C, 175 kg/cm²) in five cow's milk allergic children. All samples were positive in skin prick test (SPT) and DBPCFC with a trend to higher allergenicity of processed milk samples. In comparison there were no positive reactions with an extensively hydrolysed casein-based infant formula. The amounts of milk samples which induced symptoms during DBPCFC in the five children were between 5 ml (starting dose) and 75 ml [3]. In three adults with milk allergy amounts of 5, 50 and 250 g whole milk (pasteurized and homogenized) induced allergic reactions in DBPCFC [4]. The ingested amounts of milk refer to approximately 165 mg to 8.25 g of milk protein.

2.1.2. Boiled milk

A remarkable reduction in allergenicity of milk can be observed after boiling (100°C) for 10 min, while boiling for 2 and 5 min, respectively, induced no significant change in SPT and dot-immunoblotting experiments [5,6]. Heating of skimmed milk for 10 min resulted in a decrease of approximately 50 and 66% of IgE-binding for alpha-lactalbumin and caseins, while beta-lactoglobulin and serum albumin lost their IgE-binding potencies in scored crossed radio-immunoelectrophoresis (CRIE) [7]. Norgaard et al. [5] proved the inactivation of beta-lactoglobulin

and serum albumin after boiling milk samples for 10 min, while caseins still induced positive reactions in SPT.

2.1.3. Cheese

Several cases of allergic reactions including anaphylactic reactions after ingestion of different kinds of cheese (hard cheese, soft cheese, among others mozzarella, parmesan) are described in the literature [8,9]. Allergic reactions to goat's and sheep's cheese are described as well [10,11]. Studies of the alteration of the allergenicity during the manufacture of cheese have not been undertaken to date.

2.1.4. Lactic acid fermentation

Fermentation of sterilized cow's milk using a mixture of meso- and thermophile lactic acid bacteria resulted in a 99% decrease of antigenicity of alpha-lactalbumin and beta-lactoglobulin (ELISA, rabbit antibodies), while the allergenicity in skin tests was hardly affected [12].

2.1.5. Hydrolysis with digestive enzymes

Purified milk proteins were more likely to be hydrolyzed by digestions with duodenal fluid and human trypsin and elastase in *in vitro* experiments than proteins in milk itself. Caseins were degraded first, followed by beta-lactoglobulin and alpha-lactalbumin, which were hydrolyzed at 100 and 500 times lower rates [13,14].

Schmidt et al. [15] performed a combined *in vitro* hydrolysis of whey proteins with pepsin (90 min) followed by hydrolysis with a mixture of pancreatic enzymes (pH 7.5 for 150 min).

Simulating pH values of duodenal fluids from adults and small children, pepsin digestions were done at pH 2, 3 and 4, respectively. Residual amounts of whey proteins after hydrolysis at pH 2 and pH 3 were about 0–14%. In contrast after digestion at pH 4 48, 58 and 91% of alpha-lactalbumin, serum albumin and bovine immunoglobulin retained their allergenic activity (RAST inhibition). Beta-lactoglobulin was hardly affected by pepsin hydrolysis, but almost completely digested by pancreatic enzymes [15].

Astwood et al. [16] confirmed the high stability (>60 min) of beta-lactoglobulin against peptic hydrolysis (pH 1.2), while caseins and serum albumin

were completely hydrolyzed after 2 min and 30 s, respectively.

2.1.6. Hypoallergenic infant formulas

Hypoallergenic infant formulas are produced from caseins or whey proteins by means of heat denaturation and enzymatic hydrolysis, sometimes combined with ultrafiltration. These formulas have decreased allergenic potential and are used as milk substitutes in infant nutrition. Partially and extensively hydrolyzed formulas are available. But only extensively hydrolyzed formulas should be applied in the nutrition of cow's milk allergic infants [17]. Despite the fact that extensively hydrolyzed whey protein formulas [18,19] as well as extensively hydrolyzed casein formulas [3,20] are tolerated without any reactions by most cow's milk allergic children (immunoblot, RAST, DBPCFC), severe adverse reactions have also been reported in a few patients. Anaphylactic reactions occurred after ingestion of extensively hydrolyzed casein formulas [21–23] and extensively hydrolyzed whey formulas [24].

According to a study with 20 cow's milk allergic children 10 and 13% did not tolerate extensively hydrolyzed formulas produced from caseins and whey proteins, respectively. 45% did not tolerate a partially hydrolyzed whey formula [25].

In RAST inhibition and skin testing the allergenic potential of casein formulas was lower than that of whey formulas. Oldaeus et al. [26] identified 15% positive reactions to extensively hydrolyzed whey formula and 2.5% to extensively hydrolyzed casein formula in 45 cow's milk allergic children by SPT. Similarly Wahn et al. [27] determined the least residual allergenic activity in extensively hydrolyzed casein formulas in skin tests and oral challenge investigating six different formulas.

2.1.7. Other milk products

Severe allergic reactions after ingestion of bakery products, pastry, chocolate and sausages which contained milk proteins have been reported [28–30].

2.1.8. "Hidden" milk allergens

Gern et al. [31] described six incidents of allergic symptoms occurring in cow's milk allergic children after ingestion of tofu (frozen dessert) (two cases), hot dog with beef (two cases), bologna (two cases), a

rice frozen dessert and tuna in aqueous solution. The children had no other food allergies. The amount of caseins determined by ELISA (enzyme-linked immunosorbent assay) was up to 2202 µg/ml in contaminated products and in products containing caseinate as an undeclared additive 136 µg/ml. All products were labeled "non-dairy" or "pareve" (containing neither milk nor meat).

Malmheden Yman et al. [32] measured the casein contents in products which induced allergic reactions in nine cow's milk allergic individuals. The following contents of undeclared caseins or caseinates were determined: 1.1% in meat balls, 2.6% in recombined ham, 1.0% in sausage, 0.2% in strawberry/cream lollipops and 1.1% in meringue. Milk protein contaminated products contained 0.04% caseins (hot dog), 0.8% (dark chocolate), and 0.2% (soy based ice cream). One fatal case after ingestion of approximately 100 g of a sausage contaminated with milk proteins (60 mg caseins) was reported.

A 2-year-old and a 3-year-old boy experienced anaphylactic reactions after ingestion of sorbets contaminated with milk proteins [33,34]. In one case reactions occurred within 20 min after ingestion of approximately 113–170 g of a lemon sorbet. Traces of whey proteins (9 µg/ml) were determined in the sorbet. The ingested amount of whey proteins was estimated to be 120–180 µg (or 23–24 µl milk) [34].

In the Netherlands, a case of anaphylaxis was reported in a 30-year-old woman after ingestion of a prepackaged bread with salmon [35]. No fish hypersensitivity was present. Further investigations of the product revealed that the salmon was treated with a microbial enzyme (transglutaminase) and caseins to improve the structure of the meat by covalent linkage between casein and meat proteins. The ingested amount of caseins was estimated to range from 10 to 50 mg assuming an ingestion of 10–50 g of salmon [35].

2.2. Egg and egg products

Major allergens identified in egg white are ovomucoid (Gal d 1, 28 kDa), ovalbumin (Gal d 2, 44 kDa), ovotransferrin (Gal d 3, 77 kDa) and lysozyme (Gal d 4, 14 kDa) [36]. The major allergenic component of egg yolk is alpha-livetin (identical to chicken serum albumin, 70 kDa).

