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STATE OF THE ART ON FOOD ALLERGENS – A REVIEW

Lucjan Jędrychowski, Barbara Wróblewska, Agata Szymkiewicz

Department of Food Enzymes and Allergens, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn

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The review addresses contemporary problems linked with the incidence of food allergy, cross-reactivity of allergens of various origin, hidden allergens resulting from the use of food additives, food contaminations and infections, as well as widely-distributed allergens connected with universal functions in respective tissues, including: enzymes, lipid transport proteins (LTP), and pathogenesis-related proteins (PR-proteins). In addition, it provides characteristics of major food allergens of plant and animal origin. Extension of the array of allergenic products has been shown to be affected by globalization processes, international food turnover, extension of cultivation area and popularization of Western cultural habits. Technological processes applied to raw materials and food are likely to change allergenicity of the finished product, *i.e.* either decrease or increase it.

INTRODUCTION

Allergy is one of the most oppressive ailments of a contemporary man living in highly-developed societies. It occurs irrespective of age, race, or sex of a person affected by it and is characterised by various pathological symptoms. It is estimated that *ca.* 10-35% of human population world wide is affected by allergic conditions [Sicherer *et al.*, 2000], including 0.5-8% of the population with allergic response to ingested foodstuffs. To a greater extent, this problem affects children (4% to 8%) due to immaturity of their immune system and systems protecting mucous membranes of the gastrointestinal tract. With age, a phenomenon of an allergic march may be observed that consists in a change of pathological symptoms from allergic enteritis or atopic dermatitis (at an early age) to the development of inhalatory allergy or bronchial asthma (in adolscence). Adults are less susceptible to food allergy (from 1% to 2%) [Nowak-Węgrzyn *et al.*, 2001; Schäfer *et al.*, 2001]. Likewise in other countries, in Poland the incidence of allergies, including food allergies, has been observed to increase distinctly in recent years [Jędrychowski, 2003].

Extensive and in-depth analysis of the phenomenon of allergy epidemiology in a world wide scale has enabled determining food products that pose the greatest allergenic risk. In the Member States of the European Union, the major allergens (inducing 90% of allergic reactions) include: cow's milk, peanuts, eggs, soybeans, wheat, nuts, fish, crustacea and molluscs, celery, lupine, mustard, sesame seeds and products containing them as well as products obtained from their processing. Food producers have been obliged to indicate the presence of sulfites occurring in products in the concentration exceeding 10 mg/kg or 10 mg/L, expressed as SO_2^-. Being substances with low molecular weights, sulfites belong to a group of haptens and are incapable of inducing allergic reactions, though they may be a causative agent of food hypersensitivity [Directive 2003/89/EC, 2003].

Nowadays, allergic risk is considered in a wide context of food acquisition. In discussing issues linked with the occurrence of food allergies, particular stages of the food production process should be taken into account while stipulating standards of health safety of food (Figure 1).

The extent of incidence and epidemiological characteristics of food allergies vary between countries, namely:

– in the USA 65% of allergic patients react to milk, 45% to chocolate and Coca-cola type drinks, 33% to peanuts, 30% to cereal grains, 26% to vegetables, whereas in 26% of children allergic reactions to fish are observed to predominate;

– in France food allergies are induced mainly by egg white (46.3%), peanuts (40%), mustard (20%), and cow's milk (7.5%);

– in the Netherlands, amongst patients suffering from allergy 28% of allergic reactions are elicited by milk, 23% by broad bean, 22% by coffee, 18% by tomatoes, 16% by egg white, 14% by chocolate, 13% by fish and 11% by oranges;

– in Denmark 2.2% of the total population of children are allergic to cow's milk;

– in Poland allergy symptoms are most often induced by cow's milk (45%), egg white and poultry meat (30-37%), cereal products and other plant proteins (25%), and to a lesser extent by tomatoes, potatoes and fish [Jędrychowski, 2001; Kaczmarski, 1997].

In the Member States of the European Union, an increasing risk is observed that results from the intake of lupine and lupine-containing products, whereas 30-60% of patients with

Author's address for correspondence: prof. Lucjan Jędrychowski, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, ul. Tuwima 10, 10-747 Olsztyn, Poland; tel.: (48 89) 523 46 20; fax: (48 89) 524 01 24; e-mail: eljot@pan.olsztyn.pl

