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An overview of hidden food allergens: need for change to the priority food allergen lists?

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Key words

Hidden allergens; food allergy; food labelling; priority food allergen lists; molecular allergy.

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Impact statement

The adoption of plant-based diets and new sustainable foods is contributing to the onset of new emerging food allergens. Diagnostic implementations and priority food allergen lists revision will be needed.

Summary

Many food allergens not actually included in the European priority list of allergenic foods have the potential to cause severe allergic reactions and could escape correct identification and behave as "hidden allergens". Moreover, the adoption in recent years of novel diets based on plant products and new sustainable foods or the use of specific food additives have contributed to the onset of new emerging allergens of public health importance. The knowledge of hidden allergens is important both for physicians and for patients to improve the prevention, diagnosis and treatment of food allergies, in order to decrease eventual improper diagnosis of idiopathic anaphylaxis. In this review, the characteristics of the most frequent hidden allergens and their diagnostic tools are described. A detailed history with a careful review of the ingredient lists, an understanding of possible cross-reactions or contaminations with other foods, together with an allergological evaluation consisting of "in vivo" or "in vitro" tests and, where necessary, an oral food challenge, are recommended for the successful identification of the culprit allergen.

In future, it will be very important to implement these diagnostic tools, especially in the field of molecular allergology, and reporting allergens on labels should become mandatory.

Introduction

Dietary changes of the last years, based on the adoption of plant products and new sustainable foods with low environmental and economic impact, have contributed to the onset of new emerging allergens of public health importance (1, 2). According to the current European labeling regulations, these "new allergens" are not always labeled in food products and so they can act as "hidden allergens". A hidden allergen is defined as a substance that is unrecognized or not declared on the product label. The omission of these allergens is not always intentional, and it can be due to different reasons such as misleading labels, allergenic foods that can contaminate other safe foods, carelessness, use of uncommon terms in the food list or ingredient switching (3). The first priority list of allergenic foods for the European Union was established by the European Parliament and the Council through Directive No 1169/2011 and it currently includes fourteen allergens: cereals containing glutens, milk, crustaceans, eggs, fish, peanuts, soybeans, tree nuts (*i.e.,* almonds, hazelnuts, cashews, pecan nuts, Brazil nuts, pistachio nuts, macadamia nuts and Queensland nuts), celery, mustard, sesame seeds, sulphur dioxide and sulphites (at concentrations of more than 10 mg/kg or 10 mg/liter expressed as SO_2), lupin and molluscs. The criteria used to include these foods on the priority list are based on the following parameters: the evidence for an IgE mechanism, the adverse reactions caused by IgE-mediated reactions, the potency, the severity and

the prevalence in the general population through double-blind, placebo-controlled food challenge or other published reports in accordance with the International Life Sciences Institute-Europe (4). However, the foods included on this and on priority lists from other countries don't always fully comply with all the expected scientific criteria, probably due to a variable weighting of the importance of the aforementioned factors (*i.e.,* prevalence, severity and potency) in terms of impact on public health (5). Food allergens not included in the actual labeling regulations lists can act as "hidden allergens" and they can cause severe allergic reactions or anaphylaxis. Awareness thereof is important for both physicians and patients in order to improve the prevention, diagnosis and treatment of food allergies, leading to a decrease of eventual improper diagnosis of idiopathic anaphylaxis. Aim of this review is to describe the main hidden allergens, to summarize their characteristics, the foods and substances where they usually are found, their more common reported allergic reactions and the diagnostic tools currently available for their identification, focusing on the implementation of diagnostic strategies and the need for their future mandatory labeling on foods products. We also include a section related to complementary and alternative medicine, as the increasing use of herbal medicines or treatments in Western countries has contributed to the rise of sensitization to these substances, which in some cases can act as hidden food allergens.

Hidden allergens have been divided based on their sources in the following sections: vegetable allergens (*i.e.,* herbs and spices, legumes and seeds), additives (*i.e.,* thickeners, food dyes and prebiotics), food substitutes (*i.e.,* wheat and meat substitutes) and complementary and alternative medicine (**table I**).

Methods

In this overview, a literature search was carried out in the PubMed/Medline database using the PubMed search engine as of December 2023. The research included a combined set of keywords: "Hidden allergens", "Hidden food anaphylaxis", "Food labelling", "Food allergen priority", "Spices allergy", "Legumes allergy", "Seeds allergy", "Pine nut allergy", "Additives allergy", "Buckwheat allergy", "Oat allergy", "Millet allergy", "Quinoa allergy" and "Psyllium allergy". The search was mainly limited to articles published over the last 24 years (2000-2023). No language restrictions were adopted. The overview was performed based on the relevant literature, including case reports, case series, clinical trials, retrospective and prospective studies, retrospective series reviews, scoping and narrative reviews. From a result of more of 57,000 entries, 158 relevant articles were selected based on titles, abstracts and relevance to the topic. To obtain additional information sources, the lists of references of relevant articles were examined and a further selection of their content was assessed. Duplicated results, articles not accessible or with titles or abstract not adherent to the topic, were excluded. A priority to plants-derived "hidden allergens" or additives used as ingredients in food products and to healthy food alternatives was given. In this review, other sources of "hidden allergens" like insects, cereal mites, parasites (*e.g., Anisakis simplex*) or contaminations with known declared allergens present in non-food items, were not taken into consideration.

