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Review

Brazil nut allergy: A review

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Tree nuts and their products are highly nutritive and beneficial to health. On the other hand, some of them can lead to development of allergic reactions, protein intolerance or liver diseases in some groups of consumers. Therefore, it is important to study the risks of tree nuts ingestion as a major issue for food regulators and the pharmaceutical industry safety. A review of surveillance and correlation to possible Brazil nut allergy reactions with effects on consumers and some therapeutic potential was carried out to help in understanding the mechanisms and so preventing damages to sensitive consumer's health.

Key words: *Bertholletia excelsa*, aflatoxin, allergy, protein, immunoglobulin, risk.

INTRODUCTION

Allergies

Adverse reactions caused by the consumption of some foods have been widely studied and reported since ancient times. They can occur by several mechanisms. However, the food allergies are caused by specific immune responses (immune mediated). Food allergy is defined as an immune response to food proteins disease (Cianferoni and Spergel, 2009). For example, in the urticaria (commonly referred to as hives) the symptoms and the anaphylaxis are triggered by the immunoglobulin E (IgE), which is antibody-mediated immune responses. On the other hand, the cell-mediated diseases (eosinophilic esophagitis and enterocolitis), which are also induced by food proteins, are classified as Mixed IgE and No-IgE mediated. The allergic reactions involve immunologic mechanisms that may or may not be mediated by the IgE. They are usually associated to food allergies and hypersensitivity reactions characterized by a rapid release of mediators such as histamine (Figure 1).

On the other hand, the term food intolerance (non-immune mediated) refers to any abnormal response to (one or more than one) specific food, food substance (lactose/caffeine/fish proteins) or additive (sulfites) without the involvement of immunologic mechanisms. Allergies are characterized by the IgE synthesis against antigens that enter the body through ingestion, the inhalation (particles) or dermis contact (skin). Food allergy is an increasingly common problem in Western countries, and an effective therapeutic treatment is needed, however, it has not been

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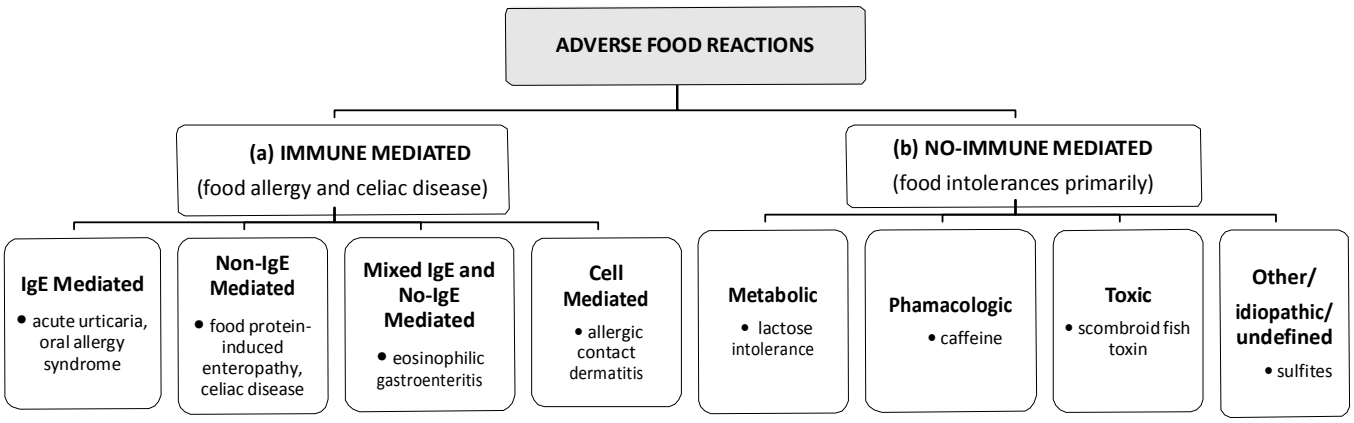

Figure 1. Types of adverse reactions developed through foods ingestion (a) Immune mediated and (b) Non-immune mediated (adapted from Boyce et al., 2010).

accomplished yet. Some therapeutic strategies have been investigated to identify the foods that most often cause severe anaphylactic IgE-mediated reactions, which commonly cause allergies in children (such as peanuts, walnuts, and shellfish or cow's milk and hen's egg). Boyce et al. (2010) strategies stated that an adverse reaction to food may be: (a) a mediated-immune response: food allergy (food protein-induced and urticaria reactions) or celiac disease; and (b) non mediated-immune response: primary food intolerance (lactose, caffeine, histamine, and sulfite intolerance).