2.2.1. Raw eggs

Norgaard and Bindsvle-Jensen [4] determined threshold concentrations which induced symptoms in DBPCFC in seven egg allergic adults. Challenges were performed with masked fresh, raw hen's eggs and dilutions of them. The ingestion of 50 mg whole egg induced symptoms of oral allergy syndrome (OAS), diarrhoea, asthma, and conjunctivitis in two patients. 500 mg and 2.5 g induced allergic reactions in an additional two patients. In the other three patients amounts of 50 g of whole egg were necessary for the induction of symptoms. The ingested amounts ranged from 5 mg to 5 g of egg protein.

2.2.2. Boiled eggs and heated egg white

Soft-boiled (100°C, 3 min) and hard-boiled eggs (100°C, 20 min) contained ovomucoid and ovalbumin with decreased, but clearly detectable antigenicity determined by radio-immunoelectrophoresis (RIEP) with rabbit antisera [37].

IgE-binding of heated egg white (90°C, 10 min) decreased about 58% in RAST [38]. In DBPCFC a decrease of positive reactions was observed after heating of egg white (90°C, 60 min) of 55% in egg allergic patients with positive DBPCFC to freeze-dried egg white [39]. Heated ovomucoid depleted egg white gave only 6% positive DBPCFC.

2.2.3. Meat products containing egg protein additives

Leduc et al. [40] investigated three experimental pork meat pastes with 2% added dried egg white. In raw and in pasteurized pastes (70°C, 2 h) egg white allergens were detected by SDS–PAGE immunoblot and EAST, while no allergens could be detected in the sterilized paste (115°C, 90 min).

2.2.4. Hydrolysis with digestive enzymes

According to Astwood et al. [16], ovalbumin and phosvitin resisted peptic digestion at pH 1.2 (>60 min), while ovotransferrin was immediately degraded and ovomucoid was degraded after 8 min of treatment.

2.2.5. Egg lecithins

Palm et al. [41] demonstrated the allergenic activity of egg lecithins in a 15-month-old girl in DBPCFC. The ingestion of 50 mg induced allergic

symptoms of the skin within an hour (erythema of neck and shoulders). The egg lecithins contained 11.3 g protein per 100 g.

2.2.6. "Hidden" egg allergens

Sampson et al. [28] reported a fatal anaphylactic reaction in a 2-year-old girl after ingestion of a hamburger containing egg proteins. Malmheden Yman et al. [32] determined contents of 0.14 and 0.16% of undeclared ovalbumin in meat balls which induced allergic symptoms in two cases. Undeclared egg (0.003–0.013% ovalbumin) added to pasta products induced adverse reactions. According to Fremont et al. [42] allergic reactions to cheese were induced by undeclared added lysozyme.

2.2.7. Microparticulated proteins

Sampson and Cooke [43] investigated the allergenic potential of Simplese, a fat substitute, by SDS–PAGE immunoblotting. Simplese consists of nanoparticles 0.1–3 mm in diameter which are coagulated by heat and high-shear processing. There was no difference in IgE-binding between the Simplese proteins and native egg proteins with 16 sera from egg and/or cow's milk allergic individuals.

2.3. Fish and fish products

"Allergen M" (Gad c 1, 12 kDa) from codfish was the first food allergen which was characterized in detail. Gad c 1 belongs to the family of Ca-binding parvalbumins. Cross-reactive Gad-c-1-homologous proteins were identified in several fish species: salmon (Sal s 1), tuna, perch, carp, eel, catfish, dogfish and snapper [44,45]. Additional fish allergens are present in the molecular mass range from 15 to 200 kDa.

2.3.1. Raw fish

Amounts of 6 mg to 6.7 g of fresh, raw codfish meat induced allergic symptoms in ten fish allergic adults challenged by DBPCFC. These amounts contained approximately 1 mg to 1 g of protein. Anaphylactic reactions were observed with relatively high amounts of 25–50 g codfish [46].

2.3.2. Boiled fish

Bernhisel-Broadbent et al. [47] demonstrated the allergenicity of boiled fish with ten fish species in DBPCFC. In SDS–PAGE analysis boiled fish showed denaturation of some protein bands and formation of high-molecular bands in comparison with protein extracts from raw fish. Immunoblotting revealed a relatively strong decrease of IgE-binding to allergens from boiled fish without completely abolishing it. The results of applied in vitro tests often did not correlate with the clinical relevance of fish hypersensitivity [47].

Hansen et al. [48] proved a high stability of fish allergens in codfish, herring, and plaice after boiling for 6 min, 1 h, and 4 h in two patients with codfish allergy. With the exception of macarel the activity of histamine release was unchanged for all protein extracts from boiled fish. In contrast protein bands >40 kDa were heat labile, while proteins with lower molecular mass were stable. Even after 4 h of boiling IgE-binding fish proteins were identified in SDS–PAGE immunoblot.

2.3.3. Canned fish products

Bernhisel-Broadbent et al. [49] investigated the allergenic potencies of canned tuna and salmon. Results of DBPCFC with canned tuna were negative in 18 fish allergic patients. Challenges with canned salmon were negative in two patients with salmon allergy.

SDS–PAGE revealed a significant loss of distinct protein bands in canned fish as compared to raw and boiled fish. The IgE-binding activity of canned fish was low in immunoblotting and estimated to be 100–200 times lower than in boiled fish (EAST inhibition) [49].

2.3.4. Surimi

Surimi is produced from minced and thoroughly washed fish meat from different species. Starch, egg white and other food additives giving a gelatinous structure after heating may be added. Testing six codfish allergic individuals, Mata et al. [50] investigated the allergenicity of surimi in comparison to fresh codfish. Only two of the patients were positive to surimi in SPT, while RAST results were positive in all six sera. Maximum inhibition of IgE-binding to codfish allergens by surimi extracts were between 60

and 94% (RAST inhibition). Allergens in fresh codfish migrated in the range of 13 to 63 kDa. In contrast, surimi retained only one 63 kDa allergen in SDS–PAGE. IgE-binding activity of surimi was confirmed in RAST inhibition with sera from fish allergic individuals by Helbling et al. [51].

2.3.5. “Texturized” fish meat

Salmon meat treated with enzymes and caseins induced allergic reactions in a cow’s milk allergic patient (see Milk and Milk Products: “Hidden” Allergens).

2.3.6. Other fish products

Helbling et al. [51] identified IgE-binding activity of uncooked and cooked pizza toppings in RAST inhibition.

2.3.7. “Hidden” allergens in used fat

Yunginger et al. [52] reported a fatal anaphylactic reaction of a fish allergic individual who ingested fried potatoes. The fat was previously used for frying fish.

2.3.8. Hydrolysis with digestive enzymes

Aas and Elsayed [53] investigated the stability against enzymatic digestion of the muscle myogene fraction from codfish. After treatment with trypsin, pepsin, subtilisin and pronase (48 h, 37°C), respectively, no allergenicity was detectable in skin testing. In contrast the elastase digested fish protein retained about 50% of its allergenic activity. Some allergenic activity was present in pronase digests after 24 h and trypsin and pepsin digests after 2 h, respectively.

2.4. Crustaceae and products of these

Shrimps, lobster, crab and crayfish belong to the family of crustaceae. The major allergens of crustaceae are tropomyosins which were identified in several shrimp species (Met e 1, Par f 1: 39 kDa, Pen a 1: 36 kDa, Pen i 1: 34 kDa), lobsters (Pan s 1, Hom a 1), and squids (Tod p 1, 38 kDa), which belong to the mollusc family [54–56].