Vegetable allergens

Herbs and spices

The definition of these terms varies in different references. In the culinary definition "spices" include aromatic seasonings obtained from the bark, buds, fruit, root, and seeds of various trees and plants, while "herbs" usually come from the leafy part of the plant (6). They belong to different botanical families: Apiaceae, Lamiaceae, Lauraceae, Leguminosae, Liliaceae, Myristicaceae, Myrtaceae, Piperaceae, Solanaceae, Zingiberaceae, *etc*. Spice allergy is more frequent in adults, due to the greater exposure in this class compared to children, with a frequency ranging from 2 to 6.4% of adults with food allergy (7, 8). According to data from French CICBAA database based on 589 cases of food allergy, the greatest sensitizations are to Apiaceae and Liliaceae families (8). Herbs and spices are commonly used in various food or commercial preparations such as soups, meat dishes, sauces, gravy, ready meals, seasoning, curries, barbeque, pizza, *etc*.

Excluding celery and mustard, which are subject to food labelling requirements in the European Union, other spices are commonly involved in allergic reactions. Among the Apiaceae botanic family coriander, fennel, parsley and cumin are the most involved in allergic reactions. Coriander is an ingredient present in curry, in Italian or Thai seasoning and in Masala powder. It is also used as a "natural" flavoring in beer even if is not always reported in alcoholic drinks and in one case it was reported to have caused anaphylaxis (9). Fennel allergy was described in the context of a pollen food allergy syndrome with mugwort involving a homologue to Api g 5 (profilin) recognized in fennel (10) or of a non-specific lipid transfer protein (nsLTP) syndrome (11), while forms of contact urticaria were described with parsley (12). Cumin was also directly involved in rare cases of anaphylaxis (13) and it can be mistaken for other allergenic foods. Moreover, undeclared contaminants such as peanuts, almonds, Brazil nuts, cashews, hazelnuts, and pistachio were found in cumin used in the manufacture of many food products in the United States (14).

Among the Liliaceae family, garlic and onion were described to be involved in clinical manifestations such as contact dermatitis, urticaria, occupational asthma, rhinitis and also anaphylaxis (15, 16). In addition to profilin and nsLTP, associations between mugwort pollen and food allergies to vegetables, herbs and spices from the Apiaceae (*e.g.,* celery, carrot, parsley, fennel, cumin, coriander, aniseed) and other botanic families (*i.e.,* Solanaceae and Liliaceae) seem to involve another family protein called defensins: ancient

Table I *- Class of food allergens and their associated botanic families, hidden allergens, sources and clinical manifestations.*

Class of allergens	Botanic family	Hidden allergen	Foods and substances containing the hidden allergen	Clinical manifestations
Herbs/spices	*Apiaceae*	Coriander	Curries, seasoning, Masala powder, beers and drinks	Anaphylaxis (9)
	//	Fennel	Curries, sausages, pizza, soups, pasta dishes	Oral allergy syndrome (10) Systemic reactions (11)
	//	Parsley	Seasoning, pasta dishes	Contact urticarial (12)
	//	Cumin	Curries, Masala powder, seasoning	Rare anaphylaxis (13)
	Liliaceae	Garlic and onion	Seasoning, ketchup, Masala powder, pizza, soups, pasta dishes	Contact dermatitis, urticaria, occupational asthma, rhinitis and anaphylaxis (15,16)
	Iridaceae	Saffron	Risotto alla Milanese, pasta dishes	Systemic reactions (18) Respiratory symptoms (rhinitis, conjunctivitis) (19)
	Piperaceae	Black, green and white pepper	Seasoning, curries, lemon pepper, pizza, sauces, soups, barbeque	Systemic reactions (19)
	Solanaceae	Paprika, Cayenne, Chili pepper	Curries, seasoning, pasta dishes, soups, barbeque	Rhinoconjunctivitis, Anaphylaxis (20-23)
	Anacardiaceae	Pink peppercorns	Meat dishes, pasta dishes, seasoning	Cross-reactivity in subject with cashew-nut allergy (24)
	//	Sumac	Mediterranean, Middle East and African dishes	Systemic reaction in subject with cashew nut allergy (25)
Legumes	*Fabaceae*	Pea, lentil, chickpea, bean, *etc.*	Prepackaged food, gluten-free pasta, vegan and meat substitutes (peas, lentils, chickpeas) Yoghurts, pizza, milkshakes, vegetarian burgers (peas)	From mild reactions to anaphylaxis (26)
	//	Fenugreek	Curry powder, spice mixes (Greek, Indian, Turkish, Iranian and Egyptian dishes)	Anaphylaxis, cross-reactivity in subjects with peanuts allergy (30)
Seeds	*Cucurbitaceae*	Pumpkin seeds	Appetizers, condiments in bread or salads, snacks	Anaphylaxis (31-33)
	Compositae	Sunflower seeds	Appetizers, oil, margarine or bread products	Rhinitis, asthma, oral allergy syndrome, urticaria, angioedema, contact dermatitis (38,39) and anaphylaxis (40,41)
	Pinaceae	Pine nuts	Both in raw or roasted form and as ingredients in breads, cakes, cookies, sauces, pesto sauce, candies, vegetable and meat dishes	Oral allergy syndrome, urticaria, angioedema and anaphylaxis (42)
	Linaceae	Flaxseeds	Bakery products	Anaphylaxis (49-53)
	Lamiaceae	Chia seeds	Bread and bread products	Anaphylaxis (56) and dermatitis (57)
	Cannabaceae	Hemp seeds	Energy bars, yoghurts, bakery products	Anaphylaxis, asthma, angioedema (60)
	Papaveraceae	Poppy seeds	Ingredients in cakes, bread and for garnish	Mild oral symptoms, contact urticaria, anaphylaxis (61-65)