Food allergy should be considered in individuals presenting anaphylaxis or any combination of symptoms that occur: within minutes to hours of ingesting food (especially in children) or after the ingestion of a specific food (on more than one occasion). The severity of allergic reactions to foods, however, is multifactorial and variable. Accordingly, the approaches being pursued are both food allergen -specific and -nonspecific. The allergen-specific approaches include oral, sublingual, and epicutaneous immunotherapy. It is carried out with native food allergens and mutated recombinant proteins. Diets containing cooked foods (milk and eggs) are an alternative approach to food *oral* immunotherapy and are already changing the paradigm of strict dietary avoidance for patients with food allergy. On the other hand, the nonspecific approaches include monoclonal anti-IgE antibodies, which might increase the threshold doses for food allergen in patients with food allergy. The variety of strategies for the treatment of food allergies increases the likelihood of success. Schreier and Wright (2014) reported that it is also necessary to consider, among the risk factors, the environmental influences on the healthy development of an immune system (during critical stages of development), particularly in the intestine. It is also important to mention that in addition to genetic predisposition, the psychological stress is known to play an important role in allergic and other inflammatory diseases, such as asthma.

Food additives such as sulfites (asthma), monosodium-glutamate and tartrazine (hives) and other chemical substances that are added to foods are also associated with adverse reactions (ASBAI, 2008). Regarding environmental aspects, the presence of mycotoxins (carcinogens that are products of fungal metabolic processes - immune systems suppressors) in the environment can be considered as a factor that increases the immune response. A family history of atopy and the presence of atopic dermatitis is already considered risk factors for sensitization to food allergens and the development of food allergy. According to Schutze et al. (2010), epidemiological studies have shown that indoor fungi, including mycotoxin producers, are associated with increased prevalence and exacerbation of respiratory diseases. Low et al. (2011) reported that the environmental growth conditions significantly influence the allergenicity of common fungi (through the differential production of allergenic proteins), and they highlight the importance of allergenicity measurements for understanding the environmental exposure to allergenic fungi. Still considering the environmental exposure, cross-reactivity may occur due to the presence of aeroallergens.

According to Souza and Rosario Filho (2012), house dust mites are the main sensitizers of atopic individuals, followed by *cockroaches* (*Periplaneta americana* and *Blattella germanica*), which caused skin reactions in 24.1% of people with asthma. Bees (*Hymenoptera)* can also cause anaphylactic reactions. According to Kohler et al. (2014) the diagnosis of allergy by bee stings is usually based on clinical history of anaphylactic reactions. The antibodies cross-reactive to conserve structures found in allergens include homologous primary structures of protein allergens (*Hyaluronidases, Dipeptidyl peptidases IV* and *Vitellogenins*) and cross-reactive carbohydrate determinants that are present in the majority of *Hymenoptera* venoms. Regarding