2.4.1. Boiled shrimps

Several studies proved the heat stability of the allergenic potential of shrimps after boiling.

Daul et al. [57] demonstrated the allergenicity of boiled shrimps in 30 patients with a history of shrimp allergy. A total of 30% were positive in open oral challenge with boiled shrimps. Six of these patients were positive in DBPCFC too. Doses of the provocation tests were 1 to 16 shrimps corresponding to 4–64 g of shrimps and approximately 0.7–12 g protein.

Naqpal et al. [58] isolated a 34-kDa allergen (Pen i 1) with unchanged allergenicity from boiled shrimps. Daul et al. [151] detected in shrimps as well as in the cooking water similar allergenic activity of the major allergen Pen a 1 in SDS–PAGE immunoblot.

A patient who reacted in SPT exclusively to boiled shrimps was described by Rosen et al. [59]. The patient experienced an anaphylactic reaction after ingestion of boiled shrimps and had negative SPT results with raw shrimps.

Yunginger et al. [52] reported a fatal case of anaphylaxis after ingestion of crab.

2.5. Meat and meat products

Allergens from meat are serum albumins (66 kDa), gamma-globulins (60 kDa), and actins [60] as well as several additional proteins (14, 18, 20, 45 and >60 kDa). Cross-reactivity to whey proteins from milk are due to serum albumins and gamma-globulins. Denatured type I collagen (beef) was identified as the major allergenic component of gelatine.

2.5.1. Poultry and mammals

A total of 64% of patients with sensitivity to poultry meat in immunoblot analysis were clinically allergic to poultry. In contrast only 29% of patients with beef sensitivity were clinically allergic to beef [61]. A total of 75% of patients sensitive to meat from mammals were simultaneously sensitized to meat from poultry, while conversely no more than 50% of poultry sensitive patients gave positive IgE-binding to meat extracts from mammals in SDS–PAGE immunoblot [61].

2.5.2. Heated meat

Fisher [62] reported a case of anaphylactic symptoms after ingestion of rare-cooked beef, while well-cooked beef was tolerated.

Werfel et al. [6] studied 11 patients aged 16 months to 14 years with beef allergy. In DBPCFC, eight patients reacted positive after ingestion of well-cooked beef (brown), the other three patients reacted to rare-cooked meat (red centrally and pink peripherally). Amounts inducing allergic symptoms were between 10 and 60 g, and 250 mg in one case. Reactions were predominantly symptoms of the skin, severe systemic reactions were not observed.

The water solubility of proteins decreased with time of heat treatment. After heating (85°C) of minced beef up to 2 h, a 17.8 and 19 kDa band and four additional bands (14, 20, 45 and >60 kDa) were weakly detected [6]. These protein bands were also detectable in well-cooked minced beef (20 min, up to 80°C) by immunoblotting. Strongest IgE-binding potential was demonstrated for the 17.8 kDa allergen in patients with positive DBPCFC to cooked beef. Bovine serum albumin and gamma-globulin were not detectable in SDS–PAGE after heating (80°C) of minced beef for 10 and 3 min, respectively. In contrast isolated serum albumin was stable at 95°C for 15 min. Isolated gamma-globulin was denatured within 15 min at 65°C [6].

In SPT 7 of 11 children with beef allergy, who had positive SPT to raw bovine serum albumin, reacted positive to heated bovine serum albumin. Four children were positive in DBPCFC with heated bovine serum albumin [63].

Ayuso et al. [61] performed in vitro tests with 57 patients with suspected meat allergy to beef, pork, lamb, rabbit, or poultry. Only two patients reacted to tropomyosins (which are the major allergens in crustaceae) one from pork and one from chicken. A total of 75% of sera showed stronger IgE-binding to allergen extracts from raw meat than to extracts from heated meat (20 min, 140°C). Chicken meat was an exception. Six of 24 sera reacted strongly to proteins in heated chicken meat, while IgE-binding to proteins from raw meat was hardly detectable. Allergens simultaneously detected in raw and heated chicken meat had molecular masses of 17, 20, 24, 28, 31 and 66 kDa in SDS–PAGE. Heat labile allergens in raw

chicken meat were 45 and 150 kDa protein bands. Neoallergens were detected in heated chicken meat at molecular mass range of 14 to 90 kDa.

2.5.3. Homogenization and lyophilization

Fiocchi et al. [63] studied the allergenicity of freeze-dried beef and homogenized beef in ten children with positive SPT to raw and heated beef and positive DBPCFC with 180 g heated beef (5 min, 100°C). In SPT all children reacted to untreated bovine serum albumin and five children reacted positive in DBPCFC with untreated bovine serum albumin.

Only one child had positive SPT to freeze-dried beef, while none of the children had positive challenge with freeze-dried beef (DBPCFC). The same tests with homogenized meat were negative in all the children. Earlier SPT results from Fiocchi et al. [64] indicated only a weak allergenic potential of homogenized and freeze-dried beef and freeze-dried sheep's meat in two of 12 children with clinically relevant beef allergy.

2.5.4. Hydrolysis of meat proteins

Fiocchi et al. [64] investigated the allergenicity of serum albumin (beef, sheep) after enzymatic digestion with pepsin. A total of 12 children with clinically relevant symptoms of beef allergy and positive SPT to native serum albumin were studied. Four of 12 children reacted positive to digested bovine serum albumin (5 min time of hydrolysis). After 2 and 4 h of hydrolysis there were only two positive SPT each. Digested ovine serum albumin preparations were positive in three children (5 min time of hydrolysis), while after 2 h of hydrolysis none of the children reacted positive in SPT. All hydrolyzates were negative in RAST.

2.5.5. DBPCFC with serum albumin

Fiocchi et al. [65] performed DBPCFC tests with 90 mg bovine and 63 mg ovine serum albumin in 12 children with clinically relevant beef allergy. Immediate allergic reactions were observed in two (bovine serum albumin) and three children (ovine serum albumin), respectively. One child experienced de-

layed reactions of severe dyspnoea, cough and asthma (ovine serum albumin).

2.6. Other food ingredients of animal origin

2.6.1. Gelatine

Anaphylactic reactions to gelatine containing vaccines were frequently reported. Seven of 26 children who experienced systemic allergic reactions induced by gelatine containing vaccines also had allergic reactions after ingestion of gelatine containing foods [66]. Cross-reactivity between gelatines from different mammals was demonstrated by RAST inhibition with sera from 12 children with hypersensitivity to bovine gelatine. Only one child was strongly sensitized to fish gelatine [67]. Using SDS-PAGE immunoblot and RAST Sakaguchi et al. [68] showed that the IgE-binding potential of type I collagen from beef is located on the alpha 2 chain.

One patient described by Wahl and Kleinhans [69] experienced OAS after ingestion of fruit gums containing gelatine. Allergens with molecular mass range of 40 to 120 kDa were detected by SDS-PAGE immunoblot.

After enzymatic treatment of pork gelatine (heat-denatured collagen) with collagenase Sakai et al. [70] described a product of peptides <10000 Da with very low IgE-binding activity.

3. Allergens of plant origin

3.1. Legumes, nuts, and seeds

3.1.1. Peanut and peanut products

Major allergens from peanuts are Ara h 1 (vicilin, 63.5 kDa), Ara h 2 (conglutin, 17 kDa) and Ara h 3 (glycinin, 57 kDa). Ara h 4 is an isoallergen of Ara h3. Minor allergens are Ara h 5 (profilin, 15 kDa) and two conglutinin-homologous proteins Ara h 6 (15 kDa) and Ara h 7 (15 kDa) [71].

