Peanut allergens

Received for publication, September 28, 2009
Accepted, October 05, 2010

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Abstract
Peanut allergies represent a threat for sensitive people in worldwide with an increasing prevalence especially in USA and Europe. Thus to know the allergenic factors is very important. A lot of studies were focused on the eleven peanut allergens established until now. The most studied allergens are Ara h, Ara h 2, Ara h 3 and Ara h 6 for which are set a series of structural, biochemical and functional data. Some of the other allergens are only identified or a very few characterized (Ara h 4, Ara h 7, Ara h 10, Ara h 11) and require extensive studies to determine their exact structure and properties. The most recently identified and partially characterized peanut allergen, Ara h 9, up by its very own properties a series of questions waiting to be solved. For this one and isoallergen Ara h 7.0201 even their level of allergenicity is subject of controversy. In addition the cross reactivity of the different allergens arise numerous as well.

Keywords: peanut, allergens, structure, biochemical properties

Introduction
At the beginning of the Third millennium, many health problems are associated with allergic manifestations. Allergic reactions to food range from digestive symptoms such as diarrhea, vomiting and abdominal pain, asthma, ear and nose infections, skin problems, anaphylactic shock and in the most severe cases death.

30 years ago, we barely mentioned about allergy but now this one represents a new enemy of the modern society. The term “allergy” was introduced in 1906 by Clemens P. Pirquet to describe both protective immunity and hypersensitivity reactions [1]. Over time the term is used for adverse reactions to an irritant that the body perceives as harmful. The allergy symptoms are wide and depend of patient from varying degrees of drippy, stuffy, achy nose, crusty or itchy until the worst, death.

The body’s reaction to food that does not involve the immune system is a food intolerance caused by some physiologic characteristics of the host such as metabolic disorders (for example lactose intolerance caused by lactase deficiency).

Food allergies are caused by more than 170 foods, but for 90% severe allergies are responsible milk, eggs, peanuts and nuts, selfish, cereals containing gluten, soybean, celery, mustard, sesame, lupin, molluscs and products thereof. Children outgrow most food allergies but peanuts, tree nuts, fish and shellfish allergies are life long and represent together other nuts the cause for 90% of lethal anaphylactic food reactions.

To protect those people who have food allergies and to protect health of citizens the European Commission has developed legislation which mandates clear labelling of specific food allergens [2-9]. The potential allergenic ingredient are listed in Annex IIIa of the Labelling Directive and are under periodical re-evaluation and is, when necessary, updated on the basis of the most recent scientific findings.

There is currently no treatment for food allergies. The only proved method to prevent a reaction is strict avoidance. In this context the people with food allergies must carefully read food labels and ask about the food ingredients in a restaurant, at a friend’s house, party or other.
The avoidance is often not possible because of hidden exposures and the consumers with food allergies can react to very small amounts of the offending food contaminating another food. The thresholds for different allergens and/or people are not precisely established yet.

The commonly treatment to relieve symptoms of oral allergy in emergency is administration of epinephrine, adrenalin, antihistamine and steroid and stabilization of airway, ventilatory and circulatory function.

The mainly causative agents of food allergy are proteins, glycoprotein and peptides. In this context the identification, characterisation and quantification of allergens play an important role concerning the impact of those on at least 2% adults and 6-8% children health [10-16].

The influence of processing treatments on the allergens was wide studied but it seems that any treatment doesn’t eliminate the allergenicity [17-19].

Some studies showed that the allergenicity of some food can be reduced using enzymes or different technologies like fermentation, even it is a strong allergen like Ara h 1 [20-24].

Nevertheless until now the only way is to avoid the offending allergen even if there are a lot of immunotherapy studies or replacement crops at present with some hypoallergenic plants [25].

Food allergens are grouped in two classes: class I when the adverse reactions appear when allergen is present in the gastrointestinal tract and class II when allergic reactions appear to inhalant allergens.

**Short characterisation of peanut.**

Among highly allergenic foods for sensitive people are included peanut too. Peanut is also one of the foods listed in Annex IIIa of Commission European legislation as allergenic ingredient which is mandatory to be mentioned on food labels it contains or can contain.

The groundnuts or peanuts are originally South American, were they were grown by Indian communities. It was introduced to West-Africa by the Portuguese in the 16th century and to Europe from Africa from the year 1830. Peanut oil was originally used as a lubricant in industrializing Europe and then for cooking.