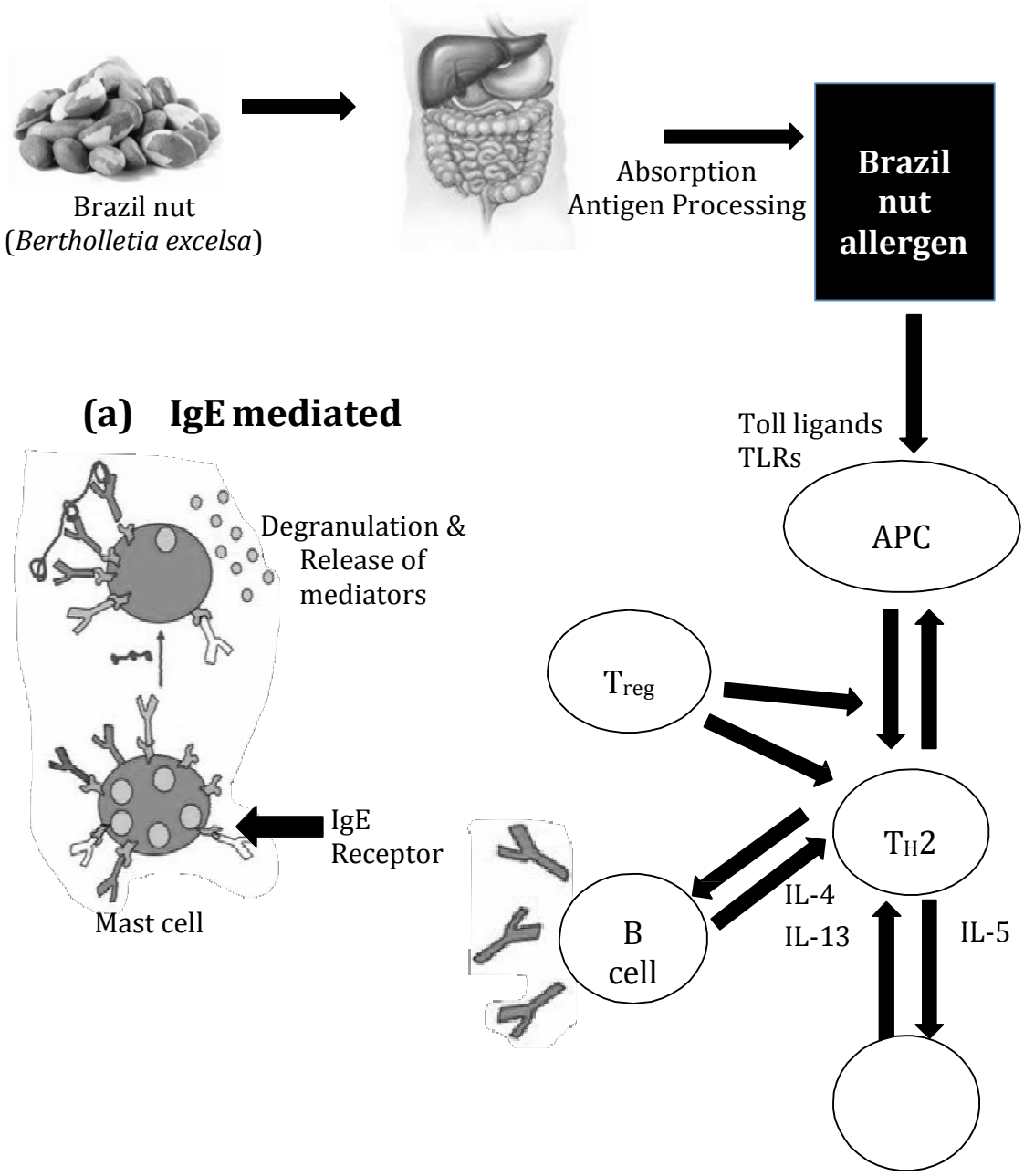

Figure 2. Mechanism of Food Allergen Sensitization (adapted from Otsu and Dreskin, 2011). APC: antigen presenting cells (APCs); T cells: that are of the Th2 variety, leading to activation of B cells; IgE, FcεRI, on mast cells and basophils; MIgE/FcεRI complexes are cross linked, leading to cell activation and release of pro-inflammatory mediators; (a) IgE Mediated; (b) Non IgE mediated.

reactions to food, Figure 2 shows types of different reactions, where initial sensitization is thought to be through the GI track.

Food allergens are absorbed and taken up by the dendritic cells or other antigen presenting cells (APCs). Peptides are then presented to T cells (Th2 variety), leading to activation of B cells, that mature into IgE-producing plasma cells. Then it (IgE) binds avidly into the high affinity receptor for IgE (FcεRI) on the mast-cells and basophils. When allergen is reintroduced, these M-IgE/FcεRI complexes are crosslinked, leading to cell activation and release of pro-inflammatory mediators (Otsu and Dreskin, 2011).

Food allergy × tree nuts

The allergic reactions to tree nuts are a growing global

Table 1. Nutrients of tree nuts and pulses including protein content

Nutrients	Tree nuts (g%)			Pulses
	Brazil nut (Freitas et al., 2012)	Cashew nut (Vicent et al., 2009)	Macadamia (Freitas and Naves, 2010)	Peanut (Freitas and Naves, 2010)
Lipid	57.94	34.95	66.1	44.57
Protein	16.30	27.31	8.40	24.03
Carbohydrate	3.36	25.39	22.18	12.01
Fiber	12.53[1]	1.42[2]	NI[3]	11.30

[1]Total dietary fiber; [2]crude fiber; [3]not informed.

concern, as the number of affected individuals continues to rise (Willison et al., 2014). Unlike some other food allergies, tree nut severe reactions can persist throughout life since they are usually consumed as snacks or used as ingredients in other products (by different consumer groups in various geographical locations). Among the tree nuts (and pulses) usually associated to food allergies are: cashew nuts, almonds, hazelnuts, pecans, Brazil nut, pistachio, peanuts and chestnut. The contact with the native allergenic proteins derived from tree nuts, which are generally difficult to isolate and purify, triggers the allergic reaction. The substances that cause food allergy belong to protein families with diverse biological functions: (a) Allergens: enzymes (mainly proteases), ligand-binding proteins or lipocalins, albumins, tropomyosins and calcium-binding proteins; (b) Pollen allergens: pathogenesis-related proteins, calcium-binding proteins, pectate lyases, bexpansins, and trypsin inhibitors; (c) Plant and animal food allergens: lipid transfer proteins, profilins, seed storage proteins and tropomyosins (Chapman et al., 2007). Tree nuts are in general energy dense (23.4 and 26.8 kJ/g), have high lipid content (45 to 75%), and are rich sources of proteins, unsaturated fatty acids, fibers, vitamins (E, B6, folic acid, and niacin), minerals (magnesium, potassium, and copper), phytosterols (stigmasterol, sitosterol, and campesterol), and polyphenols (catechins, resveratrol, etc.) (Vadivel et al., 2012). Table 1 summarizes the proximate composition of some tree nuts and peanut for comparison.