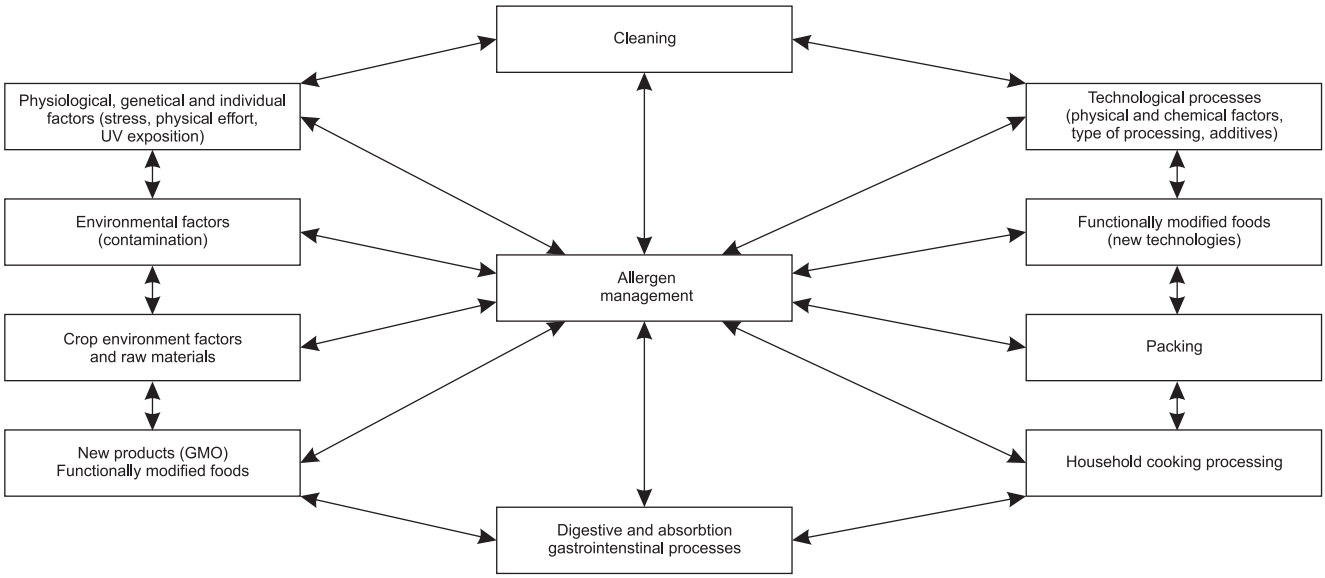

FIGURE 1. Sources of allergens at particular stages of the food production process.

allergy to peanuts display allergic reactions to lupine. Diet supplementation with molluscs (gastropods, bivalves and cephalopodes) caused that 1/5 of persons with allergic reactions to sea fruits, *i.e.* 0.4% of the total population, reacts also to molluscs [Commission Directive 2006/142, 2006].

Extension of the array of allergenic products is affected by processes of globalization, international turnover of food, increasing cultivation area and popularization of Western cultural habits [Iikura *et al.*, 1999]. Recently, attention has been paid to a variety of food additives (*e.g.* of biotechnological origin) being a potential cause of the appearance of an allergic reaction.

Quite troublesome, both from the viewpoint of clinical diagnostics, health safety of consumers as well as dietetics, is the fact linked with difficulties in precise determination of minimal doses of an allergen capable of inducing allergic reactions. The doses are highly diversified and result from biochemical and biological properties of allergen components. A threshold dose (minimal dose) of wheat proteins capable of inducing allergenic reactions accounts for 500 mg of wheat [Directive 2003/89/EC – Annex, 2003], on the other hand such a reaction may also be evoked by a few μg of nuts. For fish allergens the threshold value reaches 1 mg and for shrimps – 1 g. Exact determination of threshold doses is additionally difficult due to the fact that allergenicity of products may be affected by technological processes as well as these linked with digestion and absorption of food.

A serious problem and health risk posed to allergic persons is the occurrence of the so-called "hidden allergens". They include: egg, milk, and peanuts being constituents of complex products. There have been cases of anaphylaxy induced by milk proteins after consumption of ice creams, cottage cheese, pancakes, frankfurter-type sausages and wafers [Kurek, 2004].

Analytical difficulties in the identification of allergens result often from structural and conformational similarity of epitopes in proteins originating from various sources, cross-reactivity between proteins of different food products as well as between air-borne and food or contact allergens [Sharma *et al.*, 2001]. A severe problem is posed by an analytical issue linked with the detection of new epitopes likely to induce allergic reactions, including advanced analytical methods for investigations of spatial structures of allergens.

A severe health risk is also elicited by cross-reactivity between proteins from raw materials of similar origin. It is due to structural affinity resulting from homology of linear epitopes and similarity of spatial conformation (homology of conformational epitopes).

An important group of food allergens is constituted by panallergens, components of mainly plant origin, which – though phylogenetically distant – exhibit cross-reactivity. Their presence is usually linked with unexpected cross reactions, typical of the clinical picture of Oral Allergy Syndrome (OAS) and latex-fruit syndrome. Panallergens include: profilins, Bet v 1 homologs, class 1 chitinases, Lipid Transfer Proteins (LTPs) and pathogenesis-related proteins (PRs) occurring in vegetables, fruit and plant pollens [Jędrychowski, 2001].

The need of providing health safety to allergic patients necessitates educational activities, in-depth knowledge on a wide spectrum of allergens as well as correct labeling of food products [Commission Directive 2006/142, 2006].

ALLERGENS OF PLANT ORIGIN

Plants, vegetables, fruit and seeds are a source of a number of valuable nutrients and health-promoting components on the one hand and a vast number of allergens with diversified biological and functional properties on the other.

The knowledge on allergens as well as their data bases are subject to ceaseless and dynamic development and extension. Currently, data referring to allergen data base "AllFam" point to 831 defined allergens classified to 182 protein families. The latest studies have brought a rapid increase in the number

of isolated and characterized plant allergens [Breiteneder & Mills, 2005; Jenkins *et al.*, 2005; Mills *et al.,* 2003]. A total of 357 allergens of plant origin have been identified thus far. They have been divided into 58 families based on their functional properties and knowledge of their structure. Proteins are included into the same family once they contain at least 30% of identical amino acid residues or have lower sequential affinity but their functions and spatial structures are similar. The most widespread groups of plant proteins containing allergens are cupin superfamily and prolamin superfamily (Table 1) and families of pathogenesis-related proteins – PRs (Table 2).