3.1.1.1. Raw peanuts. Moneret-Vautrin et al. [72] determined threshold concentrations which elucidate allergic reactions after ingestion of peanut by DBPCFC. A total of 25% of 50 patients with peanut allergy reacted positive after challenge with less than

100 mg, 62.5% after challenge with 100–1000 mg, and 12.5% after challenge with amounts up to 7.1 g.

3.1.1.2. Peanut flour. Five of eight analyzed peanut flours showed no difference to extracts from raw peanuts in RAST inhibition [73]. Three other flours showed significantly different slopes which could be due to different peanut varieties. However, all eight peanut flours showed strong IgE-binding from pooled serum from five highly sensitized peanut allergic individuals. A flour produced from peanut shells showed only weak allergenic activity [73].

Extremely low doses of 10–50 mg of peanut protein applied as commercial peanut flour were tested by DBPCFC in 14 peanut allergic patients [74]. A systemic reaction was observed in one case at a challenge dose of 5 mg. In two cases 2 and 50 mg induced mild objective symptoms, while 1–50 mg induced mild subjective symptoms in five other cases. Two allergic individuals who were reactive with objective symptoms at higher doses reacted with short-lived subjective symptoms at a dose of 100 µg. Five peanut allergic patients did not react to challenge with doses up to 50 mg [74].

3.1.1.3. Heated peanuts. Nordlee et al. [73] measured almost the same IgE-binding activities in oil-roasted and dry-roasted peanuts in comparison with raw peanuts (RAST inhibition).

Keating et al. [75] identified peanut allergens in plant edible oils which were used for roasting of peanuts (radio-immuno assay (RIA), pooled serum from five peanut allergic patients). The allergen concentration could be reduced to approximately 0.1–1% after filtration and steam cleaning of the plant oil samples.

Burks et al. [76] observed no reduction of IgE-binding after heating (100°C up to 60 min) of isolated peanut protein extracts and Ara h 1 and Ara h 2 in RAST inhibition experiments (pooled serum from ten patients with peanut allergy). Extracts were prepared from roasted peanuts (13–16 min, 163–177°C).

Koppelman et al. [77] isolated Ara h 1 from ground and heated peanuts (20–140°C, 15 min). No significant changes of the IgE-binding properties could be determined in ELISA inhibition with a pooled serum from eight peanut allergic individuals.

3.1.1.4. Hydrolysis with digestive enzymes. Becker [78] demonstrated the liberation of peanut allergens during chewing of peanuts for 10 min without any detectable degradation of allergens in SDS–PAGE immunoblot using sera from ten peanut allergic patients.

Simulating digestive fluids Burks et al. [76] combined two successive enzymatic steps for digestion of peanut protein extracts from roasted peanuts. The first step simulated the gastric fluid (pepsin hydrolysis) while the second step simulated the duodenal fluid (trypsin, chymotrypsin, and intestinal mucosa peptidase). A 100-fold reduction of the IgE-binding potency was measured by RAST inhibition (pooled serum from ten patients with peanut allergy).

Vieths et al. [79] performed similar digestion studies with peanut protein extracts from roasted peanuts. After peptic treatment (2 h) several IgE-binding fragments were identified by SDS–PAGE immunoblot analysis. The allergenic activity was strongly reduced after digestion with pancreatic enzymes (45 min). However, in the EAST and RBL cell mediator release assay, the IgE-binding activity and mediator release capacity of peanut extract was only weakly influenced, indicating that IgE reactive and allergenic peptides were present, even after application of gut conditions. Astwood et al. [16] determined a high stability of Ara h 2 (>60 min) against pepsin hydrolysis (pH 1.2), while peanut lectin was stable for 8 min. Becker [78] proved the high stability of Ara h 1 against pepsin hydrolysis (80 min).

Applying a higher enzyme-substrate ratio of 1:3 (treatment 24 and 48 h), Hong et al. [80] destroyed the IgE-binding activity of peanut protein extracts after pepsin hydrolysis completely (five peanut allergic patients, SDS–PAGE immunoblot). However, these pepsin digested peanut preparations were active in T-cell proliferation. In vitro stimulation of PBMC (peripheral blood mononuclear cells) from seven peanut allergic patients revealed a significant T-cell proliferation slightly lower than by stimulation with native peanut protein extracts [80].

3.1.1.5. Peanut butter. A higher IgE-binding inhibition to crude raw peanut extract was determined for four commercially available peanut butter samples

using a pooled serum from five highly sensitive peanut allergic patients (RAST) [73].

3.1.1.6. Peanut oil. According to Nordlee et al. [73] peanut oil samples did not inhibit IgE-binding to raw peanut extracts in RAST inhibition. Moreover challenges of ten peanut allergic individuals with total doses of 8 ml refined peanut oil were negative in DBPCFC [81].

In vitro investigations with four commercial peanut oils differing in grade of processing were performed by Teuber et al. [82]. The IgE-binding potentials in dot-immunoblotting experiments with a pooled serum from 17 nut and/or peanut allergic patients decreased in the following order: unrefined peanut oil (54°C maximum temperature of processing) > unrefined peanut oil (65–93°C) >> refined, bleached, and deodorized peanut oils (230–260°C). Protein contents of unrefined oils were about 10–11 µg/ml and of refined oils about 3 and 5.7 µg/ml. In dot-blotting 1 µg protein from each sample was applied.

In a study by Hourihane et al. [83] the allergenicity of refined and unrefined peanut oils was tested in DBPCFC with 60 peanut allergic patients. No symptoms occurred after challenge with refined peanut oil, while six patients experienced allergic reactions after ingestion of 1–10 ml of unrefined peanut oil (oral and throat itching, swelling of lips, wheeze). Olszewski et al. [84] observed four positive reactions after challenge with 5 ml crude peanut oil in DBPCFC with 11 peanut allergic patients, while SPT were all negative.

A total of 14 of 62 peanut allergic patients showed a positive DBPCFC test with refined peanut oil [72]. Doses of 5–15 ml peanut oil induced symptoms of immediate reactions of the skin (facial erythema and pruritus, six cases) and respiratory tract (bronchospasm one case) as well as delayed respiratory (bronchospasm one case), cutaneous (labial oedema one case, eczema three cases, buccal itching and oral allergy syndrome two cases), and gastrointestinal symptoms (abdominal pain with nausea two cases).

Adverse reactions after ingestion of infant formulas containing peanut oil were reported in four children with atopic dermatitis (aged 4–13 months) [85]. After elimination of peanut oil from the diet

symptoms improved. All four children reacted positive to oral provocation tests with refined peanut oil and two had positive labial challenge tests. Only one child was skin tested (positive reaction).

Kull et al. [86] found a significantly higher frequency of allergic symptoms in children exposed to peanut oil in vitamin A and D preparations than in children exposed to water-based vitamin preparations after consumption of peanuts (41 children with clinically relevant peanut allergy). There was no difference in frequency of sensitization between the two groups. Protein extracts from refined peanut oil were not reactive in SPT, while extracts from unrefined peanut oil were positive in 15 of 41 children [86].