Some of the risk factors associated with food allergies are genetic inheritance, diet, and intestinal microbiota. Therefore, it is important to establish the relationship between clinical reports and the consumption of specific foods such as tree nuts, commonly associated with a higher prevalence of causes of food allergies. Some tree nuts have been studied for the bio accessibility of nutritional compounds and antioxidant activity and are part of the diet of some consumer groups and specific populations. They stand out especially due to their benefits to the cardiovascular system and to the facts that they reduce blood pressure and the risk of hypertension (Alexiadou and Katsilambros, 2011; Yazdekhasti et al., 2013) and have anti-cancer effects and promote weight loss. Table 2 shows some foods including tree nuts that are associated with allergies.

The prevalence of tree nut allergy in the United States of America (USA) in children is about 0.5% (Boyce et al., 2010). In Brazil, Guimarães et al. (2015) collected information from parents of pre-school children with allergy to different foods and found the following: milk (42.7%), pork (11.6%), fruits (10.9%), chocolate (9.4%), and eggs (9.2%). Investigating the prevalence of sensitivity to food allergens in children in Brazil, Naspitz et al. (2004) reported reaction to food allergens in subjects tested for IgE sensitivity, indicating a significantly greater proportion of sensitive individuals than non- sensitive ones.

Table 3 shows the prevalence of tree nuts allergy in patients from several countries. Unfortunately, there is still little information on the prevalence and incidence of food allergy in developing and emerging economies such as China, India, and Brazil. Brazil nut (*Bertholletia excelsa* HBK), for example, a seed native to the Amazon region, has remarkable nutritional properties, especially the contents of protein and sulfur amino acids. However, the nutrient profile of tree nuts is associated to allergic reactions. Nut allergy can be dangerous and that it may occur in highly atopic individuals of any age with a strong family history of atopy (Ridout et al., 2006). The nutritional and therapeutic potential of tree nuts and the pathology and immune reactions that influence the inherent risks associated with their consumption need to be evaluated. In order to discuss the case-reports of Brazil nut allergy this review is presented.

Brazil nut and allergy reactions

Composition

Brazil nut is an extractive product of the Amazon region, that is, the fruits are collected from the forest when they fall freely from the trees to the ground during the rainy season and from which the seeds are removed and industrially processed. It can be used as raw material for the production of various products. Its worldwide

Table 2. Animal and Vegetable food allergy prevalence rates from different countries.

Protein	Food allergy prevalence (%)				
	Canada	USA	Australia	Brazil	
	(Soller et al., 2012)[3]	(Boyce et al., 2010)	(Osborne et al., 2011)	(Naspitz et al.,2004)[1]	(Guimarães et al., 2015)[2]
Animal					
Egg	0.8	1	16.5	24.4	9.2
Fish	0.51	0.6	NI	29.5	NI
Milk	1.97	3	5.6	23.1	42.7
Pork	NI	NI	NI	NI	11.6
Shell fish	1.6	1.2	0.9	NI	NI
Vegetable					
Tree nuts	1.22*	0-4.1*	NI	NI	NI
Pulses					
Peanut	1	0.6	8.9	14	NI
Soy	0.2	0-0.6	NI	11.8	NI
Wheat	0.77	0.2-1.3	NI	20	NI
Sesame seed	0.1	NI	2.5	NI	NI

[1]Atopic children; [2]children in pre-scholar ages, [3]self-reported symptoms; [4]NI: not informed; *not specified.

production is of 78,000 tones (Freitas-Silva and Venâncio, 2011). It is an excellent source of proteins, carbohydrates, lipids, vitamins, and minerals. Its lipid content is over 60%, and the ratio of saturated, monounsaturated and polyunsaturated fatty acids is 25:41:34 (USDA, 2008). It is a source of vitamin E and sulfur amino acids. Among which, methionine and cysteine are part of the 2S albumin protein fraction, the most relevant protein fraction in Brazil nuts, which makes it more appealing than other nuts that lack sulfur amino acids, essential to human health. Brazil nut is also a source of micronutrients such as selenium (Se), phytosterols, tocopherols, squalene and phenolic compounds (Costa et al., 2010; Silva et al., 2010). Beta- and gamma-tocopherols are the most abundant tocopherol isomers present in Brazil nuts. Barium (Ba) and Radio (Ra) can also be found in Brazil nuts (Martins et al., 2012).