Peanuts and other grain legume seeds

Amongst plant products the strongest allergenic properties have been reported for peanuts and nuts. In the United States, it is estimated that 1 to 2% of the population is allergic to peanuts, tree nuts (walnut, Brazil nut, cashew, hazelnut), or both [Fleisher *et al.,* 2005; Sicherer *et al.*, 2001; 2003]. Investigations prove that *ca.* 30,000 Americans are hospitalized due to symptoms of anaphylactic shock after ingestion of peanuts. Unfortunately, from 160 to 200 of cases end with death. Allergic reaction to peanuts may be instantaneous (anaphylactic shock) or may be manifested already after a few hours. In most cases allergic reactions appear after consumption of peanuts, but less frequently may also be induced by saliva (kissing, utensils) [Maloney *et al.,* 2006] or allergen inhalation [Kilanowski *et al.*, 2006]. Peanut (*Arachis hypogea*) belongs to the Papilionacea family of the Fabales order which also includes pea, bean, soybean, lupine, chickpeas and lentil. In seeds of peanuts there have been identified 10 allergens, the major ones being Ara h 1 (conarachin) and Ara h 2 (2S albumin). These two allergens are the cause of 95% of peanut allergy reactions [Long, 2002]. The other five proteins are associated with only 50% of peanut allergic responses [Maleki *et al*., 2003]. The minor peanut allergens are arachin (legumin) – Ara h 3 and Ara h 4, profilin – Ara h 5, 2S albumins – Ara h 6 and Ara h 7, oleosin, agglutinin and Ara h 8 (Bet v 1 family).

TABLE 1. Allergens from the cupin and prolamin superfamilies [Breitender & Radauer, 2004].

Protein family	Examples
Cupin superfamily	
Vicilins	Ara h 1 (peanut), Jug r 2 (walnut)
Legumins	Ara h 3/4 (peanut), Cor a 9 (hazelnut)
Prolamin superfamily	
2S albumins	Ber e 1 (Brazil nut), Ses I 2 (sesame)
nsLTPs	Pru p 3 (peach), Cor a 8 (hazelnut)
Cereal α – amylase/ protease inhibitors	Rice diametric α-amylase inhibitor
Cereal prolamins	Tri a 19 (wheat), Sec c 20 (rye)

TABLE 2. Allergens from the plant defense system [Breitender & Radauer, 2004].

Protein family	Examples
PRs	
PR-2: endo-β1, 3-glucanases	Banana glucanase
PR-3: class 1 chitinases	Pers a 1 (avocado), Cas s 5 (chestnut)
PR-4: Win-like proteins	Bra r 2 (turnip)
PR-5: TLPs	Pru av 2 (cherry), Mal d 2 (apple)
PR-9: peroxidases	Tri a Bd 36K (wheat)
PR-10: intracellular PR-proteins	Api g 1 (celery), Mal d 1 (apple)
PR-14: nsLTPs	See Table 1
Proteases	
Papain-like cysteine proteases	Act c 1 (kiwi), Gly m Bd 30K (soybean)
Subtilisin-like serine proteases	Cuc m 1 (melon)
Protease inhibitors	
Kunitz-type protease inhibitors	Soybean trypsin inhibitor
Cereal α-amylase/ protease inhibitors	See Table 1

The major peanut allergen Ara h 1 (also known as conarachin) is a 65-kDa glycoprotein with an acidic isoelectric point. It is a homotrimeric protein belonging to the cupin superfamily that includes structurally related proteins – vicilins. The modelled vicilin monomers consist of a cupin motif made of two tandemly arrayed modules related by a pseudo-dyad axis. Each module consists of a β-barrel core domain built from two walls of antiparallel a-sheet associated to a loop domain which predominantly contains a-helices. An a-helix-containing linker region interconnects the two modules [Barre *et al*., 2005b]. Hydrophobic interactions were determined to be the main molecular force holding monomers together. Hydrophobic amino acids that contribute to trimer formation are at the distal ends of the three dimensional structure where monomer-monomer contacts occur. The majority of the IgE-binding epitopes are also located in this region, suggesting that they may be protected from digestion by the monomer-monomer contacts. On incubation of Ara h 1 with digestive enzymes, various protease-resistant fragments containing IgE-binding sites were identified. The highly stable nature of the Ara h 1 trimer, the presence of digestion-resistant fragments, and the strategic location of the IgE-binding epitopes indicated that the quaternary structure of a protein may play a significant role in overall allergenicity [Shin *at al*., 1998; Maleki *et al*., 2000; Burks *et al*., 1997], using pooled serum IgE from a population of peanut-hypersensitive individuals, mapped 23 linear IgE binding epitopes of Ara h 1.

Seeds of other legumes contain also allergens belonging to vicillin proteins [Martínez *et al.,* 2000; Mills *et al*., 2002]. An allergen called Len c 1 was identified from lentil [López-Torrejón *et al.*, 2003], whereas allergenic fractions described as Pis s 1 and Pis s 2 – from pea [Sánchez-Monge *et al.*, 2004]. Burks *et al.* [1995] demonstrated that in the amino acid sequence of vicillin proteins of pea and peanuts (Ara h1) the affinity reaches 60-65%, which may be the basis of cross-reactivity between those seeds. It is difficult to find undeniable proofs for the occurrence of clinical cross-reactivity between seeds of grain legumes. In soybean the major allergens are glycinin (11S cupin), vicillin protein referred to as Gly m Bd 28k and cysteine protease Gly m Bd 30k sometimes described as allergen P34. Glycinin belongs to 11S seed storage globulin. It is an oligomer with a molecular weight of *ca.* 320 kDa, built as typical 11 S globulins from sub-units containing acidic and basic polypeptide chains. Most of the epitopes have been localized within acidic sub-units of glycinin that are exposed outside a molecule [Helm *et al.*, 2000].

