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Almond Allergens: Molecular Characterization, Detection, and Clinical Relevance

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ABSTRACT: Almond (*Prunus dulcis*) has been widely used in all sorts of food products (bakery, pastry, snacks), mostly due to its pleasant flavor and health benefits. However, it is also classified as a potential allergenic seed known to be responsible for triggering several mild to life-threatening immune reactions in sensitized and allergic individuals. Presently, eight groups of allergenic proteins have been identified and characterized in almond, namely, PR-10 (Pru du 1), TLP (Pru du 2), prolamins (Pru du 2S albumin, Pru du 3), profilins (Pru du 4), 60sRP (Pru du 5), and cupin (Pru du 6, Pru du γ-conglutin), although only a few of them have been tested for reactivity with almond-allergic sera. To protect sensitized individuals, labeling regulations have been implemented for foods containing potential allergenic ingredients, impelling the development of adequate analytical methods. This work aims to present an updated and critical overview of the molecular characterization and clinical relevance of almond allergens, as well as review the main methodologies used to detect and quantitate food allergens with special emphasis on almond.

KEYWORDS: *food allergy, almond, tree nuts, immunological reactions, allergen detection*

INTRODUCTION

Tree nuts have attained an important place in human diets because they are considered excellent foods, mainly due to their pleasant taste and potential health benefits. They are consumed all over the world by the majority of individuals in a wide variety of forms, which are more or less related to the population habits and/or the type of tree available in the geographical region. For instance, in 2009 in Portugal, 33% of the total tree nut production corresponded to almond fruits and 5% of the annual fruit consumption was from tree nuts.[1] Almond (*Prunus dulcis* or *Amygdalus communis* L.) is one of the most commonly consumed nuts, together with hazelnuts (*Corylus avellana*), walnuts (*Juglans regia*), cashews (*Anacardium occidentale*), pecan nuts (*Carya illinoiesis*), Brazil nuts (*Bertholletia excelsa*), pistachio nuts (*Pistacia vera*), macadamia nuts (*Macadamia ternifolia*), and pine nuts (*Pinus pinea* and other *Pinus* species). Among those, almond occupied the first place in terms of global trade in 2009, followed by cashew, pistachio, and hazelnut.[2] In terms of world production of tree nuts in 2010, almond ranked third on a global basis, after cashew and walnut productions, with United States and Spain as the two major producers of almond.[2]

In Europe, tree nuts such as almond are far more consumed than peanuts or seeds.[3] As a consequence, tree nuts have occupied an important place in the economy because they are an integral part of the human food supply. Tree nuts can be consumed either raw (snacks) or processed, their edible fraction being used as an ingredient in a wide variety of food products (spreads, bakery, pastry, chocolates, and confectionary products).[3] The increasing consumption of tree nuts has been related to the potential health benefits of these foods. With the present recognition by the U.S. Food and Drug Administration (FDA) regarding the health benefits attributed to tree nuts, namely, as "heart-protective" foods, the consumption of these nuts has risen, mainly in developed countries.[4]

However, in recent years, the use of tree nuts in food has also led to concerns about the growing number of individuals sensitized to tree nuts and peanuts, especially in Western countries (Europe and the United States).[5] In the United States, by the use of random-calling telephone surveys, through a 11-year follow up study, there was an increase of tree nut allergy prevalence in children, ranging from 0.6% in 1997 to 1.2% in 2002 and 2.1% in 2008, whereas in the adult population the same prevalence remained around 1.3%.[6] In Europe, hazelnut allergy is common and often associated with birch pollinosis, whereas in the United States, allergy to walnut, cashew, almond, pecan, and Brazil nut appears to be more common than hazelnut.[7] Nevertheless, recent data from Europe, the United States, and Australia identified hazelnut as the food with the highest sensitization rate.[8]

In 1985, the Codex Alimentarius Commission first listed a set of food products, in which tree nuts were included, as likely to cause hypersensitivity in sensitized individuals, advising the obligation to label foodstuffs containing possible allergens.[9] In 1993, the same Commission included tree nuts in the group of eight foods known to be responsible for almost 90% of human food allergies. Since then, special attention has been devoted to establishing clear guidelines for food allergen labeling, compelling the European Union (EU) to first include allergenic foods in Directive 2000/13/EC.[10] Accordingly, the producers have the obligation to declare all ingredients present in prepackaged foods traded inside the EU, with very few exceptions.

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Directive 2000/13/EC has been updated several times with new amendments concerning the list of potential allergens.[10] The two most important amendments were Directive 2003/89/EC Annex IIIa[11] and Directive 2007/68/EC.[12] The former included a list of 12 allergenic foods (cereals containing gluten, crustaceans, eggs, fish, peanuts, soybeans, milk, nuts, celery, mustard, sesame, and sulfur dioxide) that must always be declared on the label of foodstuffs.[11] The latter amendment lists the 14 allergenic foods (including 2 more foods, namely, lupine and mollusks), as well as some exemptions that are not required to be labeled.[12]

This review intends to provide an updated and critical overview on almond allergens, regarding their biochemical and biological characterization, as well as clinical implications on sensitized individuals. It also aims to assemble the techniques, focusing on the recent developments in protein- and DNA-based methods, for monitoring the presence of almond allergens in food products in compliance with the labeling statements.

FOOD ALLERGY

Food allergies can occur upon the ingestion of allergenic food components that in sensitized individuals can trigger mild to severe abnormal responses mediated by the immunological system. The adverse response to food proteins (mainly glycoproteins) can be mediated by immunoglobulin E (IgE) or non-IgE (cellular) mechanisms and are estimated to affect almost 3−4% of adults and 6% of young children.[13] For reasons not yet well understood, tree nuts pose serious health problems to sensitized individuals, who frequently present negative physiological responses that can vary in intensity upon exposure to these seeds.[5] Tree nuts are known to be responsible for triggering abnormal immunological responses in allergic individuals, ranging from mild reactions to potentially fatal anaphylactic shocks. More than one-third of total anaphylactic reactions that occurred in Western countries are thought to be provoked by food ingestion and are often attributed to peanut, tree nut, or shellfish ingestion.[14] According to Sicherer,[15] there are no studies to address directly the prevalence of fatal food-allergic reactions. Fatalities have been mainly reported from allergic reactions to peanuts and tree nuts, appearing to be associated with delayed treatment with epinephrine, and occur more often in teenagers and young adults with asthma and previously diagnosed food allergy. In a population-based U.S. registry, 31 deaths were registered from 2001 to 2006, 6 of which were caused by tree nuts.[16] Pumphrey and Gowland[17] reported 48 deaths in the United Kingdom from 1999 to 2006, 9 of which were related with tree nuts, ascertain all food-related anaphylaxis.