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Class of allergens	Botanic family	Hidden allergen	Foods and substances containing the hidden allergen	Clinical manifestations
	Chenopodiaceae	Quinoa seeds	Wheat substitutes, salads, pasta dishes, burgers, meatballs	Anaphylaxis (66,67), occupational asthma (68)
Additives				
Thickeners		Pectin (*i.e.*, E440)	Jellies, jams, candies, smoothies, desserts, fruit juices, milk drinks, medicines	Occupational asthma, rhinitis, dermatitis, anaphylaxis (72-78)
		Gelatin (*i.e.*, E441)	Candies, desserts, drinks, vaccines, medications	Urticaria, anaphylaxis (82-84)
	Fabaceae	Guar gum, carob gum, arabic gum, tragacanth gum	Bakery products, thickened milk products, condiments, canned soups, medicines	Occupational rhinitis/asthma, urticarial, angioedema, anaphylaxis (88,89)
Food dyes		Carmine (*i.e.*, E120)	Sweets, ice lollies, soft drinks, syrups, colored food (*e.g.*, cakes, biscuits), cosmetics, medicines, tissues	Asthma, dermatitis and anaphylaxis (90,91)
	Bixaceae	Annatto (*i.e.*, E160b)	Dairy and bakery products, vegetable oils, drinks	Urticaria, anaphylaxis (93,94)
Prebiotics		Inulin	Naturally in artichokes, chicory, salsify, processed food (*e.g.*, ice creams, butters, margarine, candies, yoghurts, cereals), medical tests (*i.e.*, intravenous test of renal function)	Anaphylaxis (95,96)
Food substitutes				
Wheat substitutes	*Poligonaceae*	Buckwheat	Gluten-free products, noodles, bread, gallettes, pancakes, porridge, kasha, burgers, pizza, polenta taragna, pizzoccheri, buckwheat pillows, soaps	Occupational rhinitis/asthma, urticaria, angioedema and anaphylaxis (99-101)
	Poaceae	Oat	Gluten-free products, biscuits, cereals, soaps, cosmetics	Rhinitis, asthma, contact dermatitis, anaphylaxis (103,104)
	//	Millet	Gluten-free products, cereals	Asthma, systemic reactions, anaphylaxis (105-109)
	Amaranthaceae	Amaranth	Gluten-free products, soups, sauces	Anaphylaxis (112)
Meat substitutes		Mycoprotein	Vegetarian or vegan ready meals	Pruritus, urticaria, respiratory symptoms, anaphylaxis (113)
Complementary and alternative medicine	*Plantaginaceae*	Psyllium (Ispaghula)	Cereals, ice creams, desserts, laxatives	Occupational rhinitis, asthma and anaphylaxis (117)

peptides present in plants and in other live creatures, including humans, with antifungal and anti-bacterial properties highly stable to thermal and acidic treatment. Sensitization to Art v 1, the major allergen from mugwort pollen belonging to defensin-like protein family, was proven to be linked to allergic reactions to celery, horse chestnut seeds, mango and sunflower seeds due to cross-reactivity with homologous defensins discovered in these plant foods. Up to now, few food defensins have been characterized and to determine if they could have a role in the mugwort pollen-related food syndromes, further allergenic defensins in herbs and spices should be identified (17).

Saffron is a spice derived from the flowers of *Crocus sativus* and may induce allergic reactions. Among the molecular allergens identified there are an Ole e 1-like protein (Cro s 1), a profilin (Cro s 2) and nsLTP (Cro s 3). The latter two molecules were shown to be involved in respiratory and severe systemic reactions, respectively (18, 19). Allergies to paprika, Cayenne, Chili pepper and peppercorns are rare. Paprika, Cayenne and Chili pepper are of the *Capsicum* genus which belongs to the Solanaceae family and allergy symptoms such as rhinoconjuncitivitis or rare cases of anaphylaxis to these spices have been described (20-22). Ten allergens have been reported in bell pepper (*Capsicum*

annuum) allergy, but only three of these are actually certified by World Health Organization/International Union of Immunological Societies (WHO/IUIS): an osmotin, member of the thaumatin-like protein family (Cap a 1), a profilin (Cap a 2) and a gibberellin-regulated protein (Cap a 7). This last molecule was found to be involved in a case of anaphylaxis after ingestion of chili pepper in a 16-year-old Japanese girl with known allergy to Japanese cedar pollen (23). Black, green and white pepper are instead all derived from *Piper nigrum* plant which belongs to Piperaceae family; while pink peppercorns are dried berries derived from *Schinus molle* trees belonging to Anacardiaceae family. In literature, a cross-reactivity among Anacardiaceae species in subjects sensitized to cashew nuts with an involvement of seed storage proteins (albumin and legumin type) was described (24). At the same time, allergic reactions to sumac, another spice of the Anacardiaceae family, were reported in subjects known for cashew nut allergy (25).

