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Pistachio nut allergy: An updated overview

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ABSTRACT

Pistachio nut (*Pistacia vera*) is highly appreciated for its organoleptic characteristics and potential health benefits. However, this tree nut is also responsible for triggering moderate to severe IgE-mediated reactions in allergic individuals. Currently, pistachio nut allergy has gained some special attention, mainly due to its intrinsic relation with cashew nut allergy. Like for other nuts, the prevalence of pistachio nut allergy seems to be increasing at a global scale. Until now, there are five allergenic proteins officially listed for pistachio nut (Pis v 1, Pis v 2, Pis v 3, Pis v 4 and Pis v 5). Relevant data on their biochemical classification has become available, enabling establishing a correlation with the respective clinical symptoms. The establishment of an effective allergen risk assessment is a key issue for the food industry, policy makers and regulatory agencies. Thus, the availability of fast, specific and sensitive methods to detect trace amounts of allergens in processed foods is crucial. In the specific case of pistachio nut, there are some protein- and DNA-based methods for its detection/quantification in foods, which can aid to verify label information. Accordingly, all relevant research advances on this topic were summarised, updated and critically discussed in this review.

Introduction

As part of the Anacardiaceae family, pistachio nut belongs to the *Pistacia* genus. So far, there is little, or even no consensus, about the exact number of species included in this genus (Al-Saghir and Porter 2012; Yi et al. 2008). As some species present more than one scientific denomination, their correct taxonomic classification has been difficult to achieve. Considering the information available at the United States Department of Agriculture – Germplasm Resources Information Network (USDA-GRIN 2017), the genus *Pistacia* encompasses at least 12 different species, some of them with different subspecies (Table 1) (USDA-GRIN 2017). The *Pistacia vera* is the species with the highest economic interest, mainly due to its edible nuts (Al-Saghir and Porter 2012; Kashaninejad et al. 2006). However, other *Pistacia* species are also used with different purposes, namely as sources of materials (wood, gums, resins, dyestuffs and tannins), fuels and compounds for traditional medicine (Table 1). *Pistacia vera* is native to Middle (Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan) and Western (Afghanistan and Iran) Asia, but nowadays it is also cultivated in many countries of the Americas, Europe and Africa (Table 1). In 2014, pistachio nut represented 6% of the total production of tree nuts. In the same year, its main producer was Iran (48.4%) followed by the USA (27.2%), Turkey (9.3%) and China (9.0%), thus ensuring almost 94% of the global production of pistachio nuts. In terms of trade, pistachio nut occupied the third place among the other

tree nuts, just behind almond and cashew nut (FAOSTAT 2017). In 2015, Turkey presented the highest domestic consumption of pistachio nuts (128,000 tonnes), followed by the European Union (81,700 tonnes), China (70,000 tonnes) and the USA (60,000 tonnes) (IndexMundi 2017; USDA 2017).

The members of *Pistacia* genus are among the oldest flowering nut trees, being small to medium in size and wind-pollinated. These species are temperate deciduous trees with physiological adaptations to desert and saline environmental conditions, so they are well adjusted to long, hot, dry summers, moderate winters and seem to well tolerate alkalinity and salinity (Ferguson and Kallsen 2016; Kallsen et al. 2009; Kashaninejad and Tabil 2011). Owing to the fact that pistachio trees are dioecious, both female and male trees are necessary for nut production (Ferguson and Kallsen 2016). There is a large number of pistachio cultivars, but depending on the geographical region, different varieties can be found. Kerman, Red Aleppo, Joley, Trabonella, Bronte, Kastel, Rashti and Sfax, are some examples of female cultivars, while Peters, Nazareth, Randy, Ask and Chico are examples of male cultivars (Kallsen et al. 2009; Parfitt, Kallsen, and Maranto 2016). Botanically, pistachio (*Pistacia vera*) fruits are semidry drupes (like almonds) composed of the following parts: a combined exocarp and fleshy mesocarp (forming the fleshy hull), and a hard endocarp. The endocarp is designated as a shell, which encloses the edible seed or nut. Presently, pistachio nuts are well appreciated for their pleasant taste/aroma and they can be consumed roasted/

Table 1. Economic importance, geographic distribution and identified allergens of the twelve species of *Pistacia* genus.

Species	Subspecies	Economic Importance	Geographic distribution	Allergenic proteins
Pistacia aethiopica	—	—	Africa (Tanzania, Ethiopia) and Asia-Temperate (Yemen).	—
Pistacia atlantica (presents 4 subspecies)	*P. atlantica* subsp. *atlantica*, *P. atlantica* subsp. *cabulica*, *P. atlantica* subsp. *mutica*, *P. atlantica* subsp. *Kurdica* (probably a hybrid of *P. vera* × *P. khinjuk*, also known as *P. eurycarpa*)	Human food: fruit; gene sources, materials: tannins and dyestuffs.	Africa (Algeria, Libya, Morocco, Tunisia), Asia-Temperate (Afghanistan, Iran, Syria, Turkey), Asia-Tropical (India and Pakistan) and Europe (Greece).	—
Pistacia chinensis (also known as *P. philippinensis*) (presents 2 subspecies)	*P. chinensis* subsp. *chinensis* *P. chinensis* subsp. *Integerrima* (also known as *P. integerrima*)	Ornamental purposes, gene sources, human food: vegetable, fuels: potential as biomass for energy generation, materials: wood, dyestuff/tannin	Asia-Temperate (China, Taiwan) and Asia-Tropical (Philippines). Western Asia (Afghanistan) and Asia-Tropical (India, Myanmar).	—
Pistacia coccinea (also known as *P. weinmanniifolia*)	—	Gene sources	Asia-Temperate (China) and Asia-Tropical (Myanmar and Vietnam).	—
Pistacia khinjuk	—	Gene sources, materials: gum/resin.	Northern Africa (Egypt), Asia-Temperate (Tajikistan, Iran, Turkey) and Asia-Tropical (Pakistan, India).	—
Pistacia lentiscus	—	Food additives; gene sources, materials: gum/resin, traditional medicine	Africa (Morocco, Tunisia), Western Asia (Israel, Syria, Turkey) and Europe (France, Portugal, Spain, Greece, Italy).	—
Pistacia mexicana	—	Gene sources	Northern America (Mexico) and Southern America (Guatemala, Honduras).	—
Pistacia saportae (probably a hybrid of *P. lentiscus* × *P. terebinthus*)	—	Gene sources	Africa (Algeria and Morocco), Asia-Temperate (Israel) and Europe (Italy, France, Portugal).	—
Pistacia simaruba (also known as *Bursera simaruba*)	—	Ornamental purposes, fuels, materials (gum/resin), traditional medicine	Northern America (Mexico, USA), Southern America (Brazil, Venezuela,), Central America (Belize; El Salvador), Caribbean (Anguilla; Cuba)	—
Pistacia terebinthus (presents 2 subspecies)	*P. terebinthus* subsp. *Terebinthus* *P. terebinthus* subsp. *Palaestina* (also known as *P. palaestina*)	Human food (fruit), gene sources, materials (gum/resin, tannin/dyestuff)	Africa (Morocco, Tunisia), Asia-Temperate (Syria, Turkey) and Europe (France, Portugal, Spain, Italy).	—
Pistacia texana	—	Gene sources	Northern America (Mexico, United States).	—
Pistacia vera	—	Human food (nuts), gene sources	Eastern Africa (Madagascar, Mauritius), Northern Africa (Morocco, Tunisia), Western Africa (Côte d'Ivoire), Northern America (USA), Central America (Mexico), Central Asia (Kyrgyzstan, Uzbekistan), Eastern Asia (China), Southern Asia (Afghanistan; Iran, Pakistan), Western Asia (Turkey, Jordan, Syrian Arab Republic, Azerbaijan, Cyprus), Southern Europe (Greece, Italy, Spain), Oceania (Australia).	Pis v 1, Pis v 2, Pis v 3, Pis v 4, Pis v 5

salted, as a snack or processed, or as an ingredient in a large variety of foodstuffs (chocolate, ice cream and confectionary products) (Kashaninejad and Tabil 2011; López-Calleja et al. 2014).

The consumption of tree nuts has been increasing owing to their alleged health benefits and common association with "healthy" food habits/diets. However, the rise in tree nut consumption also contributes to an increased risk factor for the development of tree nut allergies. For this reason, over the past 30 years, specific laws and regulations have been created to protect the health of all consumers, but with special impact on sensitised/allergic individuals. In theory, any food can be considered as potentially allergenic, although there is general consensus that eight groups of foods (cereals containing gluten, tree nuts, peanuts, soybeans, eggs, milk, seafood and fish) are the main cause for most (90%) of the reported allergic reactions. With respect to this fact, the labelling of those foods is mandatory for most countries/regions (Gendel 2012; Taylor and Baumert 2015).