3.1.1.7. “Hidden” peanut allergens. Yunginger et al. [52] reported four cases of fatal anaphylaxis. Symptoms occurred after ingestion of “two bites” of chili containing peanut butter, cake and a cookie containing peanuts, and a Vietnamese dish with slivered peanuts atop.

Sampson et al. [28] reported three fatal anaphylactic reactions in adolescents between the ages of 8 and 16 years with severe peanut allergy after ingestion of candy, cake, and a sandwich containing peanuts in different forms. An additional case of severe anaphylaxis in a 13-year-old boy due to ingestion of cookies was reported [28].

Malmheden Yman et al. [32] reported a case of anaphylaxis after accidental ingestion of peanut containing-cake.

A systemic allergic reaction after ingestion of a soup was reported in a 33-year-old peanut sensitive woman. The dry soup preparation contained undeclared peanut flour as a component of a flavouring ingredient. Approximately 45 mg peanut protein were ingested [87].

Hogendijk et al. [88] described two cases of anaphylaxis after ingestion of pizza in a fast food restaurant. Peanut allergens were identified in the pizza sauce.

Borelli et al. [89] reported recurrent anaphylactic reactions in three patients with peanut allergy after ingestion of Asian foods, chocolate products and bakery products which contained peanuts.

Foucard and Malmheden Yman [90] reported two fatal reactions in adolescents with known peanut

allergy after ingestion of an “almond bun” with peanut flakes which were substituted for almond flakes (presumed co-factor: a cold beverage) and a self prepared beverage containing peanuts, respectively. Four near-fatal allergic reactions were observed after ingestion of peanut butter, peanut paste, and candy.

According to an American survey with 3704 peanut and nut allergic individuals, approximately 1% experienced allergic reactions to peanuts on commercial airliners [91]. In 32 of 35 cases peanuts or peanut products were served during the flight. Allergic symptoms were induced via ingestive route in 14 cases, by skin contact in seven cases, and by inhalation in 14 cases.

In five of 17 commercial food products without declaration of peanut components, between 2 and 18 mg/kg of peanut protein was detected. For the quantification a competitive ELISA with polyclonal antiserum was used [152].

3.1.2. Soybean and soybean products

Major allergens from soybean are the seed storage proteins Gly m Bd 30K (30 kDa, formerly Gly m 1), glycinin (320–360 kDa, six subunits 58–62 kDa) and beta-conglycinin (140–180 kDa, three subunits 42–76 kDa) [92]. Additional allergens are profilin (Gly m 3, 14 kDa) and the Kunitz-trypsin-inhibitor (20 kDa). Inhalative major soybean shell allergens are Gly m 1 (two isoallergens with 7 and 7.5 kDa) and Gly m 2 (8 kDa) [92]. Recently, evidence for the presence of a Bet v 1 related soybean allergen in soy protein isolate has been reported [153].

3.1.2.1. Heated soybeans. Shibasaki et al. [93] studied the allergenic potentials of the 11S-, 7S-, and 2S-globulin fractions after heating for 30 min at various temperatures. Heating to 80°C resulted in a slight increase of IgE-binding by the 2S-fraction, while the allergenic potencies of the other fractions both decreased about 42–75% in RAST. Heating to 100 and 120°C induced a reduction of IgE-binding of about 39–83% in all three fractions.

Burks et al. [76] determined no significant decrease of IgE-binding in RAST inhibition after heating (100°C up to 60 min) of whole soybean protein extracts as well as 7S- and 11S-fractions and

whey proteins (pooled sera from two patients with soybean allergy).

Müller et al. [94] compared the allergenic potentials of boiled (100°C, 2 h) and raw soybeans in EAST and EAST inhibition. Specific IgE against boiled soybean protein was detected in only three of six soybean allergic patients.

Similar results were obtained by microwave heating (700 W, 25 min) of soybeans [95]. In EAST experiments nine of 15 soybean allergic patients had detectable specific serum IgE against heated soybean protein.

3.1.2.2. Hydrolysis with digestive enzymes. Treatment of soybean protein with digestive enzymes was performed by Burks et al. [76] using two successive steps of hydrolysis ((1) gastric fluid: peptic hydrolysis; and (2) duodenal fluid: trypsin, chymotrypsin and intestinal mucosa peptidase). In comparison to native soybean protein a 10-fold decrease of IgE-binding was observed for digested soybean protein RAST inhibition (pooled serum from two patients with soybean allergy).

Astwood et al. [16] reported a high stability (>60 min) of beta-conglycinin (beta-subunit) and Kunitz-trypsin inhibitor against peptic digestion (pH 1.2). Soybean lectin was stable for 15 min, while the alpha-subunit of beta-conglycinin and Gly m Bd30K were completely abolished after 2 min and 30 s, respectively.

3.1.2.3. Processed soybean products. Herian et al. [96] studied the allergenic potentials of various soybean products by RAST inhibition of IgE-binding to a protein extract from raw soybeans (pooled serum from seven soybean allergic patients). IgE-binding of protein extracts decreased in the following order: soybean sprouts (approx. 70% max. inhibition), acid hydrolyzed soy sauce (40%), hydrolyzed soybean protein (40%), tofu (25–30%), tempeh (20%), miso (20%), mold-hydrolyzed soy sauce (10%) (in comparison self inhibition of raw soybeans: 70% max. inhibition). Therefore all examined products retained detectable IgE-binding activity.

Vieths et al. [95] confirmed the IgE-binding potential of soy milk, tofu, and texturized soybean protein in EAST inhibition.

3.1.2.4. Soybean lecithins. Three cases of severe systemic reactions due to parenteral lipid-emulsions which contained soybean lecithins as emulsifiers were reported [97].

Müller et al. [94] identified IgE-binding proteins with 27, 39 and 40 kDa in four of six commercial soybean lecithins. Awazuhara et al. [98] identified an IgE-binding protein with 31 kDa in soybean lecithins containing 2.8 mg protein per 100 g.

Palm et al. [41] demonstrated the allergenic activity of soybean lecithins in a 4-year-old boy by DBPCFC. The dose of 100 mg induced allergic symptoms of the skin (erythema) within 1 h. The protein content was 3.5/100 g.

Paschke et al. [99] identified IgE-binding proteins with 35 and 37 kDa in soybean lecithins (SDS–PAGE immunoblot (pooled serum from nine soybean allergic patients)). A further IgE-binding allergen of 16 kDa could be detected in all three investigated lecithin preparations, while the 35 and 37 kDa allergens were present in soybean protein isolate used as reference standard. The protein contents of the soybean lecithins ranged from 173 to 202 mg/100 g. Values of maximum inhibition of IgE-binding to native soybean protein by protein extracts from the lecithins were 54–84% with C_{50} -concentrations of 10–16 μ g/ml (native soybean protein 0.3 μ g/ml, EAST inhibition).

3.1.2.5. Soybean oil. Bush et al. [100] performed DBPCFC tests with seven soybean allergic patients using two refined soybean oils and one cold-pressed soybean oil. Applying a total dose of 15 ml of soybean oils no reactions occurred. Protein contents were not indicated.

An anaphylactic shock was reported in a patient treated with an infusion based on soybean oil for parenteral nutrition [101].

Paschke et al. [99] identified IgE-binding proteins with 53 and 58 kDa in three unrefined soybean oils (immunoblot, pooled serum of nine soybean allergic patients), in contrast no IgE-binding to protein extracts from two refined soybean oils was observed. Protein contents of unrefined oils were about 7–10 μ g/100 g and in the two refined oils 2.5 and 2.7 μ g/100 g, respectively. Maximal inhibition of IgE-binding to native soybean protein by protein extracts from unrefined oils was between 25–53%, while no

inhibition was observed with refined oils (EAST inhibition).