Key points: spice allergy is uncommon, even if probably under-diagnosed due to the different botanical families involved. Clinical manifestation ranges from respiratory, dermatologic and gastrointestinal symptoms to anaphylaxis. The main molecular allergens involved are pathogenesis-related class 10 proteins (PR-10), profilins, nsLTP, giberellin-regulated proteins (GRPs), seed storage proteins and defensins with the last four protein families generally associated to more severe and systemic reactions. Several spice allergens are degraded by digestion and the sensitization to these allergens mostly occurs via inhalation or through cross-reacting pollen.

Legumes

Legumes include different edibles species (peanuts, soybeans, lupins, lentils, peas, beans *etc*.) all belonging to the Fabaceae botanic family. At present, only peanut, soybean and lupin are reported on the European priority allergy list of allergenic foods, failing to include other legumes that are so-called "non-priority legumes" and can act as hidden allergens. Over the last years, there has been a notable increase of non-priority legumes allergies, probably due to the increase in the consumption of these food in Western countries. They are generally used in vegan meat substitutes or in gluten-free pasta and in other food products such as yoghurt, pizza, milkshake and chicken burgers. Allergenic symptoms are similar for all legumes and can involve the cutaneous, cardiovascular, respiratory, and gastrointestinal systems causing oral allergy syndrome, angioedema, urticaria, rhinitis, asthma or anaphylaxis and even death in extreme and rare cases. Inhalation of vapor, powder, or flour from some legumes could cause respiratory symptoms such as rhinitis, asthma, and hypersensitivity pneumonitis (26). In a recent scoping review including 47 articles about non-priority legumes food allergy, the greatest part of the articles (38.3%) were published in the last five years (2015-2020) demonstrating the growing interest in this type of problem. Of the 47 articles included, 21.3% focused exclusively on the adult population and 38.3% on children. All the articles primarily focused on reporting prevalence that ranged from 0.5% to 39.6% depending on the studies. Lentil was the most commonly analysed non-priority legume (46.8%), followed closely by pea (40.4%); while none of these articles focused on the labelling of these allergens, highlighting how more studies are needed in this area (27).

Co-sensitization among legumes is frequently seen and varies from 36.7% to 100% according to a recent study conducted in adults with a legume allergy in which 16 individual proteins from 10 legumes (peanut, soybean, green pea, chickpea, blue and white lupine, black and green lentil, fava and white bean) were compared and it seems largely due to the 7S/11S globulin fractions or individual 7S and 11S globulins. However, this is not always clinically relevant, because in peanut and soybean-allergic patients, co-allergies for other legumes were uncommon (≤ 16.7%). On the other hand, in green pea, lupine, lentil, and bean-allergic patients, co-allergy for peanut (64.7%-77.8%) or soybean (50%-64.7%) was frequently seen (28). The situation in the pediatric population is not dissimilar: in a study based on 195 peanut-allergic children, Mueller *et al*. found that 64% of them were sensitized, but only 17% had an allergy to one other legume. Most allergies were found to lentil (21%), lupin (19%) and pea (15.4%) with severe reactions affecting 50% of examined subjects (29). Among legumes it could be important to consider fenugreek (*Trigonella foenum-graecum*), often used in the culinary field as a constituent of spice mixes (*i.e.,* curry powder), which is not always correctly labelled in food products. Peanut-allergic patients can react to fenugreek due to the demonstrated cross-reactivity between homologous fenugreek and peanut allergens (30). Except for peanut, soybean and lupin, the following molecular allergens of some "non-priority legumes" are actually registered in the WHO/IUIS allergen database: Len c 1 (vicilin), Len c 2 (seed biotynilated protein) and Len c 3 (nsLTP) from lentil (*Lens culinaris);* Pis s 1 (vicilin), Pis s 2 (convicilin) and Pis s 3 (nsLTP) from green pea (*Pisum sativum*); Cic a 1 (late embryogenesis protein 4) from chickpea; Pha v 3 (nsLTP) from green bean (*Phaseolus vulgaris*); Vig r 1 (pathogenesis-related protein, PR-10), Vig r 2 (vicilin), Vig r 3 (cupin), Vig r 4 (seed albumin), Vig r 5 (fragment of Vig r 2) and Vig r 6 (cytokinin-specific binding protein, CSBP) from green gram (*Vigna radiata*).