Because of their nutritive properties, taste and low cost, peanuts became a very large used legume after First World War in the food and confectionary industry.

Nowadays, at least for Americans, peanuts and peanut butter are one of favourite foods and can be found in around 75% of American homes. In China the consumption of peanuts is almost the same as in United States but while in US the peanut allergy has an important place in sensitive people, in China is practically no peanut allergy [26]. The diversity of food preparation has an important role in the prevalence of peanut allergy. It seems that the dry roasted peanuts are more allergic than boiled or fried peanuts but in the meantime cooking methods doesn’t explain why the peanut allergy prevalence is lower in China matched up to Europe and America [18, 19, 21, 27-31].

Prevalence to peanut allergy is increasing in the last years and peanut and tree nuts were responsible for the large fatalities due to allergies (94%) in the United States [15, 32-36]. Thereby peanut and nut allergens represent nowadays a challenge for health and food manufacturers both and the threat of an adverse reaction can be present for sensitive people everywhere in food.

Peanut (**Arachis hypogaea**) is a legume but between vegetables and nuts, peanuts contain bigger amount of protein (20-26%), 40-50% oil and a lot of valuable nutrients such as vitamins, minerals, antioxidants and Coenzyme Q10. Due to the high fat content peanut seeds are used like an excellent source of oil. Some of plant proteins properties contribute to their resistance to cooking and their resistance to break down during digestion. If these protein bind to lipid their resistance increase even more. In the last years oils and margarine consumption were associated with allergic sensitization [37] too.

Total protein content of peanut is represented up to 32 different proteins of which about 18, nearly 7-10%, have been identified as capable of binding specific IgE, so to be allergenic.
General properties of peanut allergens

Peanut allergens are class I allergens, are water-soluble glycoprotein and are stable to heat, acid and enzymatic digestion. Into Allergome database, at this time the most all-inclusive collection of allergen data and on protein family database, allergens were classified in protein families. In the last few years has been an explosion in the identification and sequencing of food allergens and among these of peanut allergens. So if in October 2006 were 10 listed allergenic proteins in peanut (agglutinin, Ara h1, Ara h1, Ara h2, Ara h3, Ara h4, Ara h5, Ara6, Ara h7, Ara h8, oleosin) in October 2009, 11 peanut allergens, named Ara h1 – Ara h11, have been identified, largely characterized and accepted by Allergen Nomenclature Subcommittee of the International Union of Immunological Societies (IUIS). The last updated information regarding peanut allergen nomenclature, peanut allergens identified, in October 2009 are presented in Table 1.

Table 1. Peanut allergens identified until October 2009 [38].

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Biochemical name</th>
<th>MW(SDS_PAGE), (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara h 1</td>
<td>Cupin (Vicillin-type, 7S globulin)</td>
<td>64,0</td>
</tr>
<tr>
<td>Ara h 2</td>
<td>Conglutin (2S albumin)</td>
<td>17,0</td>
</tr>
<tr>
<td>Ara h 3</td>
<td>Cupin (Legumin-type, 11S globulin, Glycinin)</td>
<td>60,0</td>
</tr>
<tr>
<td>Ara h 4</td>
<td>Cupin (Legumin-type, 11S, Glycinin)</td>
<td>37,0</td>
</tr>
<tr>
<td>Ara h 5</td>
<td>Profilin</td>
<td>15,0</td>
</tr>
<tr>
<td>Ara h 6</td>
<td>Conglutin (2S albumin)</td>
<td>15,0</td>
</tr>
<tr>
<td>Ara h 7</td>
<td>Conglutin (2S albumin)</td>
<td>15,0</td>
</tr>
<tr>
<td>Ara h 8</td>
<td>Pathogenesis-related protein, PR-10</td>
<td>17,0</td>
</tr>
<tr>
<td>Ara h 9</td>
<td>Nonspecific lipid-transfer protein 1</td>
<td>9,8</td>
</tr>
<tr>
<td>Ara h 10</td>
<td>16 kDa oleosin</td>
<td>16,0</td>
</tr>
<tr>
<td>Ara h 11</td>
<td>14 kDa oleosin</td>
<td>14,0</td>
</tr>
</tbody>
</table>

So far, it has been identified one isoallergen for each of Ara h 1 - Ara h 6 and Ara h 11 and by twos for each of Ara h 7 - Ara h10. Belong the global MW of allergen established by SDS-PAGE, were calculated as well the molecular weight for some isoallergens as: Ara h 7.0201–17,34 kDa, Ara h 8.0201–16,28 kDa, Ara h 9.0101–9,13 kDa and Ara h 9.0201–9,04 kDa.