3.1.2.6. “Hidden” soybean allergens. A case of fatal anaphylaxis in a 10-year-old girl with concomitant sensitization to peanut and soybean (specific IgE) was reported by Yunginger et al. [102]. The reactions were induced by ingestion of a pizza sausage fortified with soybean protein.

Malmheden Yman et al. [32] reported a case of fatal anaphylactic reaction after ingestion of a hamburger containing undeclared soybean additives (2.1% soybean protein). Two additional allergic reactions occurred after ingestion of a kebab containing 7% soybean protein and crab sticks containing 0.5–0.9% undeclared soybean protein.

Severe incidences of anaphylactic reactions occurred after ingestion of Spanish sausage products (chorizo, salchichon, mortadella, and boiled ham), doughnut and soup stock cubes all containing soybean proteins (skin test, RAST, bronchial and oral challenge) [103].

Senne et al. [104] reported a case of anaphylactic reactions after ingestion of pizza containing soybean proteins.

Foucard and Malmheden Yman [90] reported four fatal reactions in adolescents with known peanut allergy, who were not aware of having a concomitant soybean allergy. Reactions were caused by ingestion of meat balls (with 3% soybean protein), a hamburger with unknown content of soybean, a hamburger with 2.2% soybean protein, and a kebab containing 7% soybean protein. Six additional life-threatening allergic reactions were induced by ice cream with soybean protein, meat balls, and soy sauce.

3.1.3. Nuts and nuts products (tree nuts)

The major hazelnut allergen is the Bet-v-1-homologous protein Cor a 1 (17 kDa). It seems that a nut-specific isoform of Cor a 1, Cor a 1.0401, is responsible for the majority of hazelnut allergies [154]. Major walnut allergens are 2S-albumin Jug r 1 and vicilin Jug r 2 (44 kDa). Brazil nut contains the major allergen Ber e 1 a 2S-Albumin (9 kDa) [54].

3.1.3.1. Heated nuts. According to Wigotzki et al. [105] the major allergens from hazelnut are very heat

stable. In immunoblot and EAST inhibition with sera from patients with hazelnut allergy there was no reduction of IgE binding observed after heating ground hazelnuts at 100°C for 90 min (dry heating oven) or after microwave heating (630 W, 10 min). A decrease in IgE binding could be observed after 15 min of conventional dry heating at temperatures above 100°C. After heating to more than 170°C the major allergens were abolished in SDS–PAGE immunoblotting. A minor allergen with <14 kDa was detectable after heating up to 185°C (15 min).

There are additional data showing that Cor a 1.0401 in hazelnut is a heat-labile protein. The allergen is deactivated after heating at 140°C for 30 min. Most patients did show a strongly reduced IgE-reactivity to extract from roasted hazelnut. By contrast, high molecular mass bands and a low molecular mass allergen (12 kDa) appeared to be stable under these conditions [155].

3.1.3.2. Hydrolysis with digestive enzymes. Vieths et al. [79] performed a combined hydrolysis with artificial gastric fluid (2 h) followed by hydrolysis with pancreatic enzymes (45 min). Thereafter IgE-binding of digested hazelnut proteins was reduced to <10% as compared to native protein extract (EAST with sera from hazelnut allergic individuals).

Wigotzki et al. [106] studied the stability of hazelnut protein extracts against various enzymes. After peptic hydrolysis for 60 min the IgE-binding was only slightly decreased (maximum EAST inhibition approx. 65%). Even after 240 min of hydrolysis two of seven sera from hazelnut allergic subjects showed IgE-binding in SDS–PAGE immunoblot. Maximum EAST inhibition was about 40% as compared to native hazelnut extract [106]. In contrast hydrolysis of hazelnut proteins with trypsin, elastase, and protease (from *Tritirachium album*) significantly decreased the IgE-binding potential after 30 min of treatment to a maximum inhibition value less than 30%. Hydrolysis of hazelnut proteins with pancreatin for 60 min reduced the IgE-binding to <30% maximum inhibition [106].

3.1.3.3. Processed nut products. Wigotzki et al. [107] investigated the IgE-binding properties of commercially available products containing declared hazelnuts. Using a pooled serum from 13 hazelnut

allergic patients the concentrations of 50% inhibition (C50-values) were ten times higher (indicating reduced IgE-binding) for protein extracts from two nougat chocolates as compared to native hazelnut extracts (EAST inhibition). Two nougat masses and two nougat-creams reached only 40% maximum inhibition. Protein extracts from five hazelnut chocolates showed a 6–25 times decreased IgE-binding potency. A 17-fold decrease of the C50-value was observed for inhibition by the protein extract from a hazelnut cookie. Two additional hazelnut cookie products did not produce a 50%-inhibition of IgE-binding. C50-values of two hazelnut crackers were 16 and 23 times higher as compared to native hazelnut protein. The IgE-binding potential of a muesli bar was comparable to native hazelnut protein, while protein extracts from a cake containing hazelnut protein did not give a 50%-inhibition in EAST [107].

3.1.3.4. “Hidden” nut allergens. Yunginger et al. [52] reported a fatal anaphylactic reaction in a 16-year old boy with known allergy to peanuts and pecan nuts after ingestion of a piece of cheesecake containing ground pecans in the crust.

Sampson et al. [28] reported two fatal anaphylactic reactions after ingestion of candies containing cashew protein.

Malmheden Yman et al. [32] analysed a chocolate which induced asthma after ingestion of a 3–6 g piece (from a Christmas calendar). It contained 0.2% hazelnut protein.

Malanin et al. [108] observed the case of a girl who experienced an anaphylactic reaction after ingestion of cookies containing pecan nuts, but was tolerant to raw pecans. Exclusive reactivity to a 15-kDa neoallergen from heated pecans was described in the patient.

Applying a sandwich ELISA with rabbit-antibodies against hazelnut protein amounts of hazelnut between 3.4 and 752 mg/kg could be detected in 15 of 26 samples of food products like chocolate spread, chocolate bar, chocolate cookie, muesli cookie, and cake, which were regarded to be free of hazelnuts. A complaint sample of chocolate spread contained 4 g/kg undeclared hazelnut [156].

With a hazelnut-specific sandwich-type ELISA based on polyclonal antisera in 12 of 28 commercial

food products without labeling of declaration of hazelnut components between 2–421 mg/kg of hazelnut-protein was detected [157].

3.1.4. Sesame seeds and sesame seed products

Little is known about sesame seed allergens. A 25-kDa and a 14-kDa allergen were identified by Kolopp-Sarda et al. [109]. Asero et al. [110] described allergens with 10 kDa and in the range of 15–20 kDa and 30–67 kDa using the serum from one patient.

3.1.4.1. Processed foods. Asero et al. [110] reported a case of anaphylactic reaction in a 16-year-old girl, who ingested sesame seeds for the first time knowingly. Symptoms occurred after ingestion of a chocolate candy rolled in sesame seeds.

3.1.4.2. “Hidden” sesame allergens. Malish et al. [111] reported four sesame allergic patients who experienced allergic reactions including anaphylaxis after ingestion of sesame seed products like hamburger, candy and salad with sesame oil.