Within the European Union, the priority list of allergenic items with mandatory food labelling includes 14 groups (cereals containing gluten, tree nuts, peanuts, soybean, eggs, milk, seafood, fish, mustard, celery, molluscs, lupine, sesame and sulphites), which are required to be highlighted from the rest of the ingredients enumerated in processed foods, regardless of their quantity (Directive 2007/68/EC 2007; Regulation (EU) No 1169/2011 2011).

This review provides an updated overview regarding different topics related to pistachio nut allergy, namely prevalence data, diagnosis, clinical relevance and available treatments. Additionally, it also addresses issues associated with the biological and biochemical characterisation of pistachio nut allergens, the effect of food processing conditions on their allergenicity, as well as the available analytical methods for monitoring the presence of pistachio nuts in foods.

Health benefits of pistachio nut consumption

In general, tree nuts are a good source of macronutrients (fat, proteins), micronutrients (minerals, vitamins), fat-soluble bioactive substances (tocopherols, phospholipids) and phytochemicals (polyphenols, flavonoids, alkaloids). Among them, pistachio nuts contain substantial levels of several potentially preventive phytochemicals, such as carotenoids (lutein), phytosterols and phenolic compounds (flavonoids and resveratrol), a high proportion of monounsaturated fatty acids, a high content of minerals/vitamins and relatively low calorie density, which are attractive and "healthy" food sources (Seeram et al. 2008).

Pistachio nuts present a relatively high content of the nonessential amino acid arginine that contributes to maintain the flexibility of the arteries and increase the blood flow by boosting nitric oxide (leading to the relaxation of blood vessels). These nuts have also been related to the improvement of blood lipid profile by decreasing the levels of triacylglycerols. Pistachio nuts seem to have cardio protective effects, thus helping to reduce the risk of coronary heart diseases. Among the tree nuts, pistachio nuts present the highest level of phytosterols (structurally similar to

cholesterol), namely the β-sitosterol that is most likely to contribute to a cholesterol-lowering effect. Additionally, pistachio nuts have been associated with an increased effect in serum antioxidants (Ros 2010; Vadivel, Kunyanga, and Biesalski 2012). These nuts have also been used in traditional medicine to treat several clinical conditions like cirrhosis, abdominal disorders, abscesses, amenorrhea, contusions, sores, trauma and dysentery (Dreher 2012; Kashaninejad and Tabil 2011; Sheridan et al. 2007; Singh and Kaur 2013; Tomaino et al. 2010).

In spite of all the health benefits that are associated with tree nut consumption, they can lead to immunoglobulin E (IgE)-mediated allergic reactions in sensitised/allergic individuals. Another major concern regards the potential contamination of tree nuts with mycotoxins (aflatoxins). They are highly toxic secondary metabolites produced by fungal species of the genus *Aspergillus* that are especially found in areas with hot and humid climates. The presence of aflatoxins is very common in tree nuts (especially in almonds, hazelnuts and pistachio nuts) and cereals (among other crops), representing a serious threat to both human and animal health because they can cause various complications such as teratogenicity, hepatotoxicity and immunotoxicity (Kumar et al. 2016). Aflatoxins are greatly resistant to most common food processing techniques (thermal, physical or chemical treatments), thus maximum levels of 8 or 10 μg/kg in pistachio nuts, as well as in almonds and hazelnuts, were recommended for dietary exposure (EFSA 2009).

General considerations about food allergies

Food allergy is defined as an adverse reaction to food in which different immunological mechanisms can be involved (Muraro et al. 2014b). Each food is composed by a complex set of proteins that can behave differently regarding their potential to sensitise and interact with the immune system. Food allergens are biochemically defined as water-soluble glycoproteins with molecular weight ranging from 10 to 70 kDa, presenting high resistance to heat, acid and protease activities (Sicherer 2011b). As a consequence, allergic reactions can occur when food is ingested raw, processed or even digested (Burks et al. 2012).

Despite food allergies being typically mediated by IgE, they can also include other immunological responses, namely non IgE-mediated (celiac disease), mixed IgE- and non IgE-mediated (eosinophilic gastroenteritis) and cell-mediated (allergic contact dermatitis) (Boyce et al. 2010; Burks et al. 2012). Accordingly, target organs or systems can be differently affected by each immunological mechanism. Cutaneous reactions are the most frequent presentations of food allergies and include IgE-mediated (angioedema, acute urticaria), cell-mediated (contact dermatitis), and mixed IgE-/cell-mediated (atopic dermatitis) disorders (Boyce et al. 2010). Immunological responses targeting the gastrointestinal tract are most typically IgE-mediated, such as the immediate gastrointestinal hypersensitivity (acute vomiting), although non-IgE (allergic proctocolitis, enterocolitis syndrome) and/or mixed IgE/non-IgE (eosinophilic gastroenteritis) mechanisms can also be related to food allergies. Respiratory manifestations of IgE-mediated food

allergy occur frequently during systemic allergic reactions, being associated with episodes of anaphylaxis. Contrarily, isolated respiratory symptoms, namely those of rhinitis and asthma, are not considered to be commonly caused by food allergy (Boyce et al. 2010).

An allergic reaction is normally characterised by pronounced type 2 inflammatory responses and circulating and cell-bound allergen-specific IgE. Its pathophysiology encompasses three main mechanisms that concern the breakdown of tolerance, allergen sensitisation and allergen reactivity (Bauer et al. 2015). The loss of tolerance promotes allergen sensitisation, which is characterised by T-helper-2 (T_H2)-dominant immune responses and B-cell class switching toward IgE and IgG. Accordingly, the T_H2-driven inflammation leads to the production of specific cytokines, namely the interleukins (IL)-4, IL-5 and IL-13. The IL-4 are responsible for T_H2 proliferation, B-cell isotype switching and mast cell development, while IL-5 induce the growth, maturation, recruitment and activation of eosinophils. The IL-13 prompt IgE synthesis, eosinophil and basophil recruitment, and epithelial cell mucus production and activity. In general, the epithelial cells at multiple sites, including the skin, lung, and gut, serve as a barrier against foreign proteins. However, an increased permeability of this epithelial barrier is associated with antigen sensitisation and allergy development (Bauer et al. 2015).

The majority of the IgE-mediated reactions appear within the first two hours after ingesting the offending food and their pathophysiology encompasses two phases, sensitisation and elicitation (Figure 1) (Pelz and Bryce 2015; Vickery, Chin, and Burks 2011). The sensitisation can occur directly via the gastrointestinal tract or indirectly via respiratory or cutaneous exposures. This phase involves T-cell priming after dendritic cell activation, resulting in a T_H2 response that is characterised by the production of different types of interleukins (IL-4, IL-5, IL-9 and IL-13) by the CD4$^+$ T-cells. Subsequently, this response leads to the production of IgE by the B-cells (Pelz and Bryce 2015; Vickery et al. 2011). The elicitation happens upon re-exposure to the allergen, when the IgE binds to its high-affinity receptor on the surface of mast cells and release the mediators, such as leukotrienes, prostaglandins and histamine that cause classical symptoms (urticaria, rhinitis, angioedema, bronchospasm, laryngospasm, or anaphylaxis) (Burks et al. 2012; Sicherer 2011b; Vickery et al. 2011). However, it is important to stress that sensitisation to a given allergen can occur without evidences of clinical manifestations, suggesting that the presence of IgE alone is not sufficient to confirm the diagnosis of food allergy (Pelz and Bryce 2015).

Lately, several internal and environmental factors have been highlighted as potential risks for the development of food allergies. Accordingly, genetic aspects (familial associations and specific genes), association with atopic diseases (atopic dermatitis) and/or pre-existing conditions (asthma), gender (male/female), time and route of exposure to allergen (topical/respiratory exposure), components of diet (reduced consumption of ω-3 polyunsaturated fatty acids and vitamin D) and geographical differences in dietary habits have been pointed out as increasing risk factors for food allergy development (Lack 2008; Sicherer 2011a; Sicherer and Sampson 2014). The severity of an allergic reaction is highly dependent not only on the physiology of each sensitised/allergic individual, but also on the amount of ingested food, on the type of processing mode, and on possible interactions with other food components (Boyce et al. 2010). Moreover, factors such as speed of food absorption, ingestion of food close to exercise-time (food-dependent exercise-induced anaphylaxis) (Barg, Medrala, and Wolanczyk-Medrala 2011) and patient's age can also enhance the severity of an allergic reaction (Sicherer 2011a). The development of food allergies also depends on when the trigger food is introduced into the diet. In the case of tree nut allergies, they often appear early in childhood, around the age of 2 years (when tree nuts are typically introduced into child's diet) and usually tend to persist throughout adult life (Kagan 2003; Savage, Sicherer, and Wood 2016).