Soybean glycinin displays high sequential affinity to allergenic proteins occurring in peanuts: Ara h 3 and Ara h 4, that have previously been described as two different allergens but currently are claimed to be one and the same allergen [Beardslee *et al.*, 2000; Xsiang *et al*., 2002]. Both the acidic and basic chains of each glycinin sub-unit have been found capable of binding IgE originating from patients with allergy to peanuts [Koppelman *et al.,* 2003].

Nuts

An important group of food allergens is constituted by nuts [Asero *et al.,* 2004]. The major hazelnut allergen is Bet-v-1-homologous protein (Cor a 1.04) with a molecular mass of 18 kDa. The epitopes of hazelnut Cor a 1.04 are less related to pollen Cor a 1 than to Bet v 1 from birch pollen [Lüttkopf *et al.*, 2001]. The 9 kDa allergen hazelnut (Cor a 8) is presumably an LTP. Other major allergens are proteins with molecular weights of 47, 32 and 35 kDa [Pastorello *et al*., 2002]. The isoform of Cor a 1, Cor a 1.0401 is responsible for the majority of hazelnut allergies [Besler *et al.*, 2001]. Cor a 1.04 is thermolabile and rapidly denatures after heating. Vieths *et al.* [1998], Schocker *et al*. [2000] and Hansen *et al*. [2003] reported that roasting nuts at 140°C for 40 min did not completely remove IgE binding but reduced it by a factor of 100. This allergen is susceptible to the activity of proteases and readily undergoes pepsin hydrolysis [Vieths *et al.,* 1999; Akkerdaas *et al.,* 2000].

Major walnut and Brazil nut allergens are 2S – albumins, Jug r 1 and Ber e 1 (9 kDa), respectively. They exhibit a 46.1% identity. The 2S family of seed storage albumin proteins have four disulfide bridges, they tend to be relatively resistant to denaturation and proteolysis. Allergens of that groups occur also in cashew (Ana o 3) [Robotham *et al*., 2005] or peanuts (Ara h 2, 6 and 7) [Barre *et al.*, 2005a]. Recently it has been demonstrated that Ara h 2 possesses epitopes binding IgE antibodies identical with those occurring in proteins of almonds and Brazilian nuts, which may explain a high degree of susceptibility to nuts in patients with allergy to peanuts [De Leon *et al*., 2007]. In addition, the 2S allergenic storage proteins are likely to be responsible for the occurrence of cross-reactivity between nuts and sesame seeds. The latter contain *ca*. 20% of protein, of which almost one fourth is constituted by 2S albumins. It has been demonstrated that the amino acid sequence of sesame allergen Ses i 2 (albumin 2S) exhibits 38% affinity to that of walnut allergen (Jug r 1), 40% affinity to that of Bre e 1 (allergen of Brazilian nuts) and 34% affinity to that of Ara h 2 from peanuts [Beyer *et al*., 2002].

Wheat

The key allergens of cereals belong to the family of prolamines. The latter are the major storage proteins of most of cereals (except for oat and rice) and are characterised by a high content of proline and glutamines. The highest allergenic activity has been reported for low-molecular glutenins of wheat, *i.e.* α-gliadins and γ-gliadins. ω-5Gliadin has been described as the major wheat allergen referred to as Tri a 19. It is extremely significant in inducing food allergy in small children. This allergen is a protein with a molecular weight of *ca*. 65 kDa, possessing 7 epitopes capable of reacting with antibodies of patients manifesting allergy to wheat. It may be a causative agent of an anaphylactic reaction after ingestion of wheat flour by especially sensitive persons. It has also been demonstrated that wheat ω-5gliadin cross-reacts with γ-70 and γ-35 secalins of rice (Sec c 20) as well as with γ-3 hordein from barley (Hor v 21) [Palosuo *et al*., 2001]. That allergen is characterised by high stability and has been confirmed to maintain its allergenic activity even after baking of bread or cooking of pasta [Varjonen *et al*., 1996].

Barley and its derivatives

The area of barley cultivation (*Hordeum sativum)* is not large (10% of cereals crop size), yet – due to its extensive application in the food industry (groats industry, brewery industry) and as a feed component, it may pose allergenic risk to allergic patients.

In Poland, barley is most commonly applied for the production of brewery and distillery malt. Barley groats constitute *ca*. 70% of the total groats produced on the Polish market.

According to the most up-to-date data of the Allergme data base, 21 allergens have been identified in barley (including forms of isoallergens and various sites of allergen occurrence *e.g.* Hor v – in pollen, bran and seeds). Barley allergens detected so far include: Hor v (occurring in seeds, pollen and bran), Hor v 1, Hor v 12, Hor v 12.0101, Hor v 13, Hor v 15, Hor v 15.0101, Hor v 16, Hor v 16.0101, Hor v 17, Hor v 17.0101, Hor v 2, Hor v 21, Hor v 21.0101, Hor v 4, Hor v 5, Hor v 5.0101, Hor v LTP, and Hor v Z4. Some of these allergens are characterised by high thermal stability and resistance to proteolysis upon proteolytic enzymes of the gastrointestinal tract. The aforementioned characteristics determined by the structure stabilized, among others, by disulfide bonds cause that also beer – being a highly-processed product of barely and containing allergenic proteins with molecular weights of 9 kDa (most likely LTP), 14 kDa and 43 kDa – may induce food allergies with severe pathological symptoms, including the anaphylactic response [Gorinstein *et al*., 1999; Garcia-Casado *et al*., 2001].