Key points: non-priority legumes allergy is a growing problem in recent years and the necessity of their labeling in food allergen lists is becoming indispensable. Clinical manifestations range from mild reactions to anaphylaxis involving different organ systems. Sensitization to seed storage proteins (especially 7S/11S globulins and partially 2S globulins) seems to be involved in most allergic reactions to non-priority legumes, even if other molecular allergens are under investigation.

Seeds

Under this term foods of various species belonging to different botanical families are included. In the European Union, only sesame and mustard seed are actually reported as allergens on food labels. However, other seeds such as pumpkin, sunflower, flaxseed, pine nuts, Chia, hemp, quinoa and poppy are increasingly present in our diets and contribute to the rise in their hypersensitivity in the general population. Prevalence data on seed hypersensitivity are scarce except for sesame or mustard seeds and are mainly based on case reports or case series. Here are summarized the characteristics of the other eight edible seeds, the more common allergic reactions caused by these seeds and their actually identified molecular allergens.

Pumpkin seeds

Pumpkin seeds belongs to the Cucurbitaceae family. It is usually consumed toasted as an appetizer, added to bread or to salads as a condiment or in various snacks. Until now, few cases of pumpkin seed allergy were reported in literature, but it was demonstrated in different situations that subjects with a pumpkin seed allergy usually tolerate the pumpkin flesh, whose sensitivity would instead seem to be associated with sensitization to peach (31-35). From a molecular point of view, two seed storage proteins were identified: a 2S albumin (Cuc ma 5) and an 11S globulin (Cuc ma 4). The prior is considered the first marker of sensitization and was linked to more severe reactions; while the latter is also considered a marker of symptom severity and cross-reactivity with other vegetable sources (melon seed, mustard seed and cashew nut) (36). Moreover, in pumpkin seeds, a homologue of birch profilin (Cuc ma 2) was identified and seems to be involved in pollen-food syndromes (37).

Sunflower seeds

Sunflower seed allergies are rare even if severe allergic reactions to these seeds are described, including anaphylaxis. They belong to the Compositae family and are consumed in the form of seeds, oil, margarine or bread products. Allergy to sunflower seeds included rhinitis, asthma, angioedema, acute urticaria, contact dermatitis, oral allergy syndrome and rarely anaphylaxis (38, 39). The main allergens identified in sunflower seeds include a defensin-like protein (Hel a 1), a profilin (Hel a 2) and a nonspecific lipid transfer protein (Hel a 3), which are involved in cross-reactions to homologous mugwort pollens proteins: Art v 1, Art v 4 and Art v 3, respectively. Hel a 2 was also found to react with profilins of ragweed, olive tree and *Mercurialis perennis* (38). Moreover, 2S albumins proteins (Hel a 15, Hel a 16 and Hel a 17) and oleosins have been identified as allergens responsible for severe reactions, even if patients with sunflower seeds allergy not always had reactions to other nuts or sunflower oil (40, 41).

Pine nuts

Pine nuts belongs to the Pinaceae family, evergreen trees belonging to the old conifers division of Gymnosperms. They have been consumed for over two thousand years in the Mediterranean region. They are frequently used in raw or roasted form and as ingredients in breads, cakes, cookies, sauces, pesto sauce, candies and vegetable or meat dishes. Until now, approximately fifty cases of pine nut allergy have been reported in literature, of which mostly anaphylactic reactions (42). Generally, it is an independent allergy with a high monosensitization rate and poor cross-reactivity with other tree nuts derived of Angiosperms (*e.g.,* peanuts, nuts, walnuts, cashew and pistachio). A 2S albumin (Pin p 1) is considered the major allergen of pine nut (*Pinus pinea*) together with a 7S globulin (Pin p-vicilin), even if other several proteins of different molecular weights (17 kDa; 30, 44, and 50 kDa; and <14 kDa) have been detected (43-46). In an Italian multicentric study including 12 patients with a history of pine nut allergy, an IgE reactivity against allergens present in the lipophilic fraction of the pine nut extract was shown in a patient with negative skin and serological tests, suggesting a potential allergenic role of oleosins in the development of pine nut allergy (47).

Flaxseeds

Flaxseeds are derived from *Linum usitatissimum*, an annual plant of Central Asia and of Arab origin belonging to the Linaceae family, and they were originally used in the textile industry for its fibers. The oil obtained from the seeds is also exploited for the production of printer inks, paints, healing agents, moisturizing creams and laxatives. Moreover, flaxseeds and derivatives are also used as animal feed (birds, dogs, cats, horse *etc*.). Recently, they are included as food in our diets for their nutritional benefits, anti-inflammatory, antioxidant, and cardioprotective properties (48). For this reason, sensitization to flaxseeds in the general population has caused a noticeable increase of different allergic reactions, including anaphylaxis, to be published in literature (49-52). Recently it was shown that seed storage proteins such as a 2S albumin (Lin u 1) and an 11S globulin were involved in severe allergic reactions to flaxseeds (53,54).