In spite of the increasing knowledge about food allergies, the severity of an allergic reaction is very difficult to predict, thus suggesting that further research is still needed regarding this topic.
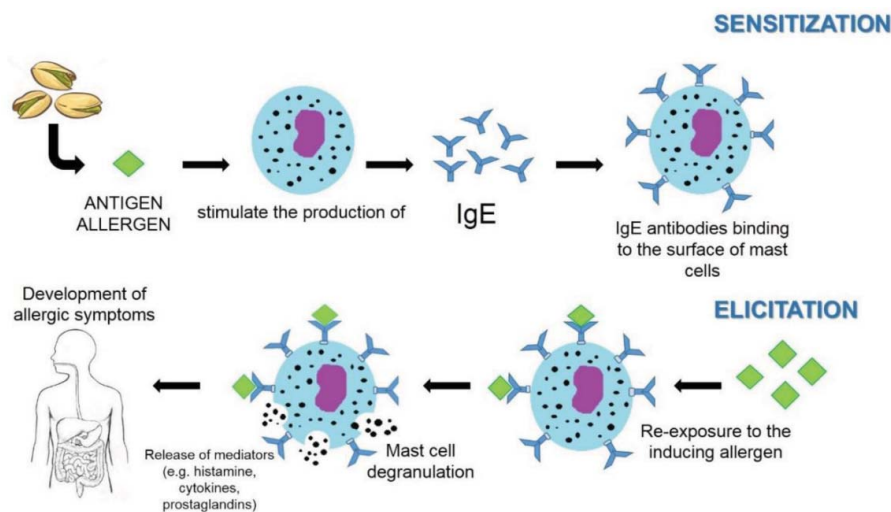

Figure 1. Mechanism of IgE-mediated allergic reaction.

Prevalence of pistachio nut allergy

Food allergies are a well-known problem of public health with special emphasis in the developed countries, but they are also starting to gain particular expression in developing nations as well (Boye 2012). The prevalence of food allergies seems to be higher in young children, though recent data highlight that food allergies are becoming more common among adolescents and young adults (Tang and Mullins 2017). So far, most of the data used to estimate the prevalence of food allergies come from surveys and questionnaires, in opposition to more objective indicators, such as provocation food tests (open food challenges – OFC and double-blind placebo-controlled food challenges – DBPCFC) (Zuidmeer et al. 2008). More recently, data from hospital admission rates, derived from national government databases in westernised countries, with diagnosis of moderate to severe food-allergic reactions have been used as indirect tools to estimate the prevalence of food allergies (Tang and Mullins 2017).

The worldwide prevalence of food-induced allergy is still far from the actual number, since it depends on numerous factors like allergy definitions, population-based studies, methodologies, geographic variation, ages of individuals and dietary exposures among others, which strongly influence the estimates (Sicherer and Sampson 2014). Currently, there are some reports about the prevalence of tree nut allergies based on objective indicators, namely on provocation food tests such as OFC and DBPCFC (Burney et al. 2010; Burney et al. 2014; Nwaru et al. 2014). However, the available literature often reports hazelnut as model of tree nut allergies, making it difficult to have access to information regarding the prevalence of allergy to other nuts.

In respect to pistachio nut allergy, its prevalence has been estimated in a few countries. In general, the prevalence of pistachio nut allergy is reported in terms of ratio of allergic patients to pistachio nut within a test-population of food allergic individuals. In Europe, four reports could be found in the literature, each one conducted in a different country, namely France, Finland, Sweden and United Kingdom (UK). Pouessel et al. (2016) reported a study based on a questionnaire performed to children and adolescents of different schools (infant school, primary school, middle school and high school) in the North of France, as part of a personalised care project to improve school integration of allergic children. According to this study, the prevalence of pistachio/cashew nuts was estimated in 27% of the 317 children/adolescents with reported food allergy, in the years of 2015–2016 (Pouessel et al. 2016). Using the clinical records of 0–18 year-old patients ($n = 371$ children) that were admitted into three paediatric hospitals in Stockholm (Sweden) with symptoms of acute reactions to food during 2007, Vetander et al. (2012) estimated a prevalence of 2% of patients with allergic reactions to pistachio nut. Based on data of skin prick tests (SPT) from the Skin and Allergy Hospital in Helsinki (Finland) between 1997 and 2013, Uotila et al. (2016) described the cross-sensitisation profiles of edible nuts in a birch-endemic area. In this study, the prevalence of pistachio nut allergy in subjects without birch sensitisation or in subjects with concomitant birch sensitisation was 14% or 55%, respectively. Accordingly, the prevalence ratio of pistachio nut

reactivity in subjects with and without sensitisation to birch pollen was about 3.90, which is lower than the prevalence ratio of sensitisation to hazelnut (15.86), almond (12.96) or peanut (8.31). In a study conducted in Leicester (UK), Luyt et al. (2016) evaluated the ethnic differences in prevalence of pistachio nut allergy considering the data from SPT made to white children (European origin) and to South Asian children in a time frame of 3 years (2012–2014). The prevalence of pistachio nut allergy was estimated in 2.6% among a test population of 2,638 subjects. Accordingly, pistachio nut was evaluated with a prevalence of 25.7% or 6.9% for South Asian or white children, respectively. The authors also reported that South Asian children are 3.7-times more likely to be allergic to pistachio than white children, since this nut is traditionally used in Asian culinary (confectionary products, sweets, curries).

In a different study conducted in 735 schools of Ancara in Turkey, children and adolescents (11–15 years) ($n = 11,500$) were tested by means of a questionnaire (Kaya et al. 2013). Individuals with clinical history consistent with IgE-mediated food allergy ($n = 107$) were further tested with SPT and serum-specific IgE. The prevalence of allergies to different foods was determined based on the results from OFC and DBPDFC. Considering pistachio nut allergy, its prevalence was estimated in 6.7% of the subjects with confirmed food allergy (Kaya et al. 2013). Within the Asian countries, a study carried out in 14 University Hospitals of South Korea during 5 years (2009–2013) reported a prevalence of pistachio nut allergy of 0.8%, considering a test population of 126 subjects with severe allergic reactions to food (anaphylaxis) (Jeong et al. 2017).

In the USA, the prevalence of tree nut allergies was determined on the basis of a survey (questionnaire) among the general USA population that evaluated a time-frame of 11 years (1997–2008). In 2008, the prevalence of tree nut allergies in the test population ($n = 12,683$) was about 1.4%. Among the tree nut allergic subjects ($n = 194$), the prevalence of pistachio nut allergy was estimated in 9.8% (Sicherer et al. 2010).

The prevalence of food allergies, such as the case of pistachio allergy, is gradually beginning to be assessed. From the available literature, most of the reports are very recent (2016), which highlight that more efforts are being conducted to improve the knowledge about the prevalence of different food allergies.

Diagnosis, co-sensitisation and cross-reactivity of pistachio allergy

In general, the guidelines for food allergy diagnosis include patient's clinical history and examination, the determination of sensitisation to food, elimination diets for diagnostic purposes and oral food challenges (Muraro et al. 2014b). A well-documented dietary history is fundamental to diagnose a food allergy, since it helps identifying potential food triggers as well as, suggesting which type of immunological mechanism might be involved (IgE versus non-IgE) (Skypala et al. 2015). Besides, it also helps determining timing and chronicity, symptoms, severity and signs, reproducibility, family history, known cofactors or coexisting medical problems (asthma). Following a definition of a clinical

history of food allergy, the *in vivo* skin prick tests and/or *in vitro* serum-specific IgE are normally the first-line tests to determine potential IgE-sensitisation to food. Either SPT or sIgE can be good indicators of IgE-sensitisation, although they cannot always predict clinically relevant food allergy (Muraro et al. 2014b). Based on the data gathered from clinical history, SPT and/or sIgE tests, an elimination diet of the food(s) suspected of inducing an allergic reaction is often recommended to significantly relief the symptoms (2–4 weeks for IgE-mediated symptoms or up to 6 weeks for non-IgE ones). The OFC (either in open or blind manner), which should be performed in controlled environment with appropriate equipment and well-trained personnel, are commonly used to confirm the diagnosis of food allergy. The diagnosis of pistachio nut allergy usually follows the same general guidelines. Like in the case of other foods, the diagnosis of pistachio nut allergy begins with the establishment of patient's clinical history followed by SPT and/or sIgE tests. The SPT with wheal size >3 mm and sIgE testing >0.35 kUA/L are normally considered as positive. Maloney et al. (2008) evaluated the use of sIgE measurements for the diagnosis of peanut, tree nut and seed allergy. Among the individuals with observable symptoms of pistachio nut allergy, the mean value for sIgE was 9.01 kUA/L but with 7% of those subjects presenting sIgE <0.35 kUA/L. In another study, describing the correlation between SPT and sIgE results in adults with suspected food allergy, Ling et al. (2016) reported a good correlation (by kappa analysis) for pistachio nut sensitisation (0.65; 95% CI). Later, Couch, Franxman, and Greenhawt (2017) reported the comparison of data from SPT and sIgE with OFC outcomes. In the case of pistachio-sensitised individuals, 31% failed the OFC, thus confirming pistachio nut allergy. From those, 75% presented sIgE values <2 kUA/L and 25% had SPT wheal <3 mm.