Fruits and vegetables

Fruits and vegetables have been identified to posses a number of allergens that play various biological functions. The most significant of them are referred to as "panallergens". They are, usually, low-molecular-weight compounds widely distributed in nature. They occur in a number of plant cells and others. Panallergens exhibit a high degree of structural affinity between one another and owing to this are the cause of strong cross-reactivity between numerous food products and tree pollens, grass pollens or latex. Panallergens include such groups of compounds as profilins, namely proteins linked with pathogenesis, or non-specific lipid transport proteins (nsLTP) [Pastorello *et al.,* 2004]. *Ca.* 62.5% of patients with food allergy display positive responses to panallergens [Ricci *et al.*, 2005].

Profilins are proteins with molecular weights of 12–15 kDa that participate in the process of actins binding in eucaryotic cells and in signal transduction on the pathway of photosynthesis. Profilin sequences of various plant species have been shown to contain as much as 70-85% of identical amino acid

residues. The key profilins inducing allergy are those which cross-react with an allergen of birch pollen (Bet v 2). In addition, they include allergens isolated from pear (Pyr c 4), cherry (Pru av 4), peach and hazelnuts (Cor a 2). Celery, banana and melon contain profilins that induce allergic reactions in patients with allergy to pollens and weeds. Profilins are relatively susceptible to heat denaturation and digestion and are usually the causative agent of the so-called Oral Allergy Syndrome (OAS) linked with the consumption of raw foodstuffs.

In contrast, the non-specific lipid transfer proteins (nsLTP) are highly resistant to proteolysis, a rapid change of pH or thermal treatment. They have been demonstrated to be capable of returning to their native form after product's chilling. Those properties of nsLTP result from their stable structure [Breiteneder & Radauer, 2004]. The nsLTP cover momoner proteins with a molecular weight of 7-9 kDa, that are linked with four disulfide bonds and constitute a form of a tunnel with hydrophobic properties. The nsLTP are major allergens of peach (Pru p 3), apples (Mal d 3), apricot (Pru ar 3), sweet cherry (Pru av 3), plum (Pru d 3), and grapefruit (Vit v 1). Although allergic reactions to lettuce are not very often, its nsLTP was the cause of an anaphylactic shock in sensitive patients and ever since has been referred to as Lac s 1 [Borges *et al.,* 2006; Breiteneder & Radauer, 2004; Fernández-Rivas *et al.*, 2006; Pastorello *et al.*, 2004].

The non-specific lipid transport proteins are usually accumulated under the skin, and that fact is usually used to explain high allergenicity of skins as compared to flesh of some fruits, especially those of the family *Rosaceae* (apple, peach, apricot, plum). Up-to-day literature indicates, however, that explicit determination of that fact is difficult. Still, persons with severe food allergy are recommended to consume peeled fruits. In addition, it is worth remembering that contents of allergens in particular varieties of fruits (apples in particular) may differ to a considerable extent [Borges *et al.,* 2006; Fernández-Rivas *et al.,* 2006].

Proteins of the defense system of plants enable their resistance to biotic and abiotic stress. They include pathogenesis--related proteins (PRs), some proteases and protease inhibitors. The pathogenesis-related proteins represent a heterogenous collection of a few families of proteins (Table 2), amongst which the strongest allergenic properties have been attributed to proteins being homologs of the major birch allergen (Bet v 1), belonging to the PR-10 family, I class chitinases (PR-3 family) or the previously-described lipid transport proteins (PR-14 family) [Asensio *et al.*, 2004]. The PR proteins are responsible for the syndrome of cross reactivity between fruits and pollens (Oral Allergy Syndrome) and between fruits and latex (latex-fruit syndrome) [Cudowska & Kaczmarski, 2003; Fernández-Rivas *et al.,* 2006]. The major allergens cross reacting with a latex allergen – hevein (Hev b 6.02) – belong to the PR-3 family that encompasses I class chitinases and occur in banana, kiwi, avocado, chestnuts, apricots and grapes. In turn, the OAS (itching, swelling and numbness of mouth, lips or throat, and sometimes rush or paroxysmal erythema around mouth) is induced by fruits of the family *Rosaceae* (apple, apricot, peach) and vegetables (celery, carrot) that cross react with allergens occurring in birch pollen, *i.e.* Bet v 1 (or sometimes with Bet v 2 homologous to profilins) [Ricci *et al*., 2005]. Allergens of that family occur, *e.g.* in cherry (Pru av 1), celery (Api g 1), and apple (Mad d 1).

Fruits contain also a vast group of allergens with the activity of proteases and protease inhibitors. Cystein proteinases (of papain type) are enzymes built of two domains, one predominated by the α-helix structure and the other by a cylindrical structure (β-barrel), that are stabilized by disulfide bonds. The name of that group derives from papain occurring in papaya. Similar proteases have been identified in other fruits: bromelain in pineapple, actinidin in kiwi and phycin in figs. Actinidin (Act c 1) is the major allergen of kiwi with a molecular weight of *ca*. 30 kDa, that constitutes nearly 50% of the total soluble protein of those fruits. Its significance as an allergen may be indicated by the fact that as much as 90% of patients allergic to kiwi responds exactly to that allergen. In addition, IgE of patients with allergy to kiwi fruits binds papain from papaya with bromelain from pineapple [Cuesta--Herranz *et al*., 2003].