So far for all peanut allergens mentioned above were established biochemical name and Molecular weight by SDS-PAGE.

Some of other properties described in Informall Database for peanut allergens are solved only partial. The main properties were solved until present for peanut allergens are presented in Table 2.

Table 2. Properties solved for peanut allergens up to October 2009

<table>
<thead>
<tr>
<th>Allergen/Isol allergen</th>
<th>Allergenicity</th>
<th>GenBank nucleotide</th>
<th>UniProt</th>
<th>Amino acid sequence</th>
<th>Sequence features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara h 1 /Ara h 1.0101</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Ara h 2 /Ara h 2.0101</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Ara h 3 /Ara h 3.0101</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Ara h 4 /Ara h 4.0101</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>NO</td>
</tr>
<tr>
<td>Ara h 5 /Ara h 5.0101</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>NO</td>
</tr>
<tr>
<td>Ara h 6 /Ara h 6.0101</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>partial</td>
</tr>
<tr>
<td>Ara h 7 /Ara h 7.0101</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>NO</td>
</tr>
<tr>
<td>Ara h 8 /Ara h 8.0101</td>
<td>NO</td>
<td>yes</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Ara h 8 /Ara h 8.0201</td>
<td>yes</td>
<td>yes</td>
<td>NO</td>
<td>yes</td>
<td>NO</td>
</tr>
<tr>
<td>Ara h 9 /Ara h 9.0101</td>
<td>NO</td>
<td>yes</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Ara h 9 /Ara h 9.0201</td>
<td>NO</td>
<td>yes</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Ara h 10 /Ara h 10.0101</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Ara h 10 /Ara h 10.0102</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Ara h 11 /Ara h 11.0101</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>
Peanut allergens are mainly seed storage proteins and form plant defence system proteins of the conglutin, vicilin and glycinin families.

In addition to their allergic properties the allergic peanut proteins have also different other properties for instance Ara h 6 is a trypsin inhibitor and the non-specific lipid transfer proteins (LTPs, 9 kDa, prolamin superfamily) seems to be responsible for high resistance to heat treatment and proteolytic digestion and an ideal candidate for cross-reactivity too. [28, 39-46].

The allergens identified so far Ara h 1, Ara h 2 and Ara h 3 are major allergens and the most peanut allergens characterized while others are considered minor allergens. Six of peanut allergens (Ara h 1 – Ara h 6) were thorough studied by the EU FP6 EuroPrevall programme and the results were published in different papers. These allergens were extracted by methods described later in their scientific papers and characterized. Thus using recombinant Ara h 8 in *Escherichia coli* and spectroscopic analyses were established amino acid sequences, details of the differences between isoforms and their generation by proteolytic processing within the seed. In addition for Ara h 1 – Ara h 3 were confirmed full sequence features and partial for Ara h 6.0101 [47].

**Ara h 1** is a major peanut allergen recognized by over 90% of peanut sensitive population and the most analyzed peanut allergen. Amino acid sequence presents high sequence similarity with other plant vicilins, member of the cupin superfamily. It is a highly stable glycoprotein homotrimer acidic complex, with mannose and complex N-glycans, consists of four domains: α helical bundle on one end, two sets of opposing antiparallel β sheets and α helical bundle on the opposite end which held together via hydrophobic interactions. Besides it is classified as conarachin because it was purified from the 40% to 85% fraction of ammonium sulphate saturation [48]. Its pI is 4.55. Ara h1 has 23 allergic epitopes of which 4 are immunodominant being recognized by more than 80% of patients. It is very abundant in peanut representing around of 12 - 16% of total protein. Ara h 1 has thermal stability and its allergenic properties are unaffected by thermal denaturation. It is resistant to proteolytic hydrolysis too [49-51].

Like Ara h 1, **Ara h 2** is a major peanut allergen being recognized by serum IgE from 90% of peanut allergic patients. Ara h 2 contains 10 epitopes of which 3 are immunodominant (epitopes 3, 6 and 7) and has a pI of 5.2. The protein structure of Ara h 2 is not enough described but it has been described to be a glycoprotein and that there are eight cysteine residues that could form up to four disulfide bonds which contribute to the overall structure, stability and allergenicity of the molecule. Ara h 2 has been shown to be resistant to acidic conditions and digestion with gastrointestinal tract enzymes and it demonstrated its trypsin inhibitor activity and this activity increases on roasting [28, 45, 52].