Pistachio nut belongs the same botanical family of cashew nut (*Anacardium occidentale*) and mango (*Mangifera indica*). Pistachio and cashew nuts are genetically closely related leading to cases of co-sensitisation and/or cross-reactivity. By definition, co-sensitisation describes the presence of specific IgE towards distinct and unique epitopes in different allergen sources, while cross-reactivity refers to the presence of IgE that recognise homologue molecules from different allergen sources (Barocci, De Amici, and Marseglia 2016). Co-sensitisation and/or cross-reactivity phenomena to pistachio and cashew nut allergens are rather high, with individuals presenting specific IgE to both nuts and/or IgE that recognised homologue proteins in pistachio and cashew nuts (Uotila et al. 2016; van der Valk et al. 2017). Using the data from SPT over a period of 17 years (1997–2013), Uotila et al. (2016) reported a prominent co-sensitisation between cashew and pistachio nuts, evidencing one of the strongest linkages among edible nuts. In a different study, concerning the improvement of diagnostic methods for allergy assessment – IDEAL a co-sensitisation between pistachio and cashew nuts was observed in 98% of a test population (*n* = 29) of cashew-sensitised individuals. However, pistachio nut sensitisation was only clinically relevant in 34% of the children and it was absent for mango (van der Valk et al. 2017).

Pistachio nut allergens

At the moment, five proteins have been identified and characterised, being all officially recognised as food allergens in pistachio nut by the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee (ALLERGEN 2017). Pis v 1, Pis v 2, Pis v 3 and Pis v 5 are seed storage proteins belonging to the prolamin and cupin superfamilies, while Pis v 4 is classified as a defence protein from iron/manganese superoxide dismutase family of proteins.

Pis v 1 (2S albumin)

Belonging to the prolamin superfamily of proteins, Pis v 1 is defined as a 2S albumin. From a botanical point of view, the 2S albumins are a major group of seed storage proteins widely distributed among mono- and dicotyledonous plants. Biochemically, they are defined as a family of water-soluble proteins at low-salt concentrations, with high contents of arginine, glutamine, asparagine and cysteine residues, which share the same common features of prolamin members. The 2S albumins present a high level of polymorphism, generally being encoded by a multigene family. They are normally synthesised as a single precursor polypeptide with 18–21 kDa that undergo sequence modifications after synthesis. Mature 2S albumins present a small (3–4 kDa) and a large (8–10 kDa) subunits linked by two inter-molecular disulphide bonds. The remaining two intra-chain disulphide bonds are within the large subunit (Moreno and Clemente 2008). 2S Albumins are involved in several biological functions, namely in seed germination and in plant defence (antifungal agents). Owing to the fact that 2S albumins can preserve their conformation even after being submitted to the harsh conditions of processing and digestion (acidic environment, proteolytic activity of digestive enzymes), these proteins have been classified as important class I allergens in several plants (Moreno and Clemente 2008). Like in other tree nuts (cashew nut – Ana o 3, Brazil nut – Ber e 1, pecan nut – Car i 1, hazelnut – Cor a 14, black walnut – Jug n 1 and English walnut – Jug r 1) and peanut (Ara h 2, Ara h 6 and Ara h 7), a 2S albumin has also been identified in pistachio nut.

Pis v 1 is the allergenic 2S albumin in pistachio nut, whose amino acid (aa) sequence is available at the NCBI database (accession number ABG73108) (NCBI 2017) (Table 2). This allergen is encoded by a nucleotide sequence of 767 base pair (bp) (NCBI accession no. DQ631675) and presents a single isoform (Pis v 1.0101) with 7 kDa (most likely corresponding to the large subunit) with a primary sequence of 149 aa (as determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, SDS-PAGE and matrix assisted laser desorption ionization-time of flight-mass spectrometry – MALDI-TOF-MS/MS) (ALLERGEN 2017). Ahn et al. (2009) reported the immunoreactivity of the 7 kDa 2S albumin (Pis v 1) with the sera of 19 out of 28 pistachio-allergic patients. Since this protein was IgE-reactive in more than 50% of the patients' sera, a classification of major allergen was suggested for Pis v 1. The MS/MS peptide sequence analysis of the native protein allowed its comparison with 2S albumins from different vegetal sources. Accordingly, three peptides (ECCQELQEVDR, CQNLEQMVR

Table 2. Identification of pistachio nut allergens according to their biochemical classification, clinical relevance and respective accession numbers.

Allergen	Isoallergens	Isoforms or variants	MW (kDa)	Length (aa)	Protein superfamily	Biochemical classification	Clinical relevance	Nucleotide (NCBI 2017)	Protein (NCBI 2017)	Protein (UniProt 2017)
Pis v 1	Pis v 1.01	Pis v 1.0101	17.3	149	Prolamin	2S albumin	Major allergen	DQ631675	ABG73108	B7P072
Pis v 2	Pis v 2.01	Pis v 2.0101	56.5	496	Cupin	11S globulin subunit	Major allergen	DQ631676	ABG73109	B7P073
	Pis v 2.02	Pis v 2.0201	53.3	472				DQ631677	ABG73110	B7P074
Pis v 3	Pis v 3.01	Pis v 3.0101	59.8	519	Cupin	Vicilin	Minor allergen	EF116865	ABO36677	B4 × 640
Pis v 4	Pis v 4.01	Pis v 4.0101	25.8	230	—	Manganese superoxide dismutase	Major/minor allergen (designation needs revising)	EF470980	ABR29644	B2BDZ8
Pis v 5	Pis v 5.01	Pis v 5.0101	53.4	473	Cupin	11S globulin subunit	Minor allergen	EU410073	ACB55490	B7SLJ1

and ELYETASELPR) from Pis v 1 were found to be homologous to sequences of Ana o 3 in cashew nut. Among others, Pis v 1 (NCBI accession no. ABG73108) presents a 66% of amino acid sequence identity in 97% of query cover with Ana o 3 (cashew) (NCBI accession no. AAL91665). The same authors were also able to produce a recombinant Pis v 1 and test it with six sera from pistachio-allergic subjects. IgE-reactivity with Pis v 1 was observed for all sera tested, suggesting that the rPis v 1 is similar to the native Pis v 1 (Ahn et al. 2009).

Pis v 2 and Pis v 5 (11S globulins or legumins)

Pis v 2 and Pis v 5 are 11S globulins (also known as legumins) that belong to the cupin superfamily of proteins. The 11S globulins are defined as bicupins owing to the presence of two cupin domains, each presenting a beta-barrel motif. Like the 2S albumins, the 11S globulins result from the expression of multiple genes, being synthesised as a single polypeptide, which is post-translationally cleaved into an acidic (30–40 kDa) and a basic (∼20 kDa) polypeptides linked by a disulphide bond. Accordingly, these proteins are non-glycosylated multimeric structures (hexamers or mixture of trimers) of 50–60 kDa bonded by non-covalent interactions, thus presenting a quaternary conformation (Dunwell, Purvis, and Khuri 2004; Mills et al. 2002). In general, the 11S globulins share a high propensity to form large thermally induced aggregates. They exhibit high thermal stability, thus maintaining their conformational structure at temperatures up to 94°C. The presence of the beta-barrel motifs appears to contribute for the stable conformational structure of legumins, thus resisting to heat denaturation and proteolysis. They are important seed storage proteins, representing the main component (often above 50%) of the protein fraction of several nuts and vegetables. Therefore, these physico-chemical properties along with the high abundance of 11S globulins in diet are thought to be main factors associated with their potent allergenicity (Mills et al. 2002). Besides Pis v 2 and Pis v 5 of pistachio nut, several legumins have been identified and often classified as major allergens, in peanut (Ara h 3) and in different tree nuts, such as Brazil nut (Ber e 2); hazelnut (Cor a 9); English and black walnut (Jug r 4 and Jug n 4, respectively); almond (Pru du 6); cashew nut (Ana o 2) and pecan nut (Car i 4) (Costa et al. 2014; Costa et al. 2012; Costa et al. 2016; Mendes et al. in press).