Out of the serine proteinases (of subtilysine type) exhibiting allergenic properties only cucumisin from melon (Cuc m 1 allergen) has been described in detail so far. It is a thermally--stable protein with a molecular mass of *ca*. 67 kDa [Breiteneder & Radauer, 2004]. Significant allergens of that group are protease inhibitors, proteins with a molecular weight of *ca*. 20 kDa, occurring in potato and classified as Sola t 2, 3 and 4 [Seppälä *et al*., 2001].

ALLERGENS OF ANIMAL ORIGIN

Cow's milk allergens

Milk allergy is an adverse reaction to proteins that are present in milk. It is one of the most common food allergies especially in early childhood. During the first 3 years most children outgrow from cow's milk allergy (CMA), nevertheless about 15% remain allergic. CMA is manifested as a IgE-mediated (I-st type of allergic reaction) or delayed reaction, according to IV-th type of allergic reaction. Milk of ruminant species (cow, goat and ewe) contains almost the same or very homologous proteins which share the same structural, functional, biochemical and immunological properties. Experts of two independent European organizations, *i.e* the European Society of Pediatric Allergy and Clinical Immunology (ESPACI) and the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), are of the opinion on prohibiting the use of milk of animal species other than cow as well as the so--called "partly" hydrolysed formula in the case of allergic reactions [Høst *et al.,* 1999].

Cow's milk (CM) contains proteins that are antigenic and capable of inducing immune responses and sensitivity. The most abundant proteins, like β-lactoglobulin (β-lg), α-lactalbumin (α-la), and casein (CN), are the major CM allergens, but also the proteins that are present in low quantities, *e.g.* bovine serum albumin (BSA), lactoferrin (LF) and immunoglobulins, can induce milk allergy.

CM contains 3-3.5% of protein divided into two main groups: caseins (80%) and whey proteins (20%). Whey proteins remains soluble in milk serum after acidic precipitation

of caseins at pH 4.6 as a coagulum. The allergic and physicochemical properties of those two classes of cow milk proteins are different.

Caseins (CN) are subdivided into subclasses: α_s1-, α_s2-, β-, κ- and γ-casein named Bos d 8 as an allergen [Bernard *et al*, 1998]. The central section of casein micelles is of hydrophobic character whereas its peripheral part is constituted by hydrophilic components with exposed C-terminus fragments of κ-casein particles. Particular casein fractions display diversified I-order structure and are characterized by various functional properties, α_S1-, α_S2-, and β-casein – unlike κ-casein – are susceptible to the action of calcium ions. A molecule of casein is resistant to the action of high temperatures but susceptible to the presence of proteinases and exopeptidases. It readily undergoes proteolytic hydrolysis in the course of digestive processes. Patients with allergy to casein are usually allergic to all four fractions of that protein. Immunological defense of a body, manifested by the production of IgE anti-casein antibodies, is linked with the phenomenon of cross reactions between epitopes of individual casein fractions. It has been demonstrated that one of the epitopes with immunoreactive potential and resistant to digestive processes is the phosphorylation site occurring both within α_S1, α_S2, as well as β-casein.

Homologies in amino acidic milk protein composition of different animal species could justify the cross-reactivity observed between individual proteins. Also the phylogenetic difference between animals could be responsible for the failed recognition of camel proteins by circulating IgEs in patients allergic to cow's milk. In our study we concluded that the affinity between goat proteins and cow α-la and β-lg was 66% and 7.2%, respectively.

Whey proteins

The strongest allergens amongst whey proteins are β-lactoglobulin (β-lg – Bos d 5) and α-lactalbumin (α-la – Bos d 4).

The β-lg protein belongs to a superfamily of lipocalins as it demonstrates capability for binding retinol, β-carotene, saturated and unsaturated fatty acids, and aliphatic bicarbonates. In cow's milk it occurs in the form of dimers with a molecular weight of 36 kDa and constitutes *ca.* 50% of the whey proteins fraction. It is represented by two genetic form: A and B, differing in mutations at positions 64 and 118 of amino acid. The A form contains aspartame acid and valine whereas the B form – glycine and alanine. Each of its molecules contain 2 disulfide bridges and 3 free cysteine groups. Such a structure enables its interactions with casein during thermal treatments. Guyomarc'h *et al.* [2003] reported that large micellar aggregates (4×10^6 Da) are formed on heating milk which contains 3:1 ratios of β-lactoglobulin: α-lactalbumin together with κ-casein and α_S2 casein. The β-lg is relatively resistant to acid hydrolysis and activity of proteases, hence its major part remains undigested during transport through the gastrointestinal tract. A highly advantageous phenomenon is the possibility of inducing organism's tolerance by administration of low doses of an antigen in the form of *e.g.* selected hydrolyzed fractions of β-lg. A partly hydrolyzed product has been demonstrated to be capable of inducing organism's tolerance, whereas hydrolyzates with a high degree of hydrolysis (built of amino acid) not to posses such properties [Isolauri *et al*., 1995].

The α-la protein is a monomeric globular whey protein composed of 123 amino acid residues with 4 disulfide bridges with a molecular weight of 14.4 kDa. It exhibits strong affinity for binding calcium ions that are capable of stabilizing the II-order structure of protein. α-Lactalbumin's folded structure is destabilized at low pH with the formation of a molten globule [Redfield, 2004]. The stability to denaturation is also strongly reduced by reduction of the disulphides [Chang, 2004]. Disulphide exchange can occur during thermal denaturation, leading to the formation of aggregates [Livney *et al.,* 2003]. The α-la is a constituent of an enzymatic system of galactoside transferase responsible for the process of lactose synthesis. In terms of structure, α-la derived from cow's milk shows high affinity to α-la of human origin. Investigations carried out with animals have demonstrated that the most active antigenic region is an amino acid loop with a disulfide bridge (60-80):S-S:(91-96).