Chia seeds

Chia seeds are a "superfood" introduced in our diets for their functional and nutritional proprieties. They are of Mexican origin and Chia (*Salvia hispanica L.*) is an annual plant belonging to the Lamiaceae family originally cultivated in South America by pre-Columbian populations for thousands of years. Since 2005, Chia seeds were introduced to the European Union as a novel ingredient in bread and bread products (55). Rare cases of anaphylactic reactions and dermatitis after ingestion of Chia seeds were described (56, 57). Albunni *et al.* (58) demonstrated that legumins and vicilins contained in Chia seed possess similar IgG or IgE binding epitopes as those in sesame proteins or other nuts (*i.e.,* hazelnuts) and legumes (*i.e.,* peanuts) and that this could translate into possible cross-reactivity reactions to these seeds in patients with other food allergies.

Hemp seeds

Hemp seeds are a variety of *Cannabis sativa* plant species, belonging to Cannabaceae, that is grown for the industrial uses of its derived products. The seeds are highly nutritious foods and they are used in various food products including energy bars, yoghurts and bakery products. Also in this case, the widespread availability and use of hemp seeds in the food industry contribute to the rise of hemp seed allergy (59). Allergic reactions have been reported after their ingestion in patients without previous direct exposure to cannabis. In these cases, a possible sensitization from an indirect exposure or a cross-reactivity with homologous pollen proteins in patients with tree or weed pollinosis is presumed (60). Sensitizations to nsLTP, PR-10 and profilin homologues (Can s 3, Can s 5 and Can s 2, respectively) appear to be involved in most allergic reactions to hemp seeds (43). Other molecular allergens registered in the official allergen database WHO/IUIS are an oxygen evolving Enhancer Protein 2 (Can s 4) and a thaumatin-like protein (Can s 7).

Poppy seeds

Poppy seeds are derived from *Papaver somniferum* and belong to Papaveraceae family. They are generally used as ingredients in cakes, breads and for garnish and are rarely considered a cause of food allergy (61). Various types of allergic reactions to these seeds were described: from mild oral symptoms and contact urticaria to anaphylaxis (61-65). Furthermore, a cross-reactivity with hazelnut, buckwheat and sesame was shown, in part explained by a sensitization to homologous seed storage proteins: Pap s 1 (7S vicilin), Pap s 2 (11S globulin) and a 2S albumin (Pap s 2S) present in poppy seeds (65). In the WHO/IUIS allergen database another protein of 10 kDa (Pap s 3) corresponding to a small hydrophilic seed protein belonging to the family of the late embryogenesis abundant protein 5 (LEA-5) was recently registered.

Quinoa seeds

Quinoa seeds are derived from *Chenopodium quinoa*, an herbaceous annual plant belonging to Chenopodiaceae family. A flour is obtained from quinoa seeds which is especially used as wheat substitute for celiac or gluten sensitive patients since it does not contain gluten. Allergy reactions to quinoa are rare and few case reports of anaphylaxis after ingestion or occupational allergy after exposure to quinoa flour are reported in literature (66-68). In a recent study conducted in rats, Ballegaard AR *et al.* showed that quinoa proteins (especially 11S globulin) were found to have an inherent medium to high immunogenicity and sensitizing capacity. Regarding the cross-reactivity with peanut and tree nuts (*i.e.,* hazelnut, walnut, cashew nut, Brazil nut) the highest percentage of identification was found for 11S globulin, profilin and oleosin. Further studies are necessary to demonstrate if this medium-high level of allergenicity of quinoa seeds is also present in humans (69).

Key points: hypersensitivity reactions to seeds range from respiratory (*i.e.,* rhinitis, asthma) and dermatologic (*i.e.,* urticaria, angioedema, dermatitis) symptoms to oral allergy syndrome and anaphylaxis. The main molecular allergens involved in severe allergic reactions are seed storage proteins (cupins, vicilins or legumins), nsLTP and oleosins, while PR-10 proteins and profilins are mainly involved in mild or oral allergy symptoms. Sensitization could occur by ingestion, contact or through pollen exposure with a cross-reaction among homologous pollen proteins. The impact of seed allergy will increase in the coming years due to their large availability and consumption in the food industry and so their entry in food allergen lists should be considered.

Additives

Additives include different compounds which are added to products to perform specific functions (*i.e.,* coloring, sweetening, thickening or preserving foods). In the European Union they are identified by letter E followed by a specific number. Product labeling must specify additive proprieties by referring to its name and E number (70). Additives are potentially involved in different kind of allergic or immunological reactions (IgE-mediated, non-IgE-mediated, and mixed IgE/non-IgE-mediated reactions) with different clinical manifestations mainly involving cutaneous, respiratory and gastrointestinal systems. However, the prevalence of these adverse reactions remain difficult to estimate due to symptom subjectivity and the lack of reliable markers of reactivity (71). Among additives, in the European Union only sulphites contained in foods above a certain concentration (≥ 10 mg/kg or 10 mg/liter expressed as SO_2) are subjected to obligatory labeling. It has been reported that other additives for which an IgE-mediated mechanism has been demonstrated that have not been subjected to mandatory labeling could potentially act as hidden allergens. The characteristics and the allergic reactions to these additives are listed below and they were divided into three categories: thickeners, food dyes and prebiotics.