The identification of immunoreactive proteins in pistachio nut with molecular weight around 30–40 kDa were firstly reported by Parra et al. (1993) in three patients with seasonal rhino-conjunctivitis and observable clinical symptoms of allergy to some tree nuts. Later, Fernandez et al. (1995) and Funes et al. (1999) also described strong IgE-binding to 30–40 kDa proteins in pistachio nut when testing sera from food allergic individuals. From the molecular size of these allergens, they are most likely to be 11S globulins of pistachio nut. Ahn et al. (2009) reported the identification of two proteins with 83.5% of identical residues and 86.7% of similarity, which were encoded by two isoforms with cDNA of 1736 bp and 1628 bp (accession no. DQ631676 and DQ631677) (NCBI 2017), respectively. Both proteins with NCBI accession no. ABG73109 and ABG73110 were evaluated by the IUIS/WHO Allergen Nomenclature Subcommittee, being further designated as Pis v 2.0101 and Pis v 2.0201. The primary sequences of Pis v 2.0101 and Pis v 2.0201 exhibit 496 aa and 472 aa, both with molecular sizes of 32 kDa and isoelectric points (pI) of 7.3 and 6.85, respectively (Table 2). These two legumins reveal 48% and 46% of sequence homology with Ana o 2, which is a major allergen in cashew nut (accession no. AAN76862) (NCBI 2017).

Fourteen out of 28 sera of pistachio allergic patients were IgE-reactive towards a band of 32 kDa, thus suggesting a classification of major allergen for Pis v 2. The same authors also described the screening of six pistachio-allergic patients with a recombinant protein (rPis v 2), exhibiting IgE-binding of all tested sera with the expressed rPis v 2 (Ahn et al. 2009).

Pis v 5 is the other 11S globulin from pistachio nut, which is included in the IUIS/WHO official list of allergens as a food allergen (ALLERGEN 2017). It presents a primary sequence of 473 aa with 36 kDa, corresponding to the acidic subunit (accession no. ACB55490), being encoded by a nucleotide sequence of 1684 bp (accession no. EU410073) (NCBI 2017) (Table 2). Pis v 5 presents 52% and 51% of sequence identity with Pis v 2.0101 and Pis v 2.0201, respectively (according to Blastp analysis). Regarding this 11S globulin from pistachio nut, little information could be retrieved from literature. Willison, Sathe, and Roux (2014) described the immunoreactivity of native Pis v 5 in ten out of the 28 sera of pistachio-allergic patients as unpublished data, thus suggesting a classification of minor allergen for Pis v 5.

Pis v 3 (7S globulins or vicilins)

Like the 11S globulins, the 7S globulins (designated as vicilins) are also bicupins of the cupin superfamily of proteins. Structurally, they are homotrimeric proteins with molecular mass of 150–190 kDa, presenting three subunits with 40–80 kDa. In terms of primary sequence, 11S and 7S globulins reveal low similarity and the aligned sequences evidence only 35–45% of identity. However, both globulins exhibit highly conserved conformational structures. 7S globulins are frequently glycosylated, with one or two N-linked glycosylation sites located at the C-terminal domain, which seems to contribute to the high structural stability of these proteins (Breiteneder and Mills 2005; Mills et al. 2002). As part of the globulin fraction, the vicilins exhibit high thermal stability, maintaining their structural integrity at temperatures up to 70–75°C. At temperatures above 75°C, these proteins seem to suffer partial unfolding that contributes to the formation of large thermally stable aggregates. Additionally, heat induces some covalent modifications that might promote the glycation process and subsequent formation of Maillard products (Mills et al. 2002). Like the 11S globulins, the 7S globulins (vicilins) are also classified as main seed storage proteins and as allergens in most vegetables (Gly m 5-soybean, Ara h 1-peanut) and tree nuts (Ana o 1-cashew nut, Cor a 11-hazelnut, Car i 2-pecan nut, Jug r 2 and Jug r 6-English walnut) (ALLERGEN 2017).

Parra et al. (1993) first reported the immunoreactivity of a pistachio nut protein with a molecular size of 52 kDa, in the sera of two out of three individuals with seasonal rhino-conjunctivitis and observable clinical symptoms of allergy to some tree nuts. When assessing the allergenicity of pistachio nut, cashew nut and mango in patients with history of food allergy

to members of the Anarcadiaceae family, Funes et al. (1999) described the IgE-reactivity of several proteins in pistachio nut, from which one corresponded to molecular size of 55 kDa. In both studies, the protein at ∼55 kDa was most likely to be a 7S globulin. Lately, Willison et al. (2008) reported the expression of a recombinant 7S globulin (molecular size ∼55 kDa) with 519 aa (accession no. ABO36677), which was encoded by a 1560 bp nucleotide sequence (accession no. EF116865) (NCBI 2017). This protein presented high homology with different vicilins from several tree nuts and vegetables, being designated as Pis v 3 in pistachio nut (Table 2). From those, Pis v 3 exhibited the highest homology with Ana o 1 from cashew nut (80% identity, 90% similarity), evidencing the likelihood of considerable cross-reactivity between these two allergens. Moreover, the authors tested the reactivity of nine murine monoclonal antibodies raised against cashew nut towards pistachio nut. From those, six monoclonal antibodies recognised rPis v 3 with different degrees on dot blots, indicating considerable epitope homology between rPis v 3 and rAna o 1. The rPis v 3 was also tested with sera from allergic individuals, evidencing IgE-reactivity in 36% of the sera from 14 pistachio-allergic patients, which suggests a classification of minor allergen for Pis v 3 (Willison et al. 2008). Confirming the high homology between Pis v 3 and Ana o 1, Rougé et al. (2011) identified an epitope in Pis v 3 (DEEQEEEDENPYVFED) that is almost identical in one epitope in Ana o 1 (DEAEEEDENPYVFED).

Pis v 4 (manganese superoxide dismutase)

The superoxide dismutases (SOD) are enzymes that play a critical function in pathological responses to oxygen toxicity. They belong to a superfamily of metalloenzymes, which are responsible to catalyse the dismutation of the superoxide radical into either ordinary molecular oxygen or hydrogen peroxide. The superoxide radical is a by-product of the oxidative phosphorylation (part of the cellular respiration) known to induce many types of cell damage. Owing to its high reactivity, the superoxide radical has to be regulate and catalyse into less reactive compounds, such as molecular oxygen or hydrogen peroxide, being further converted to water and molecular oxygen by another enzyme (catalase). The superfamily of metalloenzymes encompasses three major families of SOD, whose designation depends on the catalytic co-factor metal and on protein folding (Ni-SOD, Cu-Zn-SOD and Fe/Mn-SOD). Among those, the manganese superoxide dismutase (MnSOD) is of primal biological importance in nearly all living cells exposed to oxygen, once it is involved in defence mechanisms, such as protecting the mitochondrial DNA against oxidative damage (Finn et al. 2016; Fluckiger et al. 2002; Pfam 2017). Until now, a small number of MnSOD has been identified and characterised as allergenic proteins in fungi and plants, but only one is classified as food allergen (Pis v 4 – pistachio nut).

Ayuso et al. (2007) described the identification of an immunoreactive protein in pistachio nut with a molecular size of 23 kDa and an isoelectric point of 6. Accordingly, this protein evidenced IgE-binding with 16 out of 27 sera (59%) from well-characterised pistachio-allergic subjects, suggesting a classification of major allergen for Pis v 4. Tryptic digestion of the 23 kDa protein and subsequent MS-analysis revealed high sequence homology with MnSOD from latex, which allowed to identify this allergen as a MnSOD-like protein in pistachio nut (Ayuso et al. 2007). Accordingly, this protein with a primary structure of 230 aa (accession no. ABR29644) and an encoding sequence of 932 bp (accession no. EF470980) (NCBI 2017), was further included in the IUIS/WHO official list of allergens with the designation of Pis v 4 (Table 2). More recently, Noorbakhsh et al. (2010a) reported the successful expression of a recombinant MnSOD from pistachio nut with 201 aa and an isoelectric point of approximately 6.61, presenting a potential N-glycosylation site and four manganese ligand-binding sites: three histidine residues and in one aspartate. The recombinant Pis v 4 presented high sequence identity (88% and 84%) and similarity (93%) with MnSOD proteins from latex (Hevea brasiliensis) and grapes (Vitis vinifera), respectively, which might indicate potential cross-reactivity between Pis v 4 and MnSOD from unrelated organisms. Like in the case of MnSOD from latex, the recombinant Pis v 4 also evidenced the conserved structural motif, indicating that its conformational structure might be a homodimer or a homotetramer. Immunoreactivity of rPis v 4 was confirmed in 40% of the sera from pistachio-allergic patients (10 positive out of 25 tested sera), indicating a classification of minor allergen (Noorbakhsh et al. 2010a). The differences in the results of IgE-binding frequency (59% versus 40%) reported by Ayuso et al. (2007) and Noorbakhsh et al. (2010a), respectively, might be explained by the use of different test populations. In order to better defined a correct classification as minor or major allergen for Pis v 4, more detailed and large population studies should be conducted.