Compared to other tree nuts, Brazil nuts are known as the best source of Se (Pacheco and Scussel, 2007; Chunhieng et al., 2008; Manfio et al., 2012). Adequate intake of Se is essential for normal activity of Se-enzymes involved in the protection against oxidative stress, maintenance of the redox system, modulating the immune system, and regulation of the thyroid. Thomson et al. (2008) demonstrated that 100 µg/day of Se (equivalent to two units of Brazil nuts) ingestion for three months was effective on increasing plasma Se concentrations and glutathione peroxidase in healthy individuals. Stockler-Pinto et al. (2010) reported that the ingestion of a single serving of Brazil nut (5 g) a day for three months is effective in increasing the concentration of Se and glutathione peroxidase activity in patients with Se deficiency improving the antioxidant capacity of this mineral. Accordingly, the association between Se content and amino acids in Brazil nuts has been investigated. Silva et al. (2010) reported that Brazil nut showed the organic species se-methionine and Se- cystine after water extraction, but after simulated gastrointestinal digestion, only *Se-methionine* was found as bio accessible, corresponding to 74% of total Se (54.87 ± 4.6 $ug.g^{-1}$). Analysis of urine samples suggested the

Table 3. Tree nuts and pulses allergy prevalence in population from different countries.

References	Food	Ages[1]		Prevalence (%)		Origin
		Group	Years old	Mean	Range	
Tree nuts						
Ben-Shoshan et al. (2010)	NS[2]	Adults and children	NI[3]	1.1	NI	Canada
Fleisher et al. (2005)	NS	Adults and children	NI	0.4	NI	USA[4]
Sicherer et al. (2010)*	NS	Adults	31-40	0.3	NI	USA
	NS	Children	0-5	0.5	NI	
Shek et al. (2010)	NS	Teenager	14-16	0.8	NI	Singapore
Pulses						
Nwaru et al. (2014)	Peanut	Adults and children	NI	0.4	NI	Europe
Soller et al. (2012)	Peanut	Adults	NI	0.78	0.58-0.97	Canada
		Children	NI	1.77	1.21-2.33	
Osborne et al. (2011)	Peanut	Infants[5]	NI	8.9	NI	Australia
Sicherer et al.(2010)	Peanut	Adults	>65	0.7	0.4-1.1	USA
Lucioli et al. (2008)	Soy	Children	1	1.4	NI	USA
Oh et al.(2004)	Peanut	Children	6-12	2.8	NI	South Korea

[1]It is expressed "children" when the authors did not specify the children ages; [2]Not specified or different tree nuts involved reported by the authors; [3]not informed; [4]United States of America; 512 months old;; *2008 data.

presence of Se-cystine, and there were no significant differences between samples from men and women in terms of the concentration of this species after consumption of Brazil nuts (1 nut/day for 15 d). Therefore, food processing appears to influence the potential allergenicity associated with the protein fraction. According to Van Boxtel et al. (2008), in Brazil nuts, for example, the protein fraction 2S albumin (denominated *Ber e1)*, considered the major allergen, was stable to *in vitro* peptic digestion. Thermal denaturation (melting) can affect the digestibility of protein, since these authors found that the denaturation temperature of 2S albumin ranged from 80 to 110°C, depending on the pH value.

Under heating at temperature higher than its denaturation temperature, at pH 7.0, there was partial formation of insoluble protein aggregates and the protein dissociated into its polypeptides, while heating at pH 5.0 did not seem to induce aggregation or dissociation of protein aggregates. The denaturation temperature of about 110°C, under pH values corresponding to the general food pH values (pH 5 to 7) is high. As a result, it is likely that heat processing causes the denaturation of allergy protein fraction present in food products. Koppelman et al. (2005) investigated the high resistance of 2S albumin fraction, previously identified as an allergen, against proteolysis by pepsin. They found that although the protein denaturation temperature exceeds 110°C, at neutral pH, a fully reversible thermal denaturation was observed at 82°C at low pH. Chemical processing (the subsequent reduction and alkylation of the protein) was used to destabilize the globular fold. Far-UV circular

Table 4. Amino acid composition and total protein content in tree nuts and pulses (peanuts).