Thickeners

Pectin (i.e., E440)

Pectin is a structural heteropolysaccharide present in most primary cell walls of vegetable tissues. It is used for its emulsifying and thickening proprieties in various foods (*i.e.,* candies, jellies, jams, smoothies, fruit juices, milk drinks) but also in medications (*i.e.,* barium suspensions). Case reports of reactions to pectin present in foods or medications have been reported for many years in literature and some of these were also associated with occupational asthma or they occurred in patients with known cashew allergy (72-78). The mechanism underlying the cross-reactivity between cashew/pistachio and pectin has not been determined, but it is purported to be due to a novel carbohydrate allergen or a cross-reaction between proteins in tree nuts and apples or citrus

fruits (79-81). For this reason, pectin should always be considered in patients with cashew or pistachio allergy reporting allergic reactions to an unknown trigger.

Gelatin (i.e., E441)

Gelatin can be derived from mammalian meats or fish. It can be found as a stabilizing agent in different foods such as candies, desserts and drinks as well as in vaccines (*i.e.,* influenza, MMR, *Varicella* spp., *Varicella zoster,* rabies, typhoid, yellow fever) or in medications (*i.e.,* hemostatic agents, capsules, suppositories, erythropoietin, plasma volume expanders and colloids). Allergic reactions including anaphylaxis to desserts containing mammalian or fish derived gelatin are reported in literature, even without previous reactions to vaccines (82-84). Fish gelatin is a hydrolyzed collagen type I derived from fish skin and bones, while commercial bovine gelatin consists of denatured type I collagen derived from mammalian tendon, cartilage or skin and it has been demonstrated that they do not cross-react with each other (85). Moreover, a sensitization to galactose-α-1,3-galactose (alpha-gal) might risk the development of allergic reactions to mammalian gelatin-containing foods (86,87).

Guar gum (i.e., E412)

Guar gum is a polysaccharide extracted from guar beans (*Cyamopsis tetragonoloba*), members of the Fabaceae family, used for its thickening and stabilizing proprieties in various foods such as baked foods, thickened milk products, condiments and canned soups. Guar gum has been shown to cause occupational rhinitis and/or asthma and in one instance a case of anaphylaxis has also been described (88,89). Potential allergic reactions to other gums including carob, tragacanth and acacia gum were also reported (88).

Food dyes

Food dyes are used to enhance or change the color of many food and beverage products. Allergic reactions to natural dyes such as carmine, annatto and saffron are described.

Carmine (i.e., E120)

Carmine is a red dye extracted from dried female cochineal insects (*Dactylopius coccus*). Over 30 cases of IgE-mediated hypersensitivity reactions to carmine red were reported and most were anaphylactic reactions, as well as cases of delayed anaphylaxis (90, 91). Cutaneous exposure through cosmetics seems important to sensitization to this dye (92).

Annatto (i.e., E160b)

Annatto is a yellowish orange pigment derived from the seeds of *Bixa Orellana* and is used as an ingredient in dairy and bakery products, vegetable oils and drinks. In literature, few cases of anaphylactic reactions to this dye have been described (93, 94).

Saffron (i.e., E164)

Saffron is a yellow food coloring extracted from the dried stigma of flowers of *Crocus sativus* that may induce allergic reactions. See the section above "Herbs and spices".

Prebiotics

Inulin

Inulin is a non-digestible carbohydrate present in many natural foods such as artichokes, chicory and salsify. Because of its health benefits, it is recently used as a prebiotic added to various processed foods (*e.g.,* ice creams, butters, margarine, candies, yoghurts, cereals, *etc.*). Rare cases of anaphylaxis to inulin were reported and in one of them specific IgE antibodies directed to a specific inulin protein were shown (95, 96).

Key points: allergic reactions to food additives is not frequent, although it's overrated and often reported by patients. However, the recognition of IgE mediated allergies is important to avoid severe and life-threatening reactions. Little is known about the molecular allergens involved and needs to be investigated.

Wheat substitutes

Celiac disease affects about 1% of the general population and gluten-free diet is the primary form of treatment of this disease (97). However, during the last decade, an increment of individuals self-reporting wheat sensitivity who decide to exclude gluten from their diet was observed, despite not having a diagnosis of celiac disease. Recent observational studies from across the world suggest that about 10% (range 4.3-14.9%) of the population is self-reporting wheat sensitivity and in Italy it stands at about 12.2% (98). The progressive market promotion of gluten-free diets has notably influenced the increase in the avoidance of wheat and other cereals containing gluten (*i.e.,* barley, rye) with greater use of wheat substitutes such as oat, buckwheat, millet, quinoa and amaranth.