Clinical relevance and treatment

In terms of clinical presentation, allergic reactions to pistachio nut consumption are typically defined as immediate (within few minutes after ingestion or contact) and can induce moderate to severe clinical symptoms. Accordingly, pistachio-allergic patients often experience clinical manifestations that include hives, vomiting, abdominal pain, nasal congestion, angioedema, urticaria, pruritus, itchy throat, repetitive coughing, wheezing, red/watery eyes, dyspnea, erythema, eczema, lip swelling and hypotension (Ahn et al. 2009; Noorbakhsh et al. 2010a; Noorbakhsh et al. 2010b; Noorbakhsh et al. 2011). In some patients suffering from seasonal rhino-conjunctivitis, eczema and/or asthma, mild and severe clinical symptoms of oral allergy syndrome (OAS) upon pistachio nut ingestion have also been described (Ando et al. 2011; Fernandez et al. 1995; Jansen et al. 1992; Liccardi et al. 1996; Parra et al. 1993). Complex and severe systemic symptoms, such as the case of anaphylaxis or even specific food-dependent exercise-induced anaphylaxis, have also been reported as consequence of pistachio nut ingestion (Ahn et al. 2009; Ando et al. 2011; Porcel et al. 2006; Vetander et al. 2012).

So far, there is no effective cure for food allergy. Therefore, after the first experience of a moderate/severe allergic reaction, the patient is advised to avoid the offending food, as well as other potentially cross-reactive ones. In spite of all possible preventive measures, patients are still at risk of accidental exposure to the sensitisation/causing agent (e.g. pistachio), thus some therapeutic measures are available to treat/mitigate clinical

symptoms caused by allergic responses. In the case of a complex and systemic allergic reaction, such as anaphylaxis to pistachio nut, the first line of treatment normally involves the administration of intramuscular epinephrine (also known as adrenaline) (epipen). Accordingly, individuals with tree nut allergies and at risk of suffering anaphylactic shocks are advised to carry their own emergency epinephrine. In the event of an accidental exposure, two or more doses of intramuscular epinephrine (in intervals of at least with 5 min apart) can be applied to the patient. However, if patient does not respond to intramuscular injections, epinephrine can be administered as an infusion by appropriately experienced intensive care, emergency department and critical care physicians, with appropriate cardiac monitoring. The administration of oxygen and inhaled short-acting beta-2 agonists or glucocorticosteroids and H1/H2-antihistamines constitute the second and third lines of treatment, respectively in the case of anaphylaxis (Muraro et al. 2014a). Corticosteroids and antihistamines are the most widely used to treat the clinical symptoms of pistachio nut allergy, although the administration of epinephrine is also very frequent, especially due to the severity of the immunological responses (Fernandez et al. 1995; Garcia et al. 2000; Porcel et al. 2006).

Currently, different novel approaches have been suggested with the ultimate goal of inducing long lasting tolerance to specific allergens in allergic patients. They include IgE-blockade via treatment with omalizumab (partially humanised monoclonal antibody that links the Fc portion of the human IgE and blocks its binding to the IgE receptor on mast cells and basophils), the use of pharmaceuticals with anti-allergic properties (for example: some traditional Chinese herbal medicines) and different forms of allergen-specific immunotherapy (oral, sublingual, subcutaneous and epicutaneous or with modified allergens) (MacGinnite 2017; Yang and Chiang 2014). So far, some immunotherapies have been tested for egg, milk, peanut and some tree nuts, but none for the specific case of pistachio nut. In spite of the promising results, none of the proposed immunotherapies has yet been officially approved, since a number of issues remain to be addressed, namely optimum duration of therapy, optimal selection of patients, minimising reactions and optimising adjunctive therapies (MacGinnite 2017).

Effects of food processing on pistachio allergenicity

Physical and chemical processes that ingredients undergo during preparation/processing are known to affect differently the allergenicity of foods. In addition to the effect of food processing technologies (boiling, roasting, autoclaving), the different nature of allergens (profilins, globulins, albumins, pathogenesis-related proteins, lipid transfer proteins) is determinant to increase or reduce the allergenicity of foods. At the present, the effects of food processing technologies have been studied for several allergenic foods, namely some tree nuts (Masthoff et al. 2013). However, contrarily to other nuts, the knowledge about the effects of food processing on the allergenicity of pistachio nut is still very limited. Noorbakhsh et al. (2010b) tested two different processes, dry roasting (oven at 150°C for 8h) and steam roasting (steam blanched for 10 min under atmospheric conditions and roasted in oven at 150°C for 8 h) on raw

pistachio nuts that were previously soaked in water containing lemon juice (pH 3.2–3.5) and sodium chloride (1.6% w/v) for 12 h. The authors reported that the IgE-binding of steam-roasted pistachio was lower than raw or dry roasted pistachio nut. Moreover, both raw and processed (dried and steam roasted) pistachio nut samples were further submitted to gastric digestion, evidencing that soluble protein has been significantly decreased in steam-roasted pistachio extract. The steam-roasting process in combination with the ionic strength of soaking solution induced some structural and chemical modifications, which resulted in protein aggregation, thus contributing to reduce the solubility of the protein and subsequently affecting its IgE-reactivity (Noorbakhsh et al. 2010b). However, it is important to highlight that the conditions of processing (dry or steam roasting at 150°C for 8 h) reported in this study are hardly used at industrial scale, considering the time and potential cost of such operation, which might hamper its industrial application. Additionally, most of food industries have shared production lines for nut processing, therefore the routes associated with potential cross-contamination (namely people's handling, raw material handling, transport, processing aids, packing, rework) with other nuts or foods cannot be neglected.

Strategies for detecting/quantifying pistachio in foods

One crucial part of an effective allergen risk assessment depends on the availability of appropriate clinically validated eliciting thresholds (allergen reference doses below which the majority of allergic individuals are protected from experiencing an adverse immune response) (Reese et al. 2015). Currently, data from clinical studies using OFC and DBPCFC concerning the minimal eliciting doses for specific allergenic foods have been used for the quantification of the risk of reaction at the population level through probabilistic risk assessment approaches that can generate quantitative risk predictions. So far, the collected data to input in probabilistic risk assessment approaches is still very scarce, though reference doses for some allergenic foods have already been advanced (Crevel et al. 2014; Taylor et al. 2014). In the case of tree nuts, a reference dose of 0.1 mg of protein was proposed considering the parametric modelling of minimal eliciting doses from hazelnut-allergic populations (Taylor et al. 2014).

The other critical part of allergen risk assessment concerns the availability of fast, reliable and highly sensitive methods to detect trace amounts of allergens in processed foods (Reese et al. 2015). Based on the conventional techniques or on the most recent and advanced high-throughput technologies, in the past two decades the number of available methodologies for allergen analysis has become impressive (Costa et al. 2017). In spite of the huge number of publications, no official method has yet been proposed. In fact, no consensus has been reached regarding the choice of the best target molecule (protein versus DNA) for allergen testing.

Protein-based methods

Immunochemical assays

In the opinion of many researchers, protein-based methods are the best targets for allergen analysis. Considered as highly

sensitive, methods like the enzyme-linked immunosorbent assay (ELISA) are faced as excellent choices for allergen testing. Within the protein-based methods, the most representative and widely used assays are ELISA, lateral flow devices (LFD), dipstick tests and immunoblotting. Currently, there is a wide range of commercially available ELISA kits and LFD for almost every allergenic commodity. From the point of view of food industry, LFD are most appealing for *in situ* application, thus permitting fast screening of allergen presence. For quantitative purposes, ELISA kits are also preferred by the food industry because they only require equipment normally available at quality control laboratories, without needing specialised personnel.

For the specific detection of pistachio nut, there are some LFD and ELISA kits commercially available, which are listed in Table 3. LFD provide qualitative information regarding the presence of the pistachio nut, most of them until a limit of detection (LOD) of 1 mg/kg. The Reveal for Multi-Treenut LFD from Neogen (MI, USA) is not specific for pistachio nut since it enables detecting almond, cashew nut, hazelnut, pecan nut and walnut. The LOD reported for this LFD is 5–10 mg/kg depending on the target tree nut. Regarding the ELISA kits, those report the detection of pistachio nut protein down to 0.12–0.13 mg/kg in various matrices (Table 3). These methods are widely used probably because they are considered of fast performance, simple handling, high sensitivity (low mg/kg range) and cost-effective. Additionally, they also allow the simultaneous analysis of a large number of samples in a single plate/run. Despite the advantages, it is important to stress that the ELISA kits might be influenced by possible matrix effects and processing, leading to both false positive and negative results.

Using the MonoTrace pistachio ELISA kit (BioFront Technologies, Tallahassee, FL, USA), Liu, Chhabra, and Sathe (2015) confirmed the good performance of the kit for the specific detection and quantification of pistachio nut (no cross-reactivity with 156 food matrices) in different incurred food matrix (sponge cakes, sugar cookies and corn flakes) and commercially processed foods. The ELISA kit revealed a linear detection range of 0.5–36 mg/kg and limits of detection and quantification (LOD/LOQ) of 0.09 mg/kg and 0.30 mg/kg, respectively for pistachio full fat flour.