Egg allergens

Eggs of hens (*Gallus domesticus*) are the second, after cow's milk, xenogenic food introduced into a child's diet. Studies have shown that the major allergenic proteins of hen egg white are: ovotransferrin (53%), ovomucoid (38%), ovoalbumin (32%) and lysozyme (15%). Amongst proteins occurring in egg yolk worthy of notice is α-livitin that may induce allergic reactions through airways [Poulsen *et al.,* 2001].

Ovomucoid (Gal d 1) is a glycoprotein with a molecular weight of 28 kDa. It is built of 186 amino acid residues and has been demonstrated to be resistant to high temperature (100°C/30 min) [Hirose *et al.,* 2004] and other denaturating factors.

Ovoalbumin (Gal d 2) is a monomeric phosphoglycoprotein with a molecular weight of *ca*. 43÷45 kDa, built of 385 amino acids, belonging to the family of serpins. Three ovoalbumin fractions have been isolated, namely A_1, A_2, and A_3, that differ in the number of phosphate groups in a molecule. Usually, 100% of examined patients allergic to hen eggs display a positive reaction to the presence of ovoalbumin [Langeland, 1983a,b]. Ovoalbumin is resistant to the action of simulated digestive and intestinal juice, and preliminary denaturation with high temperature results in its increased proteolytic stability [Takagi *et al.,* 2003].

Ovotransferrin (conalbumin, Gal d 3) is a protein with a molecular weight of 77 kDa, built of 686 amino acids. It is characterised by antibacterial properties and capability for binding iron ions. Investigations of Langeland [1983b] proved that out of 68 examined sera of patients allergic to egg allergen, 48 had positive reactions to ovomucoid and 35 – to ovotransferrin.

Lysozyme (Gal d 4) is a protein with a molecular weight of 14.3 kDa, built of 129 amino acid its single polypeptide chain is linked with four disulfide bridges. It is a protein with a week allergenic potential, yet data have emerged on its capability to induce occupational inhalatory allergy as a result of inhalation.

Apovitelin (Gal d 5) is an allergen isolated from a lipoprotein fraction of hen egg yolk. It has been shown that apovietlin

I (di- or tetramer with 9 kDa sub-units) and apovitelin IV with a high molecular weight (170 kDa) may pose major allergenic risk to some patients [Walsh *et al*., 1988].

Fish allergens

Fish may be the major cause of IgE-dependent allergic reaction. In the USA cases of allergy to fish occur in 0.4% of the population, whereas in countries with a high intake of fish (Norway, Japan) that percentage is remarkably higher. Its immunodominating allergen is thermostable parvalbumin (Gad c 1), *i.e.* one of the key proteins of fish muscles. That allergen was first isolated from muscle tissue of codfish, though its presence has also been confirmed in muscles of other fish (carp or pike) or reptiles. Allergenic properties of Gad c 1 are not subject to a reduction even upon longer action of high temperature. The molecular mass of Gad c 1 allergen reaches 12.3 kDa. In terms of chemical structure, it is built of 113 amino acid residues and one glucose molecule. At least 5 sites of IgE binding have been detected in the structure of Gad c 1n [Elsayed & Apold, 1983]. Trypsin hydrolysis enabled demonstrating a highly active site with allergenic character (AA 33-44) and another site with a weaker potential (AA 88-96).

Allergy cases have also been noted as induced by the intake of fish infected with a parasite *Anisakis simplex* of the class of nematodes (*Nematoda*). Allergy may be induced by the contact of parasite larvae characterised by a relatively short life span but strong allergenic potential. Most likely, only a live larvae is capable of inducing allergy symptoms. It has been demonstrated that ingestion of a lyophylizate of over 100 larvae did not elicit clinical symptoms in patients with earlier diagnosed allergy to *Anisakis simplex* [Danek & Rogala, 2005].

Crustacea allergens

The consumption of shrimps is quite common world wide, *e.g.* in the United States of America there are differentiated *ca.* 30 edible species of crustacea, including shrimps, crabs, spiny lobsters and crayfish. Daul *et al.* [1994] isolated allergens Pen a 1 and Pen i 1 with a molecular mass of 36 kDa from an extract obtained from two species of shrimps: *Panaeus aztecus* and *Panaeus indicus*. Shrimp tropomyosin has been found to be their allergenic component. It has been estimated that the intake of 1÷2 medium size shrimps may stimulate an anaphylactic response in allergic patients. Allergies to crab allergens have mainly been observed in the environment of persons occupationally related with the processing of crab meat. The IgE-dependent reactions are mainly linked with extracts obtained during the cooking of crabs and not during the contact with unprocessed raw material. A protein with a molecular weight of 37–42 kDa has been isolated from an extract obtained after thermal treatment of crabs or from an extract of cooked meat. Tropomyosin is also the major allergen of spiny lobster (*Panulirus stimpsoni*) – Pan s 1, American lobster (*Homarus americanus)* – Hom a 1, and crab (*Chabrydis feriatus)* – Ch f 1. All those proteins have been cloned and analyses of their sequences enabled concluding that they show structural affinity to a shrimp allergen Pen a 1 [Leung *et al*., 1998; Stanley & Bannon, 1999].