Kägi and Wüthrich [112] reported three anaphylactic reactions in sesame allergic patients after ingestion of falafel burgers. This oriental speciality is made from a wheat flour bun filled with chickpea balls. It is served together with a white sauce containing freshly ground sesame seeds.

Kanny et al. [113] observed nine cases of allergic symptoms to sesame products including five anaphylactic reactions. Foods inducing symptoms were Lebanese sesame–rice-cake, bread and other pastry, Chinese food, pizza, “health” food, Turkish cake, and a hamburger sandwich.

Anaphylactic reactions occurred in a 46-year-old man after ingestion of sesame oil [114].

3.2. Cereals and cereal products

According to a study by Varjonen et al. [115] the major cereal allergens were a 26-kDa wheat protein, a 40-kDa rye protein, 26- and 46-kDa barley proteins, and a 66-kDa oat protein. A total of 16 different IgE-binding proteins were detected in all four cereals.

Other studies identified major wheat allergens: two water soluble allergens with 20 and 47 kDa [116]

and alpha-amylase inhibitor (15 kDa) [117]. Major allergens from water and salt insoluble prolamines are alpha- and fast-omega gliadins and glutelins [118].

3.2.1. Heated wheat flour and dough

Varjonen et al. [119] compared the IgE-binding potential of heated wheat flour and of a mixture of wheat flour and 50% added water. Neutral and acidic protein extracts were tested by SPT and/or RAST with sera from 12 cereal sensitive adults. IgE-binding decreased with temperature (80, 100 and 120°C) and heating time (10, 20 and 60 min). After heating at 120°C for 10 min no further decrease was observed. RAST-classes of the pooled serum decreased from class 4 against untreated wheat flour to class 2 against the heated wheat preparations. Allergenic potential of the heated dough was clearly lower than of heated flour after heating to 80 and 100°C, respectively. No differences could be observed at 120°C [119].

3.2.2. Barley malt and malt products

Neise and Sennekamp [120] performed open challenges with native barley malt in 42 subjects with suspected barley malt allergy. A total of 22 subjects reacted positive with symptoms of urticaria, eyelid oedema, pruritus of the skin and mucosa, rhinitis, dyspnoea, and oedema of pharynx and uvula. RAST results from 14 challenge positive patients were positive in only three cases. Neise and Sennekamp [120] identified beer as a common cause of relatively mild allergic reactions in barley malt allergic individuals.

Three cases of severe allergic reactions after drinking beer were reported by Curioni et al. [121]. Symptoms of generalized urticaria, laryngeal oedema, dyspnea, and facial angio oedema occurred. Emergency treatment was necessary in two cases. A 10-kDa major allergen was described in beer and barley malt, which is probably not involved in bakers' asthma [121].

Figueredo et al. [122] reported an anaphylactic reaction in a 21-year-old woman after drinking beer.

3.2.3. “Hidden” wheat allergens

Malmheden Yman et al. [32] reported an allergic reaction after ingestion of a “wheat-free” maize

pasta which was contaminated with 0.3% wheat gluten. Two additional cases with gastrointestinal symptoms occurred after ingestion of buckwheat flour containing 15–25% wheat flour (gluten content 1.3%) and a falsely “wheat-free” labeled pasta containing 7.8–11.9% wheat gluten.

3.3. Fruits and vegetables

3.3.1. Fruits of the Rosaceae family

Among others apple, pear, peach, cherry, plum and apricot belong to the plant family of Rosaceae. Cross-reactive allergens can be divided into four groups [123]:

(1) Birch pollen associated, Bet-v-1 homologous allergens with molecular masses of approximately 18 kDa, e.g. Mal d 1 (apple), Pyr c 1 (pear), Pru ar 1 (apricot), and Pru av 1 (cherry). (2) Glycoproteins in the range of 30–70 kDa including 60–69 kDa allergens cross-reactive to mugwort pollen (Art v 1). (3) Actin-regulating profilins with approximately 14 kDa, e.g. Pyr c 4 (pear). (4) Lipid-transfer proteins with 9–10 kDa, e.g. Mal d 3 (apple), Pru p 3 (peach), and Pru ar 3 (apricot).

Furthermore thaumatin-homologous allergens Mal d 2 (apple) and Pru av 2 (cherry) have been characterized.

More recently allergens belonging to the Bet v 6 family (isoflavone reductase homologous proteins) have been characterized in pear (Pyr c 5, 33.5 kDa) [54] and apple [158].

3.3.1.1. Storage of apples. The relative amount of the major allergen Mal d 1 was higher in ripened apples [124] and increased during storage at 4°C for 3 weeks [125].

3.3.1.2. Cutting and heating of fruits. During cutting or heating the allergenicity of fruits is commonly destroyed resulting in diagnostic difficulties. For example a DBPCFC test could not be performed since cutting of the fruits is necessary for blind provocation procedure [126]. Moreover it is commonly known that allergen extracts from fruits and vegetables are usually insufficient for skin testing [127–129,160] and active allergen extracts have to be produced by special extraction methods [130,131] avoiding the reactions of “enzymatic browning”

(phenolic substances and proteins, polyphenoloxidases).

Heating of apple slices (175°C, 0.5 h) abolished or strongly decreased the allergenicity in skin testing of two apple allergic patients [129].

Interestingly Vieths et al. [132] found that heating (100°C, 30 min) of the intact fruits (apple) reduced the IgE-binding activity of the protein extracts, but heating of a semi-purified allergen extract from native fruits did not destroy its immunoreactivity.

More recently the allergenic role of lipid-transfer proteins was emphasized [133–135]. Lipid-transfer proteins seem to be more stable than other fruit allergens. This fact was stressed in studies with patients who had a positive SPT to commercial allergen extracts [133]. Patients reactive to lipid-transfer proteins presented usually persisting fruit allergy without pollinosis [134].

3.3.1.3. Processed foods. In a survey 37% of 57 patients with peach allergy, who had sometimes eaten canned peach products reported symptoms to peach juices, 28% to peach syrup, and 24% to peach jam [136].

Brenna et al. [159] detected the peach allergens Pru p 3 and Bet v 1 homologous protein in four commercial peach nectars using two pooled sera from six peach allergic patients with and without birch pollinosis, respectively (SDS–PAGE immunoblot). Technological processing parameters like heating of peach nectar (121°C for 10 and 30 min) as well as treatment of a Pru p 3 containing extract semipurified from an intermediate product of peach juice with proteases from *Aspergillus* and *Rhizopus* for 60 min both failed to decrease the IgE-binding in SDS–PAGE immunoblot. Technological treatments which were able to decrease the allergenic potency of the resulting peach products were chemical lye peeling of the fruits and ultrafiltration of juice through suitable cut-off membranes.

3.3.2. Latex-associated fruits

The conserved hevein-domain (4.7 kDa) of the major latex allergen prohevein (Hev b 6.01) is a chitin-binding protein which is likely to present a cross-reactive panallergen involved in the latex-fruit syndrome. The main latex-associated fruits are avocado, banana, and chestnut (others are papaya,

passion fruit, fig, melon, mango, kiwi, pineapple, peach, and tomato) [137]. The major avocado allergen is an endochitinase with 32 kDa (Pers a 1). Two of these class I chitinases with 32 and 34 kDa were identified as major allergens in banana (proposed names: Mus p 1.1 und Mus p 1.2 [138]). The major allergen from chestnut Cas s 5 is also chitinase (32 kDa) [139].