Hypersensitivity reactions are catalogued into four groups, according to the mechanism responsible for the immunological response. Type I-hypersensitivity reactions are IgE-mediated through the activation of the mast cells, types II and III are IgG-mediated, and type IV reaction are triggered by T_H1 and T_H2 cells.[5] Food allergies are essentially included in type I category due to the specific IgE antibody production against food allergens. In this case, the allergic reactions result from a previous sensitization to the allergen, generally leading to the release of histamine and other biological mediators in subsequent exposures.[5]

Allergic reactions related to food ingestion can appear within minutes up and to 2 h after the ingestion, involving one or several target organs such as the skin and the gastrointestinal and respiratory tracts, as well as the cardiovascular system.[18] The most severe allergic manifestation is anaphylaxis, which can be fatal or near fatal, even when only traces of the allergen are ingested. Tree nuts, such as almonds, are among the food products related to this type of reaction.[14] Other less serious responses such as cutaneous reactions are the most common clinical manifestation of food allergy and are frequently observed in combination with symptoms of other target organs. The oral allergy syndrome (OAS) is another clinical manifestation associated with food allergies and generally appears within 5−15 min after the allergen ingestion. Fresh vegetables, fruits, and tree nuts are typically the agents responsible for this type of reaction.[18]

ALMOND ALLERGENS

Almond is taxonomically designated *Prunus dulcis* or *Amygdalus communis* L. (the most common denominations) and belongs to the Rosaceae family, a subfamily of the Prunoideae. The Rosaceae family also includes fruits such as peach, apricot, plum, cherry (Prunoideae subfamily), apple, pear (Pomoideae subfamily), and blackberry and strawberry (Rosoideae subfamily).[19−21]

Almond allergy in the third most commonly reported tree nut allergy in the United States (reactive in 15% of patients), behind cashew nut and walnut.[22] Some native allergens have been identified and characterized according to their biochemical function, although only a few have been cloned or tested for their reactivity with sera from almond-allergic patients.[23] Until now, eight groups of allergens were identified in almond: Pru du 1, Pru du 2, Pru du 2S albumin, Pru du 3, Pru du 4, Pru du 5, Pru du 6 (amandin), and Pru du γ-conglutin. From these eight groups, Pru du 3, Pru du 4, Pru du 5, and Pru du 6 are recognized and included in the WHO−IUIS list of allergens.[24] Their biochemical designations, clinical relevance, biological functions, and accession numbers in the NCBI[25] Database are summarized in Table 1.

Pru du 1 (PR-Proteins). One group of allergens identified in almond comprises a family of proteins named Pru du 1, included in group 1 Fagales-related protein, most commonly known as pathogenesis related-10 proteins (PR-10) (Table 1). The PR-10 family encompasses a particular set of proteins that are related to intracellular defense mechanisms and response to fungal or bacterial infections. PR-10 proteins exist in various isoforms, differing in their IgE-binding capacities.[26] The Bet v 1-homologous food allergens are thought to function as plant steroid hormone transporters,[27] and they have been identified in several Rosaceae fruits, including almond.[19,26] Bet v 1-homologues are commonly labile proteins and in general can suffer unfolding during the cooking process. The boiling process (wet processing) causes the destruction of the conformational epitopes, reducing the IgE reactivity and their ability to trigger allergic reactions in sensitized individuals.[28] In almond, seven members of PR-10 proteins have been identified. The genes encoding the putative isoallergens Pru du 1.01−Pru du 1.06A/B have already been cloned and mapped.[25] Amino acid sequences of Pru du 1.01−Pru du 1.05 possess >5% dissimilarity among these five proteins, placing them into different isoallergen groups. Pru du 1.06A and Pru du 1.06B present >95% DNA sequence identity, putting these two proteins within the same group of isoallergens. All seven proteins possess peptide sequences of 160 amino acids (aa), with the exception of Pru du 1.04, which contains 159 aa. The predicted molecular weight of the described proteins ranges between 17.1 and 17.5 kDa with isoelectric point (p*I*) values varying from 4.9 to 6.0.[19] PR-10 proteins from almond are also

Table 1. Identification of Almond Allergens According to Their Biological Function, Clinical Relevance, and Respective Accession Numbers in the NCBI Database[25]

name	biochemical designation	protein families	molecular weight (kDa)	biological function	clinical relevance	isoallergen designation	isoforms or variants	nucleotide	protein
Pru du 1	Bet v 1-homologous	PR-10 family	17 (~160 aa)	protection against pathogenic constraints and adaptation to stressful environment	mild immune reactions and related to OAS; severe allergic reactions reported in some patients with birch pollen allergy; cross-reactivity with Bet v 1 and other PR-10	Pru du 1.01	not known	EU424239.1	ACE80939.1
						Pru du 1.02		EU424241.1	ACE80941.1
						Pru du 1.03		EU424243.1	ACE80943.1
						Pru du 1.04		EU424245.1	ACE80945.1
						Pru du 1.05		EU424247.1	ACE80947.1
						Pru du 1.06A		EU424251.1	ACE80951.1
						Pru du 1.06B		EU424249.1	ACE80949.1
Pru du 2	TLP	PR-5 family	23–27 (246 aa)	thaumatins	recognized as potent allergens, but the clinical relevance is yet subject of study	Pru du 2.01A	not known	EU424256.1	ACE80956.1
			(246 aa)			Pru du 2.01B		EU424258.1	ACE80958.1
			(246 aa)			Pru du 2.02		EU424254.1	ACE80954.1
			(277 aa)			Pru du 2.03		EU424260.1	ACE80960.1
			(330 aa)			Pru du 2.04		EU424262.1	ACE80962.1
Pru du 2S albumin	2S albumin	members of the prolamin superfamily	12 (28 aa)	seed storage proteins for seed development	specific allergic symptoms not yet defined; more studies needed	Pru du 2S albumin	not known	not known	P82944.1
Pru du 3	nsLTP	members of prolamin superfamily	9 (117 aa)	lipid transfer proteins	systemic and life-threatening symptoms; cross-reactivity among Rosaceae fruits	Pru du 3.01	Pru du 3.0101	EU424264.1	ACE80964.1
			(123 aa)			Pru du 3.02		EU424266.1	ACE80966.1
			(116 aa)			Pru du 3.03		EU424268.1	ACE80968.1
Pru du 4	profilin	profilin-specific IgE usually cross-reacts with homologues from virtually every plant source	14 (131 aa)	actin-binding proteins	symptoms are mild and limited to oral cavity	Pru du 4.01	Pru du 4.0101	EU424270.1	ACE80970.1
						Pru du 4.02	Pru du 4.0102	EU424272.1	ACE80972.1
Pru du 5	r60sRP	autoimmune reactions to human P2	10 (113 aa)	intervenes in the elongation step of protein synthesis	specific allergic symptoms not yet defined; more studies needed	Pru du 5	Pru du 5.0101	AY081851.1	AAL91663.1
Pru du 6	amandin, 11S globulin, or AMP	members of cupin superfamily	360 (~1055 aa)	legumin-like protein (major storage protein)	reported to induce severe allergic reactions	Prunin-1	Pru du 6.0101	X78119.1	CAA55009.1
						Prunin-2	Pru du 6.0201	X78120.1	CAA55010.1
Pru du γ-conglutin	γ-conglutin	members of cupin superfamily	45 (25 aa)	7S vicillins	specific allergic symptoms not yet defined; more studies needed	not known	not known	not known	P82952.1