Amino acids	Tree Nuts (g%)[1]		Pulses
	Brazil nut (Venkatashalam and Sathe, 2006)	Cashew nuts (Latif et al., 2013)	Peanut[2] (Latif et al., 2013)
Alanine	4.30	4.44	3.98
Arginine	12.91	9.84	11.35
Aspartic acid	7.69	8.53	11.17
Cysteine*	0.75	0.54	1.20[3]
Glutamic acid	20.26	22.43	18.72
Glycine	4.75	4.55	5.49
Histidine	2.92	2.68	2.33
Isoleucine	3.21	4.15	3.12
Leucine	7.89	8.00	6.24
Lysine	2.95	4.59	3.35
Methionine*	8.98	2.27	1.09
Phenylalanine	4.06	4.83	5.04
Proline	5.21	5.37	4.32
Serine	4.00	5.21	4.92
Threonine	2.27	3.22	2.67
Tyrosine	2.47	2.43	3.65
Valine	4.71	5.65	3.83
Total protein (g%)	13.93	18.81	26.6

[1]g% average from crude protein; [2]untreated kernel residue data; [3]expressed by the authors as "cystine"; * most important sulfur amino acids involved in allergy reactions.

dichroism and infrared spectroscopy showed that the reduced and alkylated form lost some structures, whereas the alpha-helix structure of protein was protected. Accordingly, studies on protein digestion are important due to the potential allergenicity of Brazil nut 2S albumin.

Protein versus Brazil nut allergy

Since ingestion of some proteins is associated with allergic reactions in some consumers, Breiteneder and Mills (2005) summarized the common biochemical and physicochemical properties of food allergens. They reported that thermal stability, resistance to proteolysis, binding capacity, and lipids interactions are factors that promote the allergenic properties of food proteins. Although one or more of these factors are characteristic of the allergen proteins, there is no rule to predict the allergenicity of a given protein. It is well known that proteins denature under high pressure. Thus, several attempts have been made to change the structure of the protein allergens using high pressure processing in order to reduce its allergenicity. Several studies have been carried out on simple protein solutions and on complex food systems. Allergens have been investigated under or after high-pressure treatment using methods capable of detecting changes in the secondary and tertiary structures of proteins. When considering the protein content of the Brazil nut as potentially high (13-19 g%) and its essential amino acids, the beneficial aspects should be highlighted without disregarding food allergies. Table 4 presents the contents of protein and amino acids, including the sulfur amino acids.

In Brazil nut the methionine content is higher than cashew and peanut, and the amount of sulfur fraction has been studied in allergic reactions. Sun et al. (1987) studied the protein fractions (11S, 7S, and 2S) and registered that they contain high concentrations of glutamine/glutamic acid and arginine. However, the 2S protein contains unusually large amounts of sulfur amino acids (17.9% methionine and 8.7% cysteine). The major allergens found in Brazil nut are: (a) 2S albumin and (b) 11S legumin (Ber e 2) (Crespo et al., 2006). According to Van Bilsen et al. (2013), it is not known exactly why certain food proteins are more likely to cause reactions. One of the characteristics of most food allergens is that they are stable to the acidic and proteolytic conditions in the digestive tract. This property appears to be a risk factor for allergic sensitization. Van Bilsen et al. (2013) investigated the contribution of 2S albumin protein structure to the ability to induce *in vivo* oral sensitization using an animal model of food allergy. Disulfide bridges of albumin 2S were reduced and alkylated resulting in loss of protein structure and an increased *in vitro* pepsin digestibility. The reduced and alkylated

Table 5. Some case reports of Brazil nut allergies reported in the literature

Reference	Number of patients	Patterns of Brazil nut contact			Patient			Symptoms
		Test	Ingestion	Skin contact	Age[1]	Gender	Origin	
Nordlee et al.(1996)	04	Skin[2]	NA	NA[3]	NI[4]	NI	USA[5]	Oropharyngeal swelling and itching/facial swelling/ laryngeal edema and bronchospasm with wheezing/
Ewan (1996)	04	IgE[6]	NA	NA	NI	NI	UK[7]	Edema of tongue/ laryngeal edema/urticaria
Bartolome et al.(1997)	01	NA	01 BN[8]	NA	NI	NI	NI	Vomiting/diarrhea and loss of consciousness/
Pastorello et al. (1998)	11	NA	02 BN	NA	NI	M& F[9]	Italy	Urticaria/glottis edema/anaphylactic reaction
Borja et al.(1999)	01	NA	01 BN	NA	31	F	Spain	Pharyngeal itching/lip swelling/dysphonia/ dyspnea/ wheezing/macular exanthema.
Senna et al. (2005)	01	IgE	NA	NA	18	M	Italy	Acute wheezing/dyspnea/facial angioedema/ precipitating hypotension (max= 90 mm).
Ridout et al.(2006)	56	IgE	NA	Labial	NI	M & F	UK	Irritation/discomfort of mouth or lips/swelling of lips or tongue/ throat tightening/difficulty in swallowing/ swelling/puffiness of face or eyelids/skin rash/increased heart rate/shortness of breath/sense of doom/fear/nausea/abdominal pain/vomiting/ wheezing;/stridor/collapse/unconsciousness
Bansal et al. (2007)	01	NA	NA	Contact	NI	F	England	Urticaria and dyspnea

[1]years old; [2]IGE Skin-prick tests in response to extracts of Brazil nut and positive radioallergosorbent tests; [3]not applicable; [4]Not informed; [5]United States of America; [6]IGE measurement; [7]United Kingdom; [8]amount of Brazil nut ingested; [9]male & female.

forms of 2S albumin were administered by daily gavage dosing of 0.1 and 1 mg to rats for 42 days. Intraperitoneal administration was used as a positive control.