3.3.2.1. Heated allergens. Posch et al. [140] performed DBPCFC using stewed avocado. Challenge was positive in the two tested patients.

Gall et al. [141] heated kiwi fruits to 40, 60, 80 and 90°C by microwave treatment. The allergenicity in SPT in three kiwi allergic patients decreased with increasing temperature. Heating of kiwi fruit to 90°C resulted in negative SPT in two patients and very weak SPT in the third patient.

3.3.2.2. Hydrolysis with digestive enzymes. Posch et al. [140] treated chitin-binding avocado allergens (major band at 31 kDa, four additional bands at 50–100 kDa) with artificial gastric fluid. Proteins were labile. After 5 min of enzymatic treatment several fragments in the range of 4–6 kDa could be detected. In contrast chitinases still inhibited IgE-binding to avocado proteins completely after 30 min of digestion (RAST inhibition, two sera from avocado allergic individuals).

3.3.2.3. “Hidden” allergens. Telez-Diaz et al. [142] reported an anaphylactic reaction in a 41-year-old woman after ingestion of a meal containing avocado.

3.3.3. Celery and celery products

Bet-v-1-homologous Api g 1 (16 kDa) presents the major allergen from celery. Additional allergens are celery profilin (Api g 4, 15 kDa) and Api g 5 (55/58 kDa) as well as several proteins in the range of 30–70 kDa [54].

3.3.3.1. Heated celery. Wüthrich et al. [143] examined the allergenicity of celery tuber and celery sticks in SPT and RAST with 70 patients with positive skin test to birch and/or mugwort pollen and

celery extracts. A total of 94% had positive SPT to raw celery and 36% to cooked celery. Eight of 13 patients were reactive to cooking water. Celery–birch sensitive patients had negative or very low RAST values against heated celery tuber and celery sticks. In contrast celery–mugwort sensitive patients had clearly positive RAST values against heated celery tuber and high values against celery sticks [143].

Jankiewicz et al. [144] performed comparative SPT and EAST studies with extracts from native and heated celery tuber (microwave 750 W, 30 min, 100°C) with 46 patients of whom 14 had clinically relevant celery allergy (anamnesis). A total of 78 and 43% had at least one positive test (SPT or EAST) to native celery and heated celery, respectively. Api g 1 was demonstrated to be heat labile (100°C, 10 min), while celery profilin (up to 30 min at 100°C) and especially other allergens (>30 kDa) were relatively heat stable in SDS–PAGE immunoblot [144,145].

3.3.3.2. Preservation and other processes. In a model study Vieths et al. [146] investigated the influence of different preservation methods (gamma-irradiation with a total dose of 10 kGy, ultra high pressure 600 mPa/20°C, and commercially dried celery powder) and a high voltage impulse treatment on the allergenicity of celery using three sera from celery-sensitive patients with different specificity (to Api g 1, profilin or glycoproteins >30 kDa, respectively). None of the methods resulted in a loss of IgE-binding activity in EAST. Small changes such as slight increase or decrease in IgE-binding could be observed. In irradiated celery tuber a new allergenic protein with 18–19 kDa could be detected in SDS–PAGE immunoblot [146].

3.3.3.3. Hydrolysis with digestive enzymes. Degradation of celery proteins with artificial gastric fluid (pepsin, cathepsin, and mucin, 2 h, 37°C) revealed a loss of bands >40 kDa [146]. After successive hydrolysis with pancreatic enzymes (45 min, 37°C) the other proteins were degraded with the exception of proteins at <20 and 38 kDa. Immunoblot experiments revealed strong IgE-binding to celery proteins hydrolyzed with artificial gastric fluid using three sera from celery sensitive patients while a strong

Table 2
Stability of food allergens of animal origin and presence as hidden allergens

Allergens	Heating	Enzymic hydrolysis	Significance as hidden allergen
Milk and Milk Products	Stable	Partially stable	High
Eggs and Egg Products	Stable	Stable	High
Fish and Fish Products	Stable	Partially stable	Low
Crustaceae and Products	Stable	No data	Low
Meat and Meat Products	Partially stable	Low	Low

reduction of IgE-binding was observed after additional hydrolysis with pancreatic enzymes [146].

3.3.4. Carrot und carrot products

The major carrot allergen Dau c 1 (16 kDa) is a Bet-v-1-homologous protein [147].

3.3.4.1. Heated carrots. According to Wüthrich et al. [143] the positivity in skin tests was reduced about 50% by heating the carrots (30 min, 100°C). There were 24 positive prick tests to cooked and 53 positive tests to raw carrots in 70 patients with sensitivity to celery. Positive results to the cooking water were similar to cooked carrots.

Gomez et al. [148] reported a 34-year-old cook who experienced allergic rhinitis and contact urticaria after handling raw carrots. After inadvertant ingestion of raw carrots she experienced an anaphylactic reaction while she tolerated cooked carrots.

Quirce et al. [149] reported two cases of respiratory symptoms after ingestion of raw carrots. Cooked carrots were tolerated without any problems in one

case, while in the other case mild symptoms occurred.

4. Conclusions

The stability of allergens from animal origin and their impact as hidden allergens are summarized in Table 2. Milk and egg and products of these are characterized by high stability to heating and their common occurrence as hidden allergens in processed foods. Fish and crustaceae and products of these are heat stable while the significance as hidden allergens is low. The allergenic potential of meat and meat products is partially heat stable while the enzymic stability is relatively weak. Meat allergens are not common hidden allergens. Gelatine may be more important as a hidden allergen, while its prevalence is very low.

Table 3 summarizes the food allergens of plant origin. Heat stable and partially heat stable allergens are peanuts, tree nuts, and probably sesame seeds. Relevant allergenicity after heating is retained in

Table 3
Stability of food allergens of plant origin and presence as hidden allergens

Allergens	Heating	Enzymic hydrolysis	Significance as hidden allergen
Peanuts and Peanut Products	Stable	Partially stable	High
Soybean and Soybean Products	Partially stable	Partially stable	High
Tree Nuts and Products	Partially stable	Partially stable	High
Sesame Seeds and Products	No data	No data	High
Cereals and Cereal Products	Partially stable	No data	High
Fruits of the Rosaceae-Family	Mainly labile	Labile	Low
Latex-associated Fruits	No data	No data	Low
Celery and Celery Products	Partially stable	Mainly labile	High
Carrots and Carrot Products	Labile	No data	Low

soybeans, cereals, and celery, which are partially heat stable. Important allergenic activity after enzymic hydrolysis could be observed in peanuts, soybeans, tree nuts and avocado.

Common hidden allergens of plant origin are peanuts, soybeans, tree nuts, wheat, and celery.

Crude edible plant oils generally contain amounts of proteins which could induce allergic reactions, while refined, heated oils are usually not allergenic [150]. However even refined oils induced allergic reactions. Recent studies by DBPCFC with refined peanut oil demonstrated its allergenic potency [72].

To date there is no systematic investigation of the allergenicity of processed foods.

Future research on the basis of well-characterized food products and their intermediates is needed. Standardized manufacturing processes and reliable food specifications are mandatory. Evaluation of the allergenicity of processed foods must involve an appropriate number of patients, who are clinically allergic to the native food allergen. Ideally patient cohorts are recruited from various countries and represent child and adult population. Analytical and diagnostic characterization of food products should be performed by various methods such as DBPCFC, SPT, RAST, SDS–PAGE immunoblot, mediator release assays, and inhibition tests.

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