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very similar to those found in apple (Mal d 1),[29] pear (Pyr c 1),[30] sweet cherry (Pru av 1),[31] and apricot (Pru ar 1).[32]

Pru du 2 (TLP). Pru du 2 is a group of allergens identified in almond, with five putative isoallergen genes (*Pru du 2.01A/B*–*Pru du 2.04*) already cloned and sequenced (Table 1).[19,25] These allergens belong to the PR-5 family, also known as thaumatin-like proteins (TLP), comprising three groups of responses: to pathogen infection, to osmotic stress (osmatins), and to fungal proteins.[33] The TLP identified in almond possesses protein weights ranging from 23 to 27 kDa and different sequence sizes. Pru du 2.01A/B and Pru du 2.02 contain 246 aa, whereas Pru du 2.03 and Pru du 2.04 contain 277 and 330 aa, respectively. Like PR-10 proteins, the TLP comprise four different isoallergen groups, displaying a signal peptide of 24 aa, with the exception of Pru du 2.02 protein, which has in its signaling sequence 21 aa.[19] The TLP are very resistant to proteases, pH, or heat-induced denaturation, probably due to the presence of 16 conserved cysteine residues bonded in 8 disulfide bridges.[33] These biochemical characteristics are most likely the reason these proteins can affect sensitized individuals, because they are not significantly destroyed by the usual food-processing methods.

Pru du 2S Albumin. The 2S albumins are included in the prolamin superfamily (Table 1). This group encompasses other allergenic proteins such as the nonspecific lipid-transfer proteins (nsLTP), the α-amylase/trypsin inhibitors, and the prolamin storage proteins.[34] The 2S albumins act as seed storage proteins for seed development and as defense-related proteins.[35] They are thought to cause sensitization along the gastrointestinal tract, suggesting that 2S albumins are resistant, at least to some extent, to adverse conditions such as acidic pH, proteolytic activity of digestive enzymes, and denaturing effects of surfactants.[36] The secondary structure of the 2S albumin seems to remain unaltered below 90 °C,[28] preserving their allergenic capacity when exposed to the immune system and, therefore, inducing allergic responses in sensitized individuals.[36] 2S albumins present high structural homology. However, cross-reactivity between allergens with <50% amino acid sequence homology is rare. Cross-reaction typically requires an amino acid sequence homology of >70%.[37] 2S albumin protein identified in almond is classified as a major allergen.[36,38] Pru du 2S albumin has a peptide sequence of 28 aa and a molecular weight of 12 kDa, preserving a fraction of 6 kDa after enzyme digestion, which maintains IgE-binding activity. A second fraction of 2 kDa, belonging to the 2S albumin, was also sequenced, revealing 80% similarity with the sequences near the C-terminal of English walnut (allergen Jug r 1) and Brazil nut 2S albumin. This fact, along with the high content in methionine of the 6 kDa fraction, suggests that this protein is possibly a member of the 2S albumin allergen family.[38] Although Pru du 2S albumin exhibits >80% similarity with Brazil nut 2S albumin, no cross-reactivity has been suggested to occur between these two nuts.[39] Besides high sequence homology, shared linear epitopes among 2S albumins are apparently linked to cross-reactivity.[36]

Pru du 3 (nsLTP). Like the 2S albumins, the allergenic nsLTP belong to the prolamin superfamily, being also known as the PR-14. In almond, three nsLTP (Pru du 3) were identified and characterized.[19] The genes encoding Pru du 3.01–3.03 proteins were sequenced and made available at the NCBI database (Table 1). The Pru du 3.01, 3.02, and 3.03 isoallergens have similar molecular weights (9 kDa) and belong to the nsLTP type 1 subfamily, but have different sizes, 117, 123, and 116 aa, with distinct signal sequences of 26, 30, and 25 aa, respectively.[25] The three isoallergens exhibit eight conserved cysteine residues,[19] enabling the conformation of four disulfide bridges. Like other plant nsLTP, this subfamily of nsLTP type 1 includes small and soluble proteins to facilitate the transference of lipids (fatty acids, phospholipids, glycolipids, and steroids) between membranes. nsLTP possess an internal hydrophobic core that functions as the binding site for lipids. Besides lipid transport and assembly, they also intervene in the defense of plants against fungal and bacterial activities.[25,40] Many nsLTP1 proteins, such as Pru du 3 (Pru du 3.01–3.03), have been characterized as allergens in humans.[25]

Because nsLTP are usually accumulated in the outer epidermal layers of plant organs, they are thought to be responsible for the stronger allergenicity of the peels in comparison to the inner layers of the fruit (pulps) in the Rosaceae family. These proteins are also very resistant to abrupt pH changes, thermal treatments, and pepsin digestion, having the ability to refold to their functional structures after cooling. Belonging to the same prolamin superfamily, nsLTP are only slightly less thermally stable than the 2S albumins, possibly due to the presence of a lipid-binding tunnel.[28] This group of molecules is included in the so-called panallergens that are, by definition, allergens ubiquitously spread throughout nature. Although the molecules originated from different and unrelated organisms, they are composed by similar conserved sequence regions. The nsLTP family presents highly conserved sequences and tridimensional structures that enable IgE recognition, promoting cross-reactivity among these types of proteins.[40] In addition to these facts, nsLTP are present in diverse Rosaceae fruits and seeds such as apple, peach, plum, sweet cherry, apricot, and almond, implicating a probable cross-reactivity among them.[26]