The enzyme-linked immunosorbent assay (ELISA) and passive cutaneous anaphylaxis methods were used to analyze Sera. Oral exposure to native or reduced 2S albumin resulted in specific IgG1 and IgG2 responses. In conclusion, the study demonstrated that disruption of the protein structure of Brazil nut 2S albumin reduced the sensitization potential of the rat food allergy model, whereas the immunogenicity of 2S albumin was preserved. This observation can be useful for the development of immunotherapy for Brazil nut allergy. On the other hand, de Melo et al. (1994) investigated the oral administration of various doses Brazil nuts to rats as a possible route of immunization, but they found that instead of immunization, it induced systemic tolerance. Multiple feeding, than by a single dose feeding more effectively induced immune tolerance.

Brazil nut possible allergies symptoms

In general, the symptoms of anaphylactic reactions associated with Brazil nut can vary from urticaria to anaphylactic shock, and the diagnosis can be made based on medical history or using the skin prick test (SPT) to measure the presence of specific IgE. Table 5 shows some patients with symptoms from oral ingestion or contact with body fluids (semen) of an individual who had consumed Brazil nuts (Bansal et al., 2007). The proteomic approach was used to characterize the main isoforms of Brazil nut 2S albumin. Although most isoforms have molecular weight of approximately 12 kDa with a high amino acid sequence homology, significant heterogeneity was found (Moreno et al., 2014). Alcocer et al. (2012) stated that due to its extremely high content of sulfur amino acids, the allergen fraction has attracted much scientific attention as a target protein in transgenic biotechnology research, studies on processing of plant storage proteins and as an experimental protein in nutritional supplementation experiments. Some studies investigated the addition of Brazil nut protein to other foods such as soy. However, these experiments showed the allergen presence. For example, the nutritional quality of soy can be compromised by a relative

deficiency of methionine in the protein fraction of the seeds. In order to improve nutritional quality, the 2S albumin fraction from Brazil nut has been introduced into transgenic soybeans. An allergen can be transferred from one food to another through genetic engineering (Nordlee et al., 1996). Generally, immunological methods for detection of Brazil nut allergens in foods are based on polyclonal antibodies tested in animals. Phage display technology allows obtaining high affinity antibodies, avoiding animal immunization, and it can therefore apply the principle of replacement supported by animal welfare guidelines.

De La Cruz et al. (2013b) studied specific binders against Brazil nut employing a Brazil nut protein extract and a purified 2S globulin extract. A fragment (phage) that specifically recognizes Brazil nut proteins was isolated. The selected phage was also used as affinity probe to develop an indirect phage-ELISA for the detection of Brazil nut in experimental binary mixtures and processed foods. This study described for the first time the isolation of specific recombinant antibody fragments and showed the way for the development of immunoassays for the analysis of food that can be produced *in vitro* and do not rely on animal immunization.

Despite several previous reports on the allergenic potential of the 2S albumin protein fraction, Rundqvist et al. (2012) reported that the protein alone does not cause an allergic response in rats, but the addition of the components of a Brazil nut lipid fraction would be important. Structural details of *Ber e 1* suggest that it may contribute to the understanding of the protein allergenic properties and its potential interaction partners. The overall fold of 2S albumin is similar to that of other albumins, but the hydrophobic cavities resemble that of a homologous non-specific lipid transfer protein. The allergen proved to interact with Cu^{2+} ions. This Cu^{2+} binding has a minimal effect on the electrostatic potential on the protein surface, but the charge distribution within the hydrophobic cavity is significantly changed. Since the hydrophobic cavity is likely to be surrounded by a lipid, Cu^{2+} interaction, it can in turn affect the interaction to trigger an allergic response. On the other hand, the labeling of foods containing Brazil nut is of fundamental importance for the safety of consumers susceptible to food allergy. In order to protect sensitized individuals, reliable methods to detect trace amounts of Brazil nut should be available for the food industry and for health and food safety authorities. A TaqMan real time polymerase chain reaction (PCR) method was developed for specific detection of proteins in foodstuffs. The method uses specific Brazil nut primers, targeting the 2S albumin fraction, and a positive amplification control based on the 18S rRNA gene. The applicability of this specific system for Brazil nuts was evaluated on 66 samples of different commercial foods. The real time PCR assay proved to be a useful tool for detection of Brazil nut DNA, and it can be used as a routine analysis to assert accuracy on food labeling (De la Cruz et al., 2013a).