The authors also evaluated the effects of thermal processing (autoclaving, blanching, frying, microwaving and roasting) on the immunoreactivity of pistachio seeds using the murine anti-pistachio monoclonal antibody. The kit enabled the detection of pistachio nut proteins, even in the seeds that were submitted to harsh processing conditions. In some cases, the immunoreactivity of pistachio nut proteins seemed to increase (like in blanching and frying conditions), which can probably be explained by a better accessibility of a buried epitope. The murine anti-pistachio monoclonal antibody recognised protein fractions of 50, 40 and 31 kDa that are likely to correspond to albumin and 11S globulin polypeptides in the pistachio nut (Liu, Chhabra, and Sathe 2015).

Immunosensors

Biosensing technology composes a novel and promising approach for the detection of allergens in food products. The biosensor is composed of a receptor-transducer device, which converts the recognition event of a molecular interaction between the receptor (antibodies, aptamers or DNA probes) and target molecules (proteins or single-stranded DNA fragments) in a measurable signal. A biosensor based on the recognition of an interaction antibody-target protein is normally designated as immunosensor (Pilolli, Monaci, and Visconti 2013; Schubert-Ullrich et al. 2009). Biosensors can be classified as optical, piezoelectric or electrochemical, depending on the type of transducer used. Owing to its alleged advantages, namely fast performance, simple use, low-cost

Table 3. Commercial LFD, ELISA and real-time PCR kits for the detection and quantification of pistachio allergens.

Commercial Kit	Assay type	Brand (cat no.)	Cross-reactivity	Dynamic range	LOD (mg/kg)	Sample testing (min)
Lateral Flow Pistachio assay	Lateral flow device	R-Biopharm, Darmstadt, Germany (BL611-10/-25R)	Cashew nut (4%), Brazil nut (0.1%), hazelnut (0.1%), pumpkin seed (0.1%), walnut (0.8%)	qualitative	1	10
Reveal® for Multi-Treenut	Lateral flow device for almond, cashew, hazelnut, pecan, pistachio, walnut	NEOGEN, MI, USA (8555)	No information	qualitative	5–10	10
AgraStrip® Cashew/ Pistachio test kit	Lateral flow device	Romer Labs, Tulln, Austria (COKAL1310AS)	No information	qualitative	—	11
AllergenControl™ Pistachio Residue	Lateral Flow Test	MicroBiologique, WA, USA (PA-E09)	No information	qualitative	1	30
MonoTrace Pistachio ELISA	Monoclonal antibody-based ELISA to pistachio allergen	BioFront Technologies, FL, USA (PV1-EK-96)	Pecan nut (0.001%)	1–40 mg/kg	0.12	40
AgraQuant® ELISA Pistachio	Sandwich ELISA	Romer Labs, Tulln, Austria (COKAL2748)	Cashew (12%), hazelnut (0.17%), walnut (0.0008%), pecan nut (0.0005%), sunflower (0.0002%)	1–40 mg/kg	0.13	60
AgraQuant® Plus Pistachio	Sandwich ELISA	Romer Labs, Tulln, Austria (CO KAL2748F)	Cashew (12%), hazelnut (0.17%), walnut (0.0008%), pecan nut (0.0005%), sunflower (0.0002%)	1–25 mg/kg	1	30
SureFood® ALLERGEN ID Pistachio	Real-Time PCR	R-Biopharm, Darmstadt, Germany (S3114)	None known	qualitative	≤0.4	60
SureFood® ALLERGEN QUANT Pistachio	Real-Time PCR	R-Biopharm, Darmstadt, Germany (S3214)	None known	1–400 mg/kg	≤0.4	60
PistacKit Real Time PCR	Real-Time PCR (qualitative)	4LAB Diagnostics, Codogno, Italy (IC-02-1093/IC-02-1091)	None known	1–1000 DNA copies	≥0.45 pg	60

multi-target detection and high potential for automation, biosensing technology has been regarded with special interest in the field of allergen analysis. Currently, there are biosensors proposed for the detection and quantification of several allergenic foods, namely different tree nuts, peanut, egg, milk, fish and crustaceans (Costa et al. 2017). Regarding pistachio nut, Rebe Raz et al. (2010) described the development of an optical immunosensor (based on imaging surface plasmon resonance – iSPR) for the multiple detection of several allergenic foods (peanut, hazelnut, milk, soybean, lupine, egg, pine nut, almond, macadamia nut, Brazil nut, cashew nut, pistachio nut and pecan nut) (Table 4). The authors used a microarrayed chip coated with specific antibodies against all target foods, which enable detecting different pistachio proteins with distinct sensitivities (1 and 0.8 mg/kg in cookies and dark chocolates, respectively) (Rebe Raz et al. 2010).

Mass spectrometry platforms

The mass spectrometry (MS) methodologies have also been used for the detection and quantification of proteins. Lately, these methodologies have gain special attention in the field of food allergen analysis, since they allow the simultaneous detection, quantification and identification of multiple allergens in a single run (Sancho and Mills 2010). Contrarily to other immunoassays (ELISA, LFD or immunosensors), the detection of target analytes by MS platforms is independent from allergen or marker protein/antibody interactions, allowing the direct identification of proteins or peptides. So far, MS methodologies have been advanced as great choices for allergen analysis, since they allow the direct identification of the allergen itself (protein or marker peptides). Among several advantages, the MS methodologies are less affected by the complexity of the matrices, effects of food processing and cross-reactivity phenomena, thus allowing a better quantification of food allergens. However, when compared to other protein- or DNA-based methods, allergen analysis by MS techniques is more labours and time-consuming, hampering their application in routine analysis. Besides, the maintenance of MS platforms implicate high costs related to equipment and consumables, as well as the need for specialised personnel to work with them. Presently, there are MS approaches available in the literature for the detection and quantification of several allergenic commodities, namely egg, milk, gluten-containing cereals, peanut, tree nuts and soybean in a wide variety of food matrices (Costa et al. 2017).

Presently, there are two studies that report the development of MS-based platforms for the simultaneous detection of several allergenic foods, including pistachio nut (Table 4) (Korte and Brockmeyer 2016; Sealey-Voyksner, Zweigenbaum, and Voyksner 2016). Sealey-Voyksner et al. (2016) report a multiplex approach using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) to specifically detect 12 allergenic foods (peanut, almond, pecan, cashew, walnut, hazelnut, pine nut, Brazil nut, macadamia, pistachio, chestnut and coconut). For this study, two marker peptides for allergenic proteins (Pis v 1 and Pis v 2) of pistachio nut were used for its unequivocal identification. The method allowed defining an LOD of 1 mg/kg for all target allergens, independently on the type of food matrices (cakes, cookies, cereal bars or chocolates). At the level of 0.1 mg/kg, some peptides were detected, while others were not, suggesting some effect of food matrix. Korte and Brockmeyer (2016) also described the development of a multi-target approach based on LC-MS with multiple reaction monitoring cubed (MRM^3) technology for the detection and quantification of peanut, almond, cashew nut, hazelnut, walnut and pistachio nut. The method targeted three peptides of the Pis v 5 (11S globulin) allergen and it enabled detecting and quantifying pistachio nut down to 1 mg/kg in fortified matrices (multigrain bread, vanilla ice-cream and dairy chocolate) (Table 4). Considering the data from both studies, it is expected that multi-target approaches using MS platforms will continue to be proposed for allergen analysis.

DNA-based methods

Faced as excellent alternatives for allergen analysis, several methodologies based on DNA detection have been proposed for different allergenic commodities (Costa et al. 2017). Owing to the high stability of DNA molecules towards harsh food processing conditions, methods based on DNA markers or on sequences encoding for allergens have been highlighted as very specific and sensitive approaches for allergen analysis in processed foods (Costa et al. 2017; Monaci and Visconti 2010). The polymerase chain reaction (PCR)-based methods have been widely proposed for the detection and quantification of several allergenic commodities. They consist of the specific amplification of a DNA region by means of PCR, the specificity of which is achieved by the use of primers and, frequently, probes (in the case of real-time PCR) (Costa et al. 2012). Presently, the costs

Table 4. Summary of the protein-based methods for the detection and quantification of pistachio allergens in foods available in the literature.