Allergens of edible frogs and snails

In some countries, allergy risk may also be posed by the consumption of frogs or snails.

Allergy to protein of meat originating from edible frog (*Rana esculenta*) is restricted to cases of hypersensitivity observed in persons consuming frogs' legs, often as a result of earlier allergy to fish protein. Two major protein allergens have been isolated from frog muscles, *i.e.* Ran e 1 (α-parvoalbumin) and Ran e 2 (β-parvoalbumin) [Bugajska-Schretter *et al*., 1998].

Allergy to meat of such molluscs as snails (*Helix aspersa*) is usually accompanied by more mild symptoms in the formal of the oral allergy syndrome (OAS), nettle rash or fever, yet some cases of the anaphylactic shock have been reported as well. A predominating allergen isolated from muscles of snail is tropomyosin (Hel as 1) with a molecular weight of 36 kDa. Persons allergic to snail protein are also observed to manifest allergy responses to protein of shrimp (*i.e.* tropomyosin being an allergen of both these species) and to house dust [Sidenius *et al.,* 2001].

TECHNOLOGICAL POSSIBILITIES OF FOOD ALLERGENS REDUCTION

Food allergies are induced by antigens of foodstuffs being their natural constituents or additives applied deliberately to improve taste, aroma and consistency of a finished product. They may appear in food upon technological treatments (thermal, enzymatic and chemical ones) that result in the generation of neoallergens. Enzymatic preparations, preserving agents and contaminants, e.g. microbiological contaminants and metabolites of microorganisms, may also pose allergenic risk [Sicherer *et al.*, 2003].

The processes applied are tended reduce levels of allergens in a finished product. The most important ones are those based on removing epitopes responsible for allergenic reactions (applied mainly in the technological processing of fruit juices, *e.g.* ultrafiltration or extraction), destroying epitopes through physical processes and agents, chemical or enzymatic modifying epitopes, masking epitopes by using cross-linking enzymes (*e.g.* transglutaminase) or using genetic engineering with elements of genomics and proteomics [Soler-Rivas & Wichers, 2001].

Within the food production process, with respect to its safety in the context of possible allergenic reactions, all stages referring to environmental factors are significant, *i.e*. all links of the alimentary chain from producing materials on farms through storing, processing, distribution to home processing. At each of these stages biological properties of nutrients can be altered, also those responsible for inducing allergic responses (Figure 1).

New technologies such as genetic engineering (the source of new antigenically different materials), irradiation of food, high-pressure technologies, extrusion and modified-atmosphere packaging can all improve food production and food safety. However, the potential risks associated with their application should be objectively and rigorously assessed well before these technologies are widely introduced. The effect of technological processes, especially those involving high

temperature, high pressure and microwaves on the immunogenic properties of proteins, seems unquestionable [Soler-Rivas & Wichers, 2001; Davis *et al.*, 2001; Szymkiewicz & Jędrychowski, 2002; Wróblewska & Jędrychowski, 2002; Mierzejewska & Kubicka, 2003]. Changes within both antigens themselves and the matrices in which they are localised may definitely differentiate their biological properties, biological activity, immunoreactivity, antigenicity as well as allergenicity. Antigen changes during technological processes (mainly protein-saccharides bonds) may be significant enough for some sensitizing agents to be destroyed or inactivated. There are also the possibilities of forming new antigens and allergens (neoantigens, neoallergens) during technological processes.

During technological processes various compounds can be separated, added or combined. In this context health threats resulting from possible food contamination, even in minimum amounts (detection limit of the assay – 1 ppm) during the production process gain in significance [Hourihane, 2001]. This concerns mainly products posing high allergenic risk, such as peanut proteins, during processing of which it is significant whether a given product was manufactured on "shared equipment" or "in a facility that also processes peanut" [Hourihane *et al.*, 2000]. Thus, allergenic threat may be also caused by products obtained from such materials as oils which can contain trace amounts of this allergen [Hourihane *et al.*, 1997].

As the immune system is involved in producing allergic reactions also the changes proceeding during culinary processing, digesting and absorbing nutrients in the organism as well as internal factors determining intercellular transmission are significant. Some possibilities of extending studies in this field are provided by gut perfusion techniques (on animal models) or by using complex computerised systems of bioreactors imitating digestive processes in the alimentary tract of monogastric animals *in vitro*. In this aspect, the problem of food allergies is highly complex; moreover, the degree of complexity increases when several (4-6) kinds of mechanisms according to which such specific reaction of the immune system can be triggered are taken into account.

At reducing allergenic threats caused by foods a significant position is held by technological solutions meant to modify, reduce or eliminate immunoreactive or allergenic properties [Soler-Rivas & Wichers, 2001].

CONCLUSIONS

Currently the key problems linked with the incidence of food allergies are still: hidden allergens whose occurrence results from the use of food additives as well as food contaminations and infections, and widely distributed allergens linked with universal functions in respective tissues (enzymes, lipid transfer proteins – LTP, pathogenesis related proteins – PR-proteins).

Newly encountered problems referring to allergic reactions are: globalism in the area of international trade, wide commodity exchange and merging of cultures (greater consumption of new foodstuffs), implementation of new technologies in the cultivation and processing of food products, *e.g.* GMO, high-pressure technologies, products with modified functional properties, and formation of neoallergens.

On the global scale, a need may be observed for the establishing of computer data bases of allergenic cross reactions linking health risk posed to consumers and the occurrence of other types of allergies (inhalatory and contact ones).

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