**Ara h 3** consists of a series of polypeptides ranging from approximately 14 to 45 kDa. These polypeptides are acidic and basic subunits. The molecular organization of Ara h 3 is typical to glycinin family and the amino acid sequence shows homology to 11S seed storage proteins. The Ara h 3 protein is synthesized as a single chain with a peptide bond between the N-terminal and C-terminal domains. In the mature stage this bond is cleaved and the native form at the maturity is a hexamer formed by a head-to-head association of two trimers. For this allergen were identified four epitopes on monomer, consisting of 10 – 15 amino acids, but with no obvious sequence motif shared by the peptides. From these epitopes only one is recognized by more than 35% allergic patients [53-55]. In addition because the epitopes are partially exposed on the surface of the native allergen it seems that these are recognized by the IgE antibodies after digestion followed by the allergen degradation. Consequently, it is necessary more information than more investigation in this field [56].

**Ara h 4** is considered as minor allergens even it is recognized by serum IgE from 53% of peanut allergic individuals. Ara h 3 and Ara h 4 sequences are 91% identical but were
named independently. Both Ara h 3 and Ara h 4 have a pI of 5.5. Normally these would be named as isoallergens [57, 58].

Ara h 5 is a plant profilin with a calculated pI of 4.6 and act as actin-binding proteins responsible for cytoskeleton formation in plant cells. It is a minor allergen but has a highly cross-reactivity with birch pollen, wheat and grass [58]. Ara h 5 is present only in low quantities in peanut and only 13% to 16% allergic peanut patients are sensitized to it.

Ara h 6 yields a protease resistant core on digestion with trypsin and chymotrypsin which has a native-like structure, contains five disulfides bonds and is homologous of Ara h 2 mainly in the middle part and C-terminal part of the protein. It has been demonstrated that at least parts of the Ara h 6 epitopes are cross-reactive with Ara h 2 epitopes although the first one is a minor allergen and the second one a major allergen [59, 60]. Also, even if in the database some allergens are present only in one form, the literature presents isoforms even for other peanut allergens such as Ara 2 or Ara 6 [61].

Ara h 7 seems to be the least studied allergen although in a study had been shown that from 40 peanut allergic patients it was recognized by 43% [57].

Ara h 8, unlike Ara h 1, Ara h 2, Ara h 3, Ara h 4, Ara h 6 and Ara h 7 which are seed storage proteins, is a member of the pathogenesis – related protein family PR – 10. Its pI was estimated at 5.03 and it has low stability to heat and proteolytic digestion. The peanut allergen Ara h 8 is homologous with birch pollen allergen Bev v 1 being important for birch pollen allergic patients because of its cross-reactivity [58, 62, 63].

Ara h 9 was clearly identified in 2009. In the last few years a large attention has been given peanut oil allergenicity because the allergenicity of refined peanut oils is not so clear [64-66]. Some studies showed the allergenicity, some not of edible peanut oils. However it is obviously that the refining process may affect the allergenicity and the thresholds for adverse reaction vary according to sensitive patient. Edible oils undergo usually extensive processing which removes virtually all the protein from the oil. Scientific studies showed that refined oils don’t contain allergic protein in detectable amounts [67]. But some studies showed that vegetable oils/fats, crude or even refined, can contain proteins - in peanut case peanut allergens - even that these were hot-pressed processed [68-70]. Usually by mechanical or cold press the allergic proteins are not removed in totality, have been considered impurities. These oils aren’t used domestically but are often found in healthy food, with increased nutritional value, or in gourmet food stores. Therefore the restaurants and food service facilities have to specify what kind of oil was used. In the meantime vegetable oils, and obviously the oil from peanut, are used for preparing margarine and spreads, and if oils used contained allergic protein the product would contain it too. According to the last researchers the refined oils don’t mean free from allergens.

In addition in the case of bottled peanut oil, a small quantity of unrefined oil may be mixed together with refined oil to endow with a peanut flavour and sealed as peanut oil. In addition other oleosins (18 kDa oleosin) has been suggested to be involved in allergic cross-reactivity to peanuts [43, 44].