Pru du 4 (Profilins). Pru du 4 proteins belong to the profilin family and are encoded by the putative allergen genes *Pru du 4.01* and *Pru du 4.02* (Table 1).[23,25] *Pru du 4.01* and *4.02* genes exhibit fragments of different sizes, 1041 and 754 base pairs (bp), respectively, encoding two proteins with identical sequences (131 aa), molecular weights of approximately 14 kDa, and acidic properties (p*I* of approximately 4.6). Profilins participate in the binding of a monomeric actin (G-actin) that is responsible for establishing a high-affinity complex with actin, regulating the polymerization of actin into filaments.[41] Like the nsLTP, profilins are also classified as panallergens. These proteins display a high degree of similarity and identity with several other profilins from diverse plant and tree species, revealing cross-reactivity due to the highly preserved amino acid sequences as well as the shared IgE-reactive epitopes.[23,42] IgE cross-reactivity is related to the general three-dimensional profilin fold, being composed of a five-stranded antiparallel β-sheet and two α-helices.[43] According to Tawde et al.,[23] allergens Pru p 4.01 and Pru av 4 from peach and sweet cherry, respectively, are the two proteins presenting the highest identity and similarity (99 and 98%, respectively) with almond profilins. Even apparently nonrelated species such as soybean (*Glycine max*) or olive (*Olea europeae*) exhibit >80% identity and 90% similarity with Pru du 4 allergen.[23] Therefore, it is not unexpected that a profilin from one plant species can cross-sensitize an individual to other plant tissues, such as pollen profilins sensitizing individuals to food profilins.[44] The sensitization to these proteins can result in allergic reactions to proteins from a wide range of fruits and vegetables, including fruits or seeds of the Rosaceae family such as Mal d 4 in apple,

Pru p 4 in peach, Pru av 4 in sweet cherry, or Pru du 4 in almond, among many others.[23,45]

Unlike other food allergens such as nsLTP or 2S albumins, profilins seem to display moderate structural stability,[23] because adverse conditions contribute to the denaturation of profilins and subsequent loss of conformational structure. The labile character of Pru du 4 profilin and the low levels of this protein in almond explain the difficulty of detecting it by immunoblot screens. Profilins are generally defined as minor but rather important allergens in many plant foods. The positive detection of almond profilin in 44% of patients' sera suggests the classification of Pru du 4 as a minor but important allergen.[23]

Pru du 5 (60s Acidic Ribosomal Protein P2). Almond allergen Pru du 5, also known as 60S acidic ribosomal protein P2, is encoded by the *P. dulcis* 60S acidic ribosomal protein gene (*AL60SRP*), with a size of 604 bp (Table 1).[24,25] The ribosomal P2 proteins occur in the ribosome as multimers appearing as sets of heterodimers. P2 proteins seem to be more externally located and subsequently more likely to interact with other cellular components. The biological function of this protein is based on the successive addition of amino acid residues to a polypeptide chain during protein biosynthesis.[25] The expression of a recombinant 60S ribosomal protein of almond (r60sRP) enabled the calculation of a molecular mass of approximately 11.4 kDa with a deduced peptide sequence of 113 aa, being reported for the first time in 2009 as an almond allergen.[46] This protein exhibits 81% identity and 94% homology with the recently described protein ARP60S from tomato,[47] which may indicate possible cross-reactivity between them. The presence of IgE antibodies for r60sRP in 50% of sera of patients sensitized to almond seems to classify this protein as a major allergen in almond,[46] according to the allergen nomenclature guidelines specified by the International Union of Immunological Societies (IUIS) Allergen Nomenclature Subcommittee.[24] Nonetheless, this classification must be supported with more studies regarding the IgE reactivity of patients' sera to this allergen.

Pru du 6 (Amandin). Amandin or almond major protein (AMP) is normally referred to as a member of the cupin superfamily, specifically belonging to the 11S seed storage globulin family.[26,48] The globulins are highly abundant proteins, accounting for >50% of the total seed proteins in various legumes and tree nuts. The globulins are divided in two groups, namely, the 7S vicilin type and the 11S legumin type, in which the amandin protein is included (Table 1). The functional 11S legumins are hexameric proteins, comprising six subunits with a total molecular weight of about 360 kDa.[26] Isolation and sequencing of cDNA clones from almond enabled the inference that the cDNA encoded two seed storage proteins of 61.0 and 55.9 kDa, designated prunin-1 (Pru-1) and prunin-2 (Pru-2), respectively (Table 1).[49] Both Pru-1 and Pru-2 have two polypeptides linked by disulfide bonds with 551 and 504 aa, respectively. Pru-1 is composed of an acidic α-chain of 40.1 kDa with a p*I* of 5.4 and a basic β-chain of 20.9 kDa with a p*I* of 9.6. Pru-2 is divided into two subunits of 34.5 kDa (p*I* 4.6) and 21.4 kDa (p*I* 9.5), corresponding to the α- and β-chains, respectively.[49] Pru-1 is highly water-soluble and readily cold-precipitable like other proteins from the 11S family and was recently identified as a major component of almond amandin. Pru-1 and Pru-2 are assembled in a functional protein (amandin) by means of disulfide bonds, conferring an elevated thermal stability to the entire protein.[50]

Amandin is classified both as a major protein component and as a major allergen in almond,[51,52] although the IgE epitopes of Pru-1 or amandin have not yet been identified.[53] 11S globulins, such as amandin, are thermally stable proteins known to suffer partial unfolding only at temperatures >94 °C, aggregating to form different structures within foods. The denaturation process of this type of protein, which consequently decreases their allergenicity, involves the presence of water. Almonds are often thermally treated with low-water systems, such as roasting, that rather increase the thermal stability of these proteins.[54] Until now, amandin has been the most widely studied allergen in almond with regard to its molecular structure and biochemical function.[50−53,55]

Pru du γ-Conglutin. The γ-conglutin proteins belong to the vicilins (7S globulins) of the cupin superfamily. These proteins have trimeric structures with a molecular weight of approximately 150−190 kDa, with each subunit ranging from 40 to 80 kDa (Table 1). The composition of each subunit diverges considerably, essentially due to their differences in the extent of post-translational processing (proteolysis and glycosylation).[56] Like in other fruits and seeds in which conglutins have been identified and characterized, such as in peanut,[57] soybean,[58] cashew,[59] or lupine,[60] γ-conglutin was also identified in almond with a peptide sequence of 25 aa and a molecular weight of 45 kDa.[38] This protein comprises IgE-binding epitopes located in the 30 kDa N-terminal region of the sequence. Because seed conglutins are processed in two subunits, one small C-terminal subunit of 17 kDa and a heavy chain N-terminal subunit of 28−30 kDa, it was advanced that the 30 kDa almond peptide corresponded to the heavy chain of the γ-conglutin protein.[38] A sequence identity of about 40% and homology of 60% were found between the mature form of γ-conglutin from white and narrow-leafed blue lupine and γ-conglutin from almond. High similarity (50%) was also observed between the 7S globulin from soybean and the conglutin-like protein from *Arabidopsis*,[38] contributing to a probable cross-reactivity among these seeds.