Other issues

Despite its beneficial nutritional properties, the mycobiota prevailing in Brazil nuts may include mycotoxins-producing fungi. Since these substances can also be associated with environmental allergies and have deleterious effects, especially on the liver, the associated risks should be considered. Brazil nut production involves several producing countries and a global supply chain. Due to the presence of aflatoxins (AFLs) produced by toxigenic fungi during storage and production of Brazil nuts, there is strong need for contamination control and monitoring (Baquião et al. 2013; Pacheco et al. 2013; Pacheco and Scussel, 2009). In samples collected in the early stages of the production chain (communities/forest), the frequency of aflatoxin positive samples was lower than that in samples that were processed or obtained from retail centers (Pacheco and Scussel, 2007). Mycotoxins have been extensively studied in terms of their mechanism of action, mutagenicity and carcinogenic activity (Andrade et al., 2012; Freitas-Silva and Venâncio, 2011). The knowledge of these mechanisms has led to the development of biomarkers such as biotransformation products and macromolecular adducts (Bando et al., 2007).

AFLs ingested through contaminated foodstuffs are absorbed in the gastrointestinal tract and are bio-transformed in the liver being activated and can therefore exert carcinogenic effects. The AFB1 activated form (AFB1 epoxide) can covalently bond to DNA, RNA and proteins. These bonds form adducts, which are primary biochemical lesions produced by AFB1 epoxide. The formation of adducts is a characteristic of liver carcinomas (Redzwan et al., 2012; Pereira and Santos, 2011). Therefore, DNA adducts reflect the variation in the aflatoxin intake daily diet, whereas albumin adducts integrate the exposure over several weeks. Quantification of exposure to AFB1 using precise and accurate laboratory methods is a relatively recent development. As an indicator of weeks or months of exposure, AFB1 Lysine (AFB1- lys) can be hydrolyzed with the patient serum. It can be quantified using isotope dilution liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) and quantified as a biomarker.

In a study conducted in the USA, for example, about 1% of the USA population had detectable levels (≥ 0.02 mg/L) of AFB1-lysine. Of those with detectable levels, the geometric mean was 0.038 (0.024 to 0.060 g/L), equivalent to 0.842 (0.530 to 1.34 pg/mg) albumin (Schleicher et al., 2013). In addition to the high protein level of some foods, such as Brazil nuts, several studies strongly indicate that there is need for guidance during post-harvesting and food handling steps and interventions in the preparation of food intended to reduce exposure to AFLs in different populations and ethnic groups (Leong et al., 2012; Jolly et al., 2006). The identification and measurement of biomarkers to assess human exposure to mycotoxins by simple, rapid,

precise and accurate analytical methods can help prevent or minimize the health harms that arise from human exposure to these substances. In conclusion, the risk assessment of Brazil nut consumption, concerning allergy, is necessary in order to provide actual information to consumers and improve the food safety and public health.

Concerning the therapeutic potential from Brazil nuts, their benefits are well known. According to Yang (2009), epidemiologic studies have shown inverse association between the intake of nuts and chronic diseases such as cardiovascular diseases and cancer. The content of lipids, minerals and phytochemicals and their health benefits associated with the consumption of Brazil nuts have been continuously reviewed. Their nutritional composition seems to have beneficial effects due to their antioxidant properties that are associated with a reduced risk of developing atherosclerosis and cancer. Still regarding their influence on human metabolism, Cominetti et al. (2012) demonstrated that obese people who consume Brazil nut daily could improve Se status and lipid profile, especially high-density lipoprotein and cholesterol levels, thereby reducing cardiovascular risks. The consumption of 3 to 5 nuts a day for 16 weeks, improved the lipid profile and microvascular function in obese adolescents, possibly due to the high content of unsaturated fatty acids and bioactive substances (Maranhão et al., 2011). In addition, it was reported a long-term decrease in inflammatory markers after a single intake of Brazil nuts in healthy individuals (Colpo et al., 2014). Nevertheless, the long-term effect of their consumption on the inflammatory markers should be further investigated.

CONCLUSION

The Brazil nut allergy is a clinical status dependable of prevention. Some strategies for consumers such as observe the ingredients content of food products and complete information for clinical treatments are necessary. More effort by the medical assistance is important to report clinic cases related to Brazil nut allergy to government agencies in order to apply the risk assessment concepts and to communicate the population about the risks of Brazil nut consumption by sensible individuals.

Conflict of Interest

The authors have not declared any conflict of interest.

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