Plants lipid transfer proteins (LTP) facilitate transfer of lipids between membranes in vitro, have various properties in common such as: molecular masses of 9 to 10kDa, high Isoelectric points, presence of eight cysteine residues, the ability to bind fatty acids and their derivatives and a defense role against pathogens. [71]. LTPs were discovered in 1990 years and correspond to two categories of plant proteins: LTP1 with long chain and LTP2 with shorter chain which have allergic properties, are rich in basic amino acids which gives to these allergens a pI, generally around 9 [72].

Protein held responsible for these effects in oil, named Ara h 9, were just in the last two years, identified and partially characterized. The two isoforms established of Ara 9 sharing 90% amino acid sequence identity. The two Ara h 9 isoforms share 60 -70% amino
acid sequence with LTPs from a large number of commonly foods such as hazelnut, chestnut, almond, peach, pear, plum, cherry, strawberry, lentils, lupin, sunflower, beans, pea and so on. Both of them displayed similar IgE reactivity. [73-75].

A study concerning characterisation of the non-specific lipid transfer protein, Ara h 9, and his allergenic potency in peanut-allergic patients from Mediterranean area showed that Ara h 9 was found to be a major allergen at least for the sensitive people from this area [75, 76]. In the meantime, another study revealed that Ara h 9 is a minor allergen but because in this study only 4.8% of subjects with peanut allergy had reactions to the classic major peanut allergens and up to 50% had reaction to Ara h9, it had been established that this is a relevant allergen [74]. It seems necessary to get more detailed information about clinical effects of peanut allergens.

Because LTPs manifested high resistance to proteolytic digestion and food processing is believed to LTP may reach the intestinal tract in an almost unmodified form [77-79]. This behaviour is determinate by the fact that LTPs are defence plant proteins against the attack of bacteria, fungi and viruses. The literature shows that the LTP allergy can be present at any age [80]. Nevertheless the last researches showed a large heterogeneity regarding the sensitivity against different allergens from peanut. Thus the allergenicity for isoallergens Ara h 7.0201 has not been established clearly yet and for isoallergens of Ara h 9 the opinions are enough controversies.

Ara h 10 and Ara h 11 are oleosins with MW (SDS-PAGE) of 16 kDa respectively 14 kDa. These were obtained from oil bodies from peanut and until now are not studied very much.

Conclusions

So far the focus has been straightened on studying the major allergens, but in recent years has highlighted the different properties of minor allergens given their involvement in allergic reactions. As we can see from this short peanut allergens presentation a number of uncharacterised allergens have been detected in Peanut.

In addition peanut shares many cross-reacting proteins with other members of leguminous family and that makes more difficult to detect low quantities in food. The presence of other abundant proteins can mask low abundance proteins especially in food like cookies, chocolates, sauces etc. where the matrix it is complex. Nonetheless, individuals are sensitised to a number of allergens in a heterogeneous way, rather than stereotypically to only 1 or 2.

There is no doubt that the methods useful for allergen characterisation are complex, as allergen characterization is a difficult and troublesome task.

Recently it was build a database of allergen families into AllFam based on structural classification of allergens. It has been established that only a small number of protein families contain allergens and because functional distribution of most allergens is narrow it confirmed the existence of yet unknown factors that render proteins allergenic [81].

Nonetheless, food allergies have established as major risk issues that the food industry can no longer ignore. Allergen labelling regulations require companies to label all pre-packed food if they contain any of the 14 listed allergenic foods as an ingredient. The mainly methods used to detect quantitatively or semi-quantitatively the allergens, respective peanut allergens are immunological or molecular methods like ELISA and PCR. Nowadays there are commercial available competitive ELISAs and DNA-based test kits for allergen detection in food products on market but only a few are validated by inter-laboratory studies. Still at the time of writing there is little information and published data to be found concerning commercially available dipstick [82-86].

In conclusion, further researches for a better understanding and knowledge of peanut allergens are absolutely requisite, both in terms of biochemical and clinical aspects. Besides, knowing all the issues which make from some proteins of peanuts threats to sensitive human health will allow to find solutions to fight with this menace of this century.
Acknowledgments

This study is supported by a contract funded by CNCSIS – Ideas Program, ID project code 1465, Grant 1014: Studies Regarding the Possibilities to Separate and Characterize the Peanut Allergens from Lipid Matrix, CNCSIS-UEFISCSU – Ideas Program, Bucharest, 1 Schitu Magureanu Street, postal code 050025, sector 5.

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Peanut allergens


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