CLINICAL SYMPTOMS ATTRIBUTED TO ALMOND ALLERGENS

According to the clinical manifestations, the physical/chemical characteristics of plant-derived food allergens, and the underlying immunological mechanisms, two different classes of IgE-mediated food allergies can be distinguished. In class 1, food allergy sensitization occurs through the gastrointestinal tract and is often caused by stable allergens. This class of food allergy is more frequent in children. In contrast, class 2 food allergy is more likely to appear later in life, affecting mostly adolescents and adults. This allergy is most probably developed as a consequence of sensitization to inhaled allergens. The basis for class 2 food allergy is immunological cross-reactivity due to high amino acid sequence identity and structural homology between food and inhaled allergens.[61]

Almond allergy is frequently associated with allergies to other fruits from the Rosaceae family in patients sensitized to birch pollen. This pattern of sensitization is more common in northern European countries in the context of a cross-reactive syndrome to PR-10 proteins, where multiple sensitizations to different pollens, fruits, nuts, and other vegetables can occur. In most cases, immunological reactions are typically mild and the prominent clinical manifestation is related to the OAS. However, severe allergic reactions have been attributed to members of the PR-10 protein family in patients allergic to

birch pollen.[62,63] This type of reaction arises from the homology among Pru du 1, Bet v 1, and other PR-10 allergens.[32,64,65]

Food allergy related to almond and other Rosaceae fruits can also happen without previous relevant pollen sensitization and is often attributed to allergens from the nsLTP family, in which Pru du 3 is included. The symptoms are frequently systemic and life-threatening, and cross-reactivity among nsLTP of different Rosaceae fruits has been described.[66,67] This pattern of sensitization is more recurrent in Mediterranean countries, where fruits from the Rosaceae family are widely cultivated. The nsLTP allergens are usually accumulated in the outer epidermal layers of plant organs; thus, patients displaying Rosaceae nsLTP-specific IgE antibodies often tolerate peeled fruits and certain foods, such as carrots, potatoes, bananas, and melon. Even so, sensitized individuals may be at risk of developing severe allergenic symptoms upon ingestion of nuts.[40]

TLP or PR-5 proteins include the almond allergen Pru du 2 and other fruit proteins from the Rosaceae family such as apple (Mal d 2),[33] peach (Pru p 2),[19] or cherry (Pru av 2).[68] Additionally, this group of proteins has also been described in other fruits belonging to different botanic families, such as kiwi from the Actinidiaceae family[69] and banana from the Musaceae family.[70] The clinical relevance of sensitization to distinct TLP continues to be a matter of debate, although TLP found in edible fruits have been recognized as being potent food allergens, liable to trigger allergic reactions in sensitized individuals.[71] The presence of these proteins in almond may be responsible for some of the allergic responses associated with this seed, so further studies should be conducted to establish its relevance.

A broad spectrum of cross-reactivity between profilins of inhaled and nutritive allergenic sources has been described because homologue profilins can be virtually found in almost every plant source.[40,72,73] Considering that almond contains the panallergen Pru du 4, the risk of sensitization to multiple foods and pollens in a patient allergic to profilins is elevated.[40] Fortunately, the clinical manifestations associated with profilin allergy are considered to be mild and mainly limited to the oral cavity. Profilins are not very resistant to heat denaturation and gastric digestion; thus, they cannot cause sensitization through the gastrointestinal tract, behaving as class 2 food allergens.[61] Many profilin-sensitized patients do not exhibit symptoms.[40,44] In contrast, Asero et al.[74] demonstrated that profilins can be considered as clinically relevant food allergens in specific food-allergic patients. The overall impression from clinical studies is that patients displaying profilin-specific IgE antibodies can be either asymptomatic or at risk of developing multiple pollen-associated food allergy.

Amandin (Pru du 6) has been defined both as a major storage protein and as a major allergen in almond, being one of the first allergens to be studied in almond. Roux et al.[35] reported amandin as a major allergen related to severe reactions to almond upon ingestion. Polypeptides from amandin are highly resistant to different heat treatments during food processing,[75] and the contamination of food with this allergen can lead to a significant risk of increasing the number of sensitized patients. In a recent study from Holden et al.,[76] it was suggested that amandin can possibly cross-react with α-conglutin from lupine, because this protein is another 11S globulin. To establish the clinical significance of this cross-reactivity, oral challenge tests in almond- and lupine-allergic patients should be performed.

The seed storage proteins 2S albumin and γ-conglutin identified in almond were characterized as IgE-binding proteins.[38] The availability of sera from patients allergic to almond, who were reactive to skin prick tests and positive-responsive to almond in oral challenge tests, permitted the isolation of these two almond allergens. However, it was emphasized that the IgE binding and the serological reactivity of these proteins do not imply the clinical symptoms of the allergy, and further studies of clinical reactivity, particularly regarding food challenges, are needed.

Pru du 5 was described recently in the literature as an almond allergen. The immunoreactivity of the r60sRP was evaluated with dot blot analysis using pooled and individual sera of allergic patients, showing that the expressed Pru du 5 proteins possess the ability to bind the IgE antibodies. However, to classify it as a major allergen, further investigation is still required involving a large number of sera from almond-allergic patients.[46]

The recent research based on the characterization of allergenic components has opened new perspectives in the diagnosis of food allergy. The possibility of using a large number of single allergenic proteins, either in vivo or in vitro, in diagnosing food allergy at a molecular level will have a considerable impact on the clinical management of food allergies in the near future. More collaborative studies between clinicians and researchers should be encouraged, because those would certainly enable better knowledge of the mechanisms of reaction of each specific group of allergens, their clinical manifestations, and the best preventive treatments for allergic patients.

DETECTION OF ALMOND AND OTHER FOOD ALLERGENS

The need for adequate methodology to detect food allergens has been rapidly increasing over recent years, especially in response to the demands imposed by current legislation. The food industry has been addressing with special interest the necessities of food allergic consumers, not only concerning the proper food labeling but also minimizing allergen cross-contamination among foodstuffs. Therefore, suitable analytical methods are required to detect allergenic proteins, as they are mostly present at trace levels.[77] The requirements needed for detecting allergenic ingredients in food involve appropriate specificity and sensitivity to trace minute amounts of the target allergens or the correspondent markers in complex food matrices, including processed foods.