Method	Antibody (immunization)/ Target protein	Cross-reactivity	Sensitivity level	Applied food matrices	References
Immunosensor (antibody-microarrayed chip using iSPR)	Polyclonal antibodies against pistachio nut	Strong cross-reactivity with cashew	Cookies: LOD: 1 mg/kg LOQ: 6.1 mg/kg Chocolate: LOD: 0.8 mg/kg LOQ: 4.3 mg/kg	Commercially cookies and dark chocolates	Rebe Raz et al. (2010)
MRM^3-based LC-MS	Pis v 5 (3 peptides: AMISPLAGSTSVLR, ITSLNSLNLPILK and GFESEEESEYER)	Not verified	LOD: $\leq 0.3\ \mu$g/g LOQ: $\leq 1\ \mu$g/g	Bread, milk chocolate, chocolate confectionary, muesli with fruit and berries, ice cream	Korte and Brockmeyer (2016)
LC–MS/MS	Pis v 2 (TNGLSQTSQLAGR) Pis v 1 (LQELYETASELPR)	Not verified	LOD: 1 mg/kg	Cookies, cakes, flours, bars	Sealey-Voyksner, Zweigenbaum, and Voyksner (2016)

Table 5. Summary of the DNA-based methods for the detection and quantification of pistachio allergens in foods available in the literature.

Method	Target gene (NCBI acc. no.)	Cross-reactivity	Model mixtures	Applied food matrices	Sensitivity level	References
Qualitative PCR	Dehydrin gene and rbcL gene	No cross-reactivity with 17 plant species for dehydrin gene. Cross-reactivity with cashew and Brazil nut for the rbcL gene.	Model mixtures of pistachio in mortadella	Mortadella samples	100 mg/kg of pistachio in mortadella	Barbieri and Frigeri (2006)
Real-Time PCR with hydrolysis probes	Internal transcribed spacer (ITS) between 18S ribosomal RNA and 5.8S ribosomal RNA genes	No cross-reactivity with 23-plant and 3-animal species tested	Model mixtures of pistachio nut in cookies	Chocolates, instant pudding, meat paté, wafer pralines, snacks	Absolute LOD: 0.012 pg Relative LOD: 4 mg/kg of pistachio in cookies	Brežná, Dudášová, and Kuchta (2008)
Ligation-dependent probe amplification (LPA) (multiplex system)	Dehydrin (Y07600)	No cross-reactivity with 50 plant and animal species	Not reported for pistachio	Chocolates, cookies, sausages, mortadella, pesto	Not reported for pistachio	Ehlert et al. (2009)
Multiplex PCR with hydrolysis probe	Dehydrin (Y07600)	No cross-reactivity with the samples tested	Boiled sausages and rice cookies spiked with pistachio	Spreads, parfaits, sauces, sandwiches, spaghetti bolognese, chocolates	Relative LOD: 0.1%	Köppel, Velsen-Zimmerli, and Bucher (2012)
Real-time PCR (TaqMan probe)	Internal transcribed spacer (ITS1) (AY677201)	No cross-reactivity with 49 plant species and 4 animal species	Model mixtures of raw or heat treated pistachio nut in wheat flour	Chocolates, cereals, ice creams, meat products, biscuits	0.1 mg/kg of pistachio nut DNA	López-Calleja et al. (2014)
Multiplex PCR coupled to capillary electrophoresis	Pis v 1 (DQ631675.1)	No cross-reactivity with hazelnut, oat, sesame, peanut, cashew nut, barley, wheat, soybean and pecan.	Maize powder matrix spiked with pistachio	Cakes, chocolates, cookies, waffles, noodles	Absolute LOD: 20 DNA copies Relative LOD: 0.005% of pistachio in maize powder	Cheng et al. (2016)
Real-time PCR (SYBR-Green) and real-time PCR with locked nucleic acids (LNA probe)	Pis v 1	No cross reactivity with nuts and plants used on food industry	Model mixtures of defatted raw pistachio in spelt wheat	Cereal bars, chocolates, cookies, pesto sauce, jam with pistachio	Real-time PCR SYBR-Green LOD/ LOQ:100/1000 mg/kg Real-time PCR LNA probe LOD/ LOQ:10 mg/kg	Sanchiz et al. (2017)

associated with equipment and consumables, the time per sample analysis, as well as the need for specialised personnel, are very similar to those required for immunoassays, such as ELISA. Therefore, PCR-based methods are easily implemented for routine analysis and can act as confirmatory tool for the unequivocal identification of the target allergenic food.

Up to now, there are several PCR-based approaches for the detection and quantification of different allergenic ingredients in foods (Costa et al. 2017), either based on commercial kits or in-house developed methods. For the specific case of pistachio nut, there are some commercial kits available based on real-time PCR technology. Contrarily to the ELISA kits, the commercially available real-time PCR kits are less common and normally they are only able to provide qualitative information (Table 3). The time per analysis and sensitivities reported for these kits are similar to ELISA, with the advantage of not presenting known cross-reactivities.

Along with commercial kits, there are several methods for the detection and quantification of pistachio nut by PCR, both in single or multiple analysis systems (Table 5). The proposed PCR systems were applied to a great variety of processed foods like chocolates, cereal bars, ice creams, meat products, among others, allowing detecting/quantifying pistachio nut at different levels. In general, PCR methods targeting unicopy genes present higher LOD/LOQ (Sanchiz et al. 2017) than the systems detecting multicopy genes (Brežná, Dudášová, and Kuchta 2008; López-Calleja et al. 2014). Accordingly, PCR methods amplifying multicopy genes or regions (ITS) allowed detecting/quantifying pistachio nut down to 0.1–4 mg/kg in different matrices, respectively in wheat flour and cookies (Brežná, Dudášová, and Kuchta 2008; López-Calleja et al. 2014). Multiplex PCR systems have also been described as efficient tools for the multiple detection/quantification of different allergenic commodities, namely pistachio nut in processed foods (Cheng et al. 2016; Ehlert et al. 2009; Köppel, Velsen-Zimmerli, and Bucher 2012). Those multiplex methods were based on different detection systems. Ehlert et al. (2009) and Cheng et al. (2016) reported the development of multiplex PCR systems coupled to capillary electrophoresis analysis. Cheng et al. (2016) detected pistachio nut in maize powder at the level of 50 mg/kg, while Ehlert et al. (2009) did not report the sensitivity of the method for pistachio nut in model mixtures. Köppel, Velsen-Zimmerli, and Bucher (2012) proposed two hexaplex systems for the simultaneous detection of 12 allergenic commodities. For pistachio nut, the LOD/LOQ was 32 mg/kg in boiled sausages and 5 mg/kg in rice cookies. The described PCR-based methods present high sensitivities and specificities for pistachio nut identification, detection and quantification, which can be easily applied to a wide variety of commercial foods at a routine basis.

Conclusion

Within the tree nut group, pistachio nut allergy has attained an impressive expression especially due to its intrinsic relation with cashew nut allergy, both in terms of high co-sensitisation and cross-reactivity phenomena. Similarly, to the cashew nut allergy, the prevalence of pistachio nut allergy seems to be increasing at a global scale, not only as consequence of the rise in the consumption of tree nuts but also as a result of changes in population food habits/diets. In terms of clinical relevance, pistachio nut allergy is commonly related to moderate to severe clinical symptoms, often leading to anaphylactic reactions. As the data from hospital admission rates have become available in some regions/cities/countries, the number of individuals experiencing severe allergic responses to foods (pistachio nut) seems to be increasing.

Recently, relevant data on the biochemical classification of pistachio nut allergens enabled establishing a correlation with respective clinical symptoms elicited. Accordingly, there are 5 groups of allergenic proteins identified in pistachio nuts. Four of those are seed storage proteins, belonging to the cupin (Pis v 2, Pis v 3 and Pis v 5) and prolamin (Pis v 1) superfamilies, while one is classified as a plant defence protein (Pis v 4). The classification as major (Pis v 1, Pis v 2 and Pis v 4) and minor (Pis v 3 and Pis v 5) allergens in pistachio nuts have been proposed, although their rating could be revised in a near future. All proteins have been included as food allergens in the official list of allergens.

The establishment of an effective allergen risk assessment is currently a key issue for food industry, policy makers and regulatory agencies. As an integral part of the allergen risk assessment, the availability of fast, reliable and highly sensitive methods to detect trace amounts of allergens in processed foods are of utmost importance. To date, there are some protein- and DNA-based methods for the detection/quantification of pistachio nut at trace levels in foods, which can aid in the verification of label information. At individual level, pistachio-allergic patients still need to avoid pistachio nut and other cross-reactive foods, since no cure is available for pistachio nut allergy nor for other food allergies. The preventive measures are the only effective way of protecting themselves from accidental exposures to the offending foods. Presently, some preliminary clinical studies suggest the use of immunotherapies to induce desensitisation and subsequent tolerance to specific foods, although none of those studies concern pistachio nut allergy.

In spite of the knowledge about pistachio nut allergy, as well as other relevant tree nut allergies, is continuously becoming available, much research concerning all the above topics is still needed.

Conflict of interests

The authors declare that they have no conflict of interest.

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