The determination of upper limits for allergenic noningredient food components would be important progress for the protection of allergic consumers. Nevertheless, these limits are meaningful only with the development of adequate analytical methodologies to verify compliance.[78] According to Poms et al.,[79] the ideal limit of detection (LOD) for allergens in food products should range between 1 and 100 mg/kg. The "food allergy" working group of the German Society for Allergology and Clinical Immunology and the Association of German Allergologists proposed upper limits of 10−100 mg/kg of the allergenic food or 1−10 mg/kg of the protein fraction of the allergenic food, depending on its allergenicity, which would protect most allergic consumers from severe allergic reactions.[78] The study performed by Morrisset et al.[80] to establish the thresholds of clinical reactivity to milk, egg, peanut, and sesame in allergic patients suggested that detection tests should ensure sensitivities of 10, 24, and 30 mg/kg for egg, peanut, and milk proteins, respectively, to guarantee 95% safety for patients who are allergic to the referred foods, on the basis of the

Table 2. Commercial Immunoassays for the Detection of Almond Allergens

commercial kit	assay type	brand	LOD (mg/kg)	time for sample testing (min)	catalog no.
Rapid-3-D Almond Test Kit	lateral flow device (positive/negative)	Tepnel	1	10	902086G
Reveal 3-D Almond Test	lateral flow device (positive/negative)	NEOGEN Corp.	5	10	902086G
BioKits Almond Assay Kit	polyclonal antibodies to almond protein, noncompetitive sandwich type ELISA	NEOGEN Corp.	0.1	90	902083N
BioKits Almond Assay Kit	polyclonal antibodies to almond protein, noncompetitive sandwich type ELISA	Tepnel	0.1	90	902083N
Alert Almond Assay Kit	sandwich ELISA	NEOGEN Corp.	5	30	8441
RIDASCREEN FAST Mandel/Almond	polyclonal antibody specifically for almond protein detection, sandwich ELISA	R-Biopharm AG	1.7	30 (sample extraction)	R6901
ELISA Systems Almond	ELISA	ELISA Systems		35 (sample extraction)	95200 ESARD-48
Veratox for Almond Allergen	sandwich ELISA	NEOGEN Corp.	2.5	30	8440

consumption of 100 g of food. In the specific case of oil allergies, the limit of sensitivity should fall to 5 mg/kg.

One major prerequisite for the development of analytical methods, including allergen detection techniques, is the availability of certified reference materials (CRM). The existing materials from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) for peanut testing (IRMM-481) consist of five distinct types of peanut powders of different varieties and geographical origins, but they are not reference or certified materials. With regard to tree nuts, there are no test materials yet available supplied by the IRMM. Presently, an accredited Greek laboratory[81] has released a set of testing reference materials for the detection of some tree nut allergens such as almond, hazelnut, and walnut, but appropriate stability assays are lacking.

Several methods for almond detection have been developed, mainly relying on immunochemical and DNA-based techniques. The immunochemical methods include enzyme-linked immunosorbent assays (ELISA), lateral flow devices (LFD), dipsticks tests, immunoblotting, and biosensors. These methods have been successfully applied for the detection of allergens in food with the specificity based on the precise binding between epitopes present on the target protein and an immunoglobulin. Nevertheless, the use of immunoassays has numerous problems mainly due to the cross-reactivity with nontarget proteins and the low resistance of proteins to food processing, because it can cause conformational changes in the tridimensional structure of the epitopes (e.g., heat induced denaturation) and/or protein cleavage, affecting linear epitopes (e.g., fermentation).[82] During the thermal processing of foods, several interactions between food allergens and other molecular components can occur, such as protein modifications induced by Maillard reactions. Until now, little is known about how thermal processing, Maillard reactions, and other possible chemical modifications can influence the performance of commercially available immunoassay kits for the detection of allergens in foods.[83,84] In addition, it is important to remember that the solubility of a protein is also affected by chemical modifications (progressive Maillard reactions), conditioning the extractability of this analyte from the food matrix. All of these factors may, consequently, contribute to the low reproducibility, as well as increased chances of false-negative results observed with immunoassays (ELISA kits) because they are based on analyte−receptor binding.[84] Another interesting point about the immunoassays is the type of allergens used to produce the antibodies. Some authors suggest that the antibodies used in ELISA kits are produced in different conditions, and the accuracy of these methods can be affected by them.[84]

Lately, DNA-based methods have been increasingly used as highly sensitive and specific alternatives for allergen detection, taking advantage of the greater thermal stability of DNA molecules compared to proteins. These techniques rely on the use of polymerase chain reaction (PCR), either as qualitative endpoint PCR or as quantitative real-time PCR assays. The specificity is achieved using primers and probes specifically designed for the gene encoding the allergen or marker protein. Methods combining both PCR and ELISA have been developed for the detection of food allergens to fit the labeling requirements imposed by the legislation.[85]

Immunochemical Methods. ELISA is probably the immunoassay most widely used for the detection of food allergens. It relies on the specific interaction between the antibody and the antigen, which is the allergen or marker protein in the case of food allergen detection. There are different types of ELISA tests (sandwich, competitive, and indirect) available for food analysis, but the most commonly chosen is the sandwich ELISA. Immunoassays can provide qualitative or quantitative results. In qualitative tests, the results are expressed simply as positive or negative, whereas in quantitative ELISA, the optical or fluorescent signals of the unknown samples are compared with standard curves consisting of known quantities of target proteins serially diluted. Table 2 presents a set of commercially available ELISA kits for the detection of almond allergens. These tests present the advantages of rapid performance and versatility, being extensively applied with reliable results, and LOD down to 0.1 mg/kg of almond protein in food samples within 30−35 min.[86−88] In almond, almost 95% of the total protein content is water-soluble, making it easily accessible, which contributes to the frequent use of ELISA tests for almond protein detection.[88]

Roux et al.[51] reported the development of a competitive ELISA for the detection of the major allergen of almond, amandin, because this protein accounts for approximately 65−75% of total almond protein, presenting high thermal stability. The proposed method was considered to be very sensitive (detection of 5−37 mg/kg of AMP in several spiked foods) and specific, presenting only minor cross-reactivity with some globulins and albumins from other nuts and legumes.[51] Rejeb et al.[89] developed a multiresidue methodology based on competitive indirect ELISA, which allowed the simultaneous determination of almond, peanut, hazelnut, Brazil nut, and cashew nut with a LOD of 1 mg/kg of target protein in chocolate samples. Garber et al.[87] compared three commercial

sandwich ELISA test kits for the detection of hazelnuts and almonds. The determined LOD and dynamic ranges for almonds spiked into cooked oatmeal, dipping chocolate, and muffins (baked) varied from 3 to 39 mg/kg, depending on the food matrix and the tested ELISA kit.[87]

Lateral flow devices or dipstick assays are another type of immunochemical test applied to the detection of allergens in food. They are based on the same principle as ELISA, but with simpler and faster performance (~10 min), making them quite often used by the industry for rapid food screening.[88] The results are mainly qualitative or semiquantitative and can be interpreted visually. Like the ELISA tests, there are two types of LFD, the sandwich and competitive formats. Some drawbacks associated with this type of assay can be pointed out due to the susceptibility of these devices in providing false-negative results as well as the lack of quantitative information.[90] Recently, several commercial kits have become available for the quick on-site detection of food allergens, including the LFD tests that provide rapid information about the presence of certain allergens within a few minutes. The application of LFD to foods can allow the detection of almond protein down to 1 mg/kg in <10 min.[88] Table 2 lists the commercially available LFD and ELISA kits for the detection of almond allergens in raw and processed foodstuffs.

Another protein-based method for food allergen detection consists of the use of immunoblotting as a very reliable tool, although not adequate for routine analysis. It constitutes a choice for confirmatory testing of the presence of allergens in food, allowing the characterization of IgE from sensitized individuals and the evaluation of antibody specificity. Scheibe et al.[91] have described a sensitive protocol for the detection of almond in chocolates using SDS immunoblot with a chemiluminescence detection method with a LOD of 5 mg/kg of almond protein in chocolate.

Biosensors, because of their characteristics of fast response time and low cost, are very attractive platforms for new applications in different emerging fields such as allergen detection. They are analytical devices consisting of a biological recognition element (e.g., cells, proteins, and oligonucleotides) in direct contact with a transducer that produces the signal. Immunochemical sensors are able to measure interactions between different molecules in real time and can be applied for the detection and quantitation of food allergens.[92] The antibody–allergen interaction can be detected by different types of transducers (optical, acoustical, amperometrical, or potentiometrical), producing a signal that is further processed to give a proportional output to the concentration of a specific analyte. The optical biosensors base their function on the phenomenon of surface plasmon resonance (SPR). Their application was demonstrated for the detection of peanut[93,94] and other allergens from milk, egg, hazelnut, shellfish, and sesame, reaching levels of detection comparable to those of the most sensitive ELISA.[94] More recently, Bremer et al.[95] developed a rapid and sensitive direct biosensor immunoassay based on a highly specific monoclonal antibody to identify the presence of hazelnut proteins in olive oils. Biosensors have several potential applications, although only a few have been proposed for food allergen detection and, to our knowledge, none of them target almond allergens. More studies are still required to fully understand their potential utilization for monitoring the presence of allergens in foods, namely, tree nut detection.

Mass Spectrometry (MS)-Based Methods. One of the current problems associated with the detection of allergenic proteins and peptides is their identification. In this regard, MS-based methodologies have demonstrated their usefulness in obtaining information for the identification of allergenic proteins.[96] MS methods can overcome the drawbacks of cross-reactivity phenomena of immunoassays and the inability of DNA techniques to directly detect the allergenic protein. The advantages of MS rely on the unambiguous confirmation by proteins/peptides. Information about molecular mass is provided, and protein identification can be carried out by means of database search algorithms using the number of matching sequences, fragments, and peptides.[96] The identification of proteins by MS technology is usually performed using the "bottom up" approach, which is conducted on the basis of the digestion of proteins with a specific protease, commonly trypsin. Mass spectra are recorded after the separation of proteolytic fragments by reversed-phase HPLC.[97] Considering the diversity of allergenic molecules, the process of purification is specifically developed to guarantee unambiguous recognition of the molecule by the generation of a peptide mass fingerprinting. Additionally, in the case of processed foods, which may have altered patterns of proteins/peptides, the MS approach often provides insights into the nature of protein modifications readily elucidated by MS and MS/MS spectra.[96]

Some applications using liquid chromatography coupled to MS have been reported to detect hidden food allergens mainly from peanut.[98−101] Only very recently was the detection of food allergens from tree nuts reported. Bignardi et al.[102] successfully applied a method based on liquid chromatography–electrospray–tandem mass spectrometry (LC-ESI-MS/MS) for the simultaneous detection of five allergens (Ana o 2, Cor a 9, Pru 1, Jug r 4, and Ara h3/4) from cashew, hazelnut, almond, walnut, and peanut, respectively, in food matrices. The assays allowed the detection and quantitation of Pru 1 protein down to levels of 17 and 58 mg/kg, respectively, in biscuits. Another approach including the detection of tree nut allergens consisted of a multimethod to detect seven allergens based on liquid chromatography and triple-quadrupole tandem MS.[103] The use of marker peptides implemented in multiple detection mode was capable of simultaneously identifying milk, egg, soy, hazelnut, peanut, walnut, and almond in concentrations ranging from 10 to 1000 mg/kg in incurred bread material. With regard to almond detection, four different marker peptides were used to target prunin as the target allergen, from which one enabled a LOD of 3 mg/kg of almond in bread material.

DNA-Based Methods. These techniques consist of the specific amplification of a gene fragment encoding a protein from the allergenic ingredient by means of PCR, the specificity of which is achieved by the use of primers and, frequently, probes. Although these methods do not target directly the offending proteins, they are considered to be very sensitive and specific, taking advantage of the elevated stability of DNA molecules at high temperatures and their resistance to high pH values. In addition, the DNA-based methods can be included in routine analysis and act as a confirmatory tool, when adequate immunoassays do not exist.

Despite the advantages of DNA-based methods, PCR is still much contested because when detecting a gene encoding for an allergen, it does not necessarily imply its expression. Consequently, the results obtained by DNA detection do not account for the actual allergenic potential. However, the same happens with some, if not most, ELISA tests that do not necessarily detect the allergenic proteins, but rather species-specific protein markers. In fact, the detection of a molecular

marker gives indirect information of the allergenic potential, but provides the presence of the allergenic ingredient.[104]

Recent reviews have demonstrated the increased number of applications of DNA-based methods and their suitability to detect food allergens.[82,96,105] In these methods, the specific target is amplified either by end-point PCR, being distinguished on the basis of their differential migration through agarose gel electrophoresis, or by real-time PCR using fluorescent labeled probes or dyes. Other PCR-based approaches such as ligation-dependent probe amplification (LPA)[106] and the combination of PCR amplification with ELISA have also been successfully implemented to detect food allergens.[85] Nevertheless, real-time PCR has been so far the most widely applied PCR strategy to detect food allergens. Several real-time PCR approaches have been proposed to detect food allergens from peanuts,[107−112] celery,[107,113] mustard,[113] lupine,[114,115] sesame,[109,113,116] and tree nuts including hazelnut,[107,104,109,116−119] walnut,[109,120] macadamia,[121] pecan,[122] pistachio,[123] cashew nut,[109,124] Brazil nut,[125,126] and almonds.[107,109,127−129]

Pafundo et al.[127] developed two systems for the detection of almond allergens using SYBR GreenER real-time PCR. The systems specifically targeted the genes encoding for the allergenic protein Pru 1 (prunin), the major component of amandin, and the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbc*L) of *Prunus persica*, as marker for *Prunus* detection, because it is a multicopy chloroplastic sequence. The development of the two systems allowed the detection of Pru 1 in biscuits containing processed almond down to 1 DNA copy. In another work, the same authors reported the development of a multiple-target assay based on SYBR GreenER real-time multiplex PCR to detect sesame, peanut, cashew nut, hazelnut, walnut, and almond.[109] This method enabled the detection of low quantities of almond DNA (5 pg), with LOD ranging from 1 to 100 mg/kg of almond in spiked biscuits.[109] Köppel et al.[107] presented two tetraplex real-time PCR systems for the detection of eight allergens in foods based on the application of TaqMan probes. The proposed systems were called "AllAll A" and "AllAll B". "AllAll A" allowed the simultaneous detection of DNA from peanut, hazelnut, celery, and soy, whereas "AllAll B" enabled the detection of milk, sesame, egg, and almond in food. The assays exhibited good specificity and sensitivity in the range of 0.01% of target ingredient in rice cookies. Concerning the specific detection of almond, a LOD of 10 mg/kg was obtained for almond spiked in rice cookies. In the same work, the PCR results, when compared to ELISA, seemed to indicate a correlation between both methods, although more investigation is needed to support this suggestion.[107] Röder et al.[128] have also developed a method based on real-time PCR system with TaqMan probes to detect almond allergen nsLTP (Pru du 3) down to a LOD of 5 mg/kg of almond in a variety of food matrices. In this study, the PCR results were matched with those of ELISA within the known limits of variation for these tests in spike levels >100 mg/kg, allowing the establishment of a qualitative correlation between the developed real-time PCR system and two commercial ELISA kits.[128] Another study regarding the detection of a different almond allergen (Pru du 5) was proposed by Costa et al.[129] by means of high-resolution melting (HRM) analysis in a real-time PCR system with Evagreen DNA binding dye. The authors reported the detection of the gene encoding for Pru du 5 allergen with a relative LOD of 50 mg/kg of almond in walnut material and an absolute LOD of 10 pg of almond DNA. The application of HRM analysis for almond detection allowed almond to be distinguished from other fruits of the Rosaceae family such as peach, apricot, and nectarine.[129]

An important issue concerns the effect of matrix on allergen detection. A comparative evaluation of ELISA and real-time PCR techniques in detecting and correctly quantitating hazelnut in food model systems was recently described by Platteau et al.[117] These authors demonstrated that food processing has an impact on hazelnut detection in cookies and cookie ingredients using real-time PCR as well as ELISA. They further indicated that both methods lacked robustness with regard to food processing, without drawing any firm conclusion about the technique most suited to the detection of hazelnut in processed foods, highlighting the need for adequate reference materials.

SUMMARY

In recent years, some studies have been performed to characterize the allergenic proteins present in almond. To our knowledge, currently, eight groups of allergens have been identified and characterized, as well as the respective allergenic isoforms. Although some of them have not yet been well-defined with regard to their clinical implications in sensitized individuals, most are known to trigger severe adverse reactions and are susceptible to cross-reactivity with homologous allergens among other fruits from the Rosaceae family. Furthermore, an adequate characterization of the allergenic components of almond could provide new insights in the diagnosis of almond allergy and facilitate the development of preventive treatments.

Almonds are frequently subjected to harsh processing conditions prior to or during their incorporation into foods. Protein denaturation, aggregation, and structure disruption can be promoted by thermal/chemical treatments, having a potential to modify allergenic properties of almond proteins. In this context, molecular characterization studies on almond allergens are also important issues because the functionality and immunoreactivity of a protein are closely linked to its conformation. Thus, structural changes induced by thermal and/or chemical denaturation should be studied to provide important information regarding its global stability, which may help explain changes in allergenicity that occur as a result of food processing.

Food allergy with respect to almond is an important health problem due to its wide use in the food industry and, consequently, considered as a potential source of hidden allergens derived from the incorrect labeling or unintentional inclusion via improper cleanup and cross-contamination in the processing system. On the other hand, to comply with legislation, excessive labeling about the presence of potential allergens in foodstuffs may also contribute to restrict the range of adequate foods for allergic individuals.

As a consequence of the established clear guidelines on food allergen labeling, an increasing need for the development of suitable analytical methods has arisen. Immunoassays, such as ELISA, for the detection of food allergens are probably the most widely used techniques due to their high sensitivity and specificity to target the offending proteins. To overcome the problems associated with the immunochemical assays, namely, cross-reactivity, reduced protein solubility, and degradation caused by food processing, DNA-based methods have emerged as proper alternatives to detect food allergens without the need for adequate antibodies. However, recent reports have demonstrated that DNA analysis is also affected by food matrix and processing, leading to incorrect quantitation. In our opinion the

effect of food processing on the recovery and actual levels of detection for both DNA and protein methods should be adequately addressed in more future research. Another important issue, in the case of DNA methods, concerns the choice of adequate extraction protocols to obtain DNA extracts from complex food matrices, free of PCR inhibitors, maximizing the assay sensitivity.

With regard to the major requirement for allergen identification, MS-based methods through the combination of liquid chromatography with MS detection have emerged as reliable tools for unambiguous identification of proteins or peptides from allergenic foods with potential for quantitative analysis and for detecting changes after food processing. MS methods overcome the biggest problems of ELISA (cross-reactivity issues) and PCR (indirect identification of the target allergen), allowing direct detection of proteins without the need for antibodies and with potential for the simultaneous analysis of multiple allergens.

Several protein- and DNA-based methodologies have become available for the detection of allergenic ingredients in food, but the question about the most appropriate technique for allergen detection and quantitation is yet a matter of debate. Opinions continue to diverge about the best target analyte (protein or DNA molecules) to be used and on the best methods to detect them on a routine analysis basis. Official guidelines should be implemented shortly, regulating limits for the presence of potentially allergenic ingredients in prepackaged food and the recommended methodology for their monitoring.

Because the only effective method to manage food allergies for sensitized consumers at present is the avoidance of foods containing the provocative proteins, analytical methodologies to detect food allergens at trace levels have gained utmost importance. They range from well-documented protocols to newly developed tools, but reference methods, which are always needed to standardize procedures in the development of other analytical assays, are still lacking. To support this requirement, the rapid development of reference materials is of high priority.

Finally, clinicians and food chemists should work more closely on harmonization of procedures that can provide better understanding in clinical allergy tests and food analysis.

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Notes

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