Buckwheat

Buckwheat is considered a pseudocereal, because unlike other cereals it does not belong to the Poaceae family but to the Poligonaceae family instead, even if its proprieties and its food use are similar to other cereals. Two species of cultivated buckwheat are known: common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*). Buckwheat is used in different types of food such as noodles, bread, pancakes, porridge and kasha, pre-boiled and dried whole buckwheat grain. In Italy, it is also used for famous traditional dishes as polenta taragna (a hot porridge from the north of Italy) and pizzoccheri (a type of pasta from Valtellina, a small region near Como's lake in Northern Italy). Allergic reactions to buckwheat are described after the ingestion of buckwheat food products, in the work environment or when sleeping on pillows containing

buckwheat husks. Clinical manifestations range from occupational rhinitis or asthma, dermatitis, urticaria and angioedema to anaphylaxis (99). Buckwheat allergy is more common in Asian countries (*e.g.,* China, Korea and Japan) and for this reason buckwheat is included in their priority allergenic food lists. However, in Europe it could be considered a potential cause of hidden anaphylaxis (99-101). Nowadays, Fag e 1 (13S globulin), Fag e 2 (2S albumin), Fag e 3 (alpha-hairpinin), Fag e 4 (hevein-like antimicrobial peptide), Fag e 5 (vicilin-like protein) are the molecular allergens identified of *Fagopyrum esculentum* and Fag t 1 (legume-type protein), Fag t 2 (2S albumin) and Fag t 3 (seed storage protein of cupin superfamily) are those of *Fagopyrum tataricum.* Sensitization to Fag e 2 (a highly stable 2S albumin) is often related with severe reactions including anaphylaxis and is thus considered an important allergen in buckwheat anaphylaxis. In addition, an oleosin (Fag t 6) was recently implicated in allergic reactions to tartary buckwheat (*Fagopyrum tataricum*) (43,102). However, in the official allergen database WHO/IUIS, only Fag e 2, Fag e 4, Fag e 5 of *Fagopyrum esculentum* and Fag t 2 and Fag t 6 of *Fagopyrum tartaricum* are actually registered.

Oat

Oat (*Avena sativa*) is a cereal from the Poaceae family generally well tolerated by the majority of patients with Celiac disease. Oat is occasionally involved in forms of occupational allergies (*i.e.,* rhinitis or Baker's asthma) or contact dermatitis in children with atopic dermatitis who regularly used oat-based creams (103). Moreover, rare cases of anaphylaxis after ingestion were also reported (104). To date no major molecular allergens have actually been identified.

Millet

Millet is another cereal belonging to the Poaceae family, which is usually consumed in Western countries as a "healthy" alternative cereal or as a gluten-free substitute for wheat. Millet allergy is rare and has been mainly recognized in industrialized countries (*i.e.,* USA, Japan and Central Europe) where reactions especially included severe anaphylaxis (105-109). Sensitization to this cereal seems to occur mostly with inhalation through millet containing birdseed. Millet allergens have not yet been identified in detail; however, a potential cross-reactivity with homologues allergens present in other cereals (*i.e.,* rice, wheat and corn) has been reported (110). Recently a case of wheat-induced anaphylaxis in a bird-keeper caused by an early sensitization to millet was also described (111). No specific molecular allergens have been identified to date.

Amaranth

Amaranth seeds are also used as a wheat substitute and they have also been reported to cause anaphylaxis (112).

Quinoa

See the section above "Seeds".

Meat substitutes

Over the last 20 years, meat substitutes (*i.e.,* Quorn) composed of mycoproteins derived by a fungus called *Fusarium venenatum* have been marketed. They are usually part of composite dishes within which they may not always be obvious as allergens because they could take on the flavor of herbs or spices or could be confused for other types of meat. In a web survey conducted in USA, Jacobson *et al.* reported 312 allergic reactions to mycoproteins (12,5% anaphylaxis) of which 72,4% occurred at the first individual exposure (113). This last data suggests a possible cross-reactivity with other foods or allergens. In this regard, a possible link between sensitization to mold and allergic reactions to mycoproteins was found (114, 115).

Complementary and alternative medicine

Complementary and alternative medicine include a huge range of therapies, practices and healthcare systems mainly based on the use of herbal medicines or products. In Western countries, during the last decades its prevalence has noticeably increased. Allergic reactions and anaphylaxis after the use of herbal treatments that include Phleum pretense (6.5%), Andrographis paniculata (5%), Echinacea purpura (3.8%), Ginkgo biloba (3.6%) and bee products have been described in literature (116).

Psyllium (ispaghula)

Psyllium, or ispaghula, is a natural hydrophilic mucilloid of the genus *Plantago.* It was traditionally used as a laxative and recently for its lipid-lowering property. Psyllium was known to be associated with a form of occupational allergy well described in health care workers and pharmaceutical plant employees with clinical manifestations ranging from rhinitis and asthma to anaphylaxis (117). Psyllium is also used as a natural thickener in many foods (*e.g.,* cereals, ice creams, desserts) and in sensitized subjects it can act as an hidden allergen.

Key points: the growing use of food substitutes or complementary medicine in Western countries has led to a rise of hypersensitivity reactions to these allergens. Clinical manifestations range from respiratory (*i.e.,* rhinitis, asthma) and dermatologic (*i.e.,* urticaria, dermatitis) symptoms to anaphylaxis. Therefore, the occupational exposure of these substances seems to play an important role for their sensitization.

Also, for these allergens, with the exception of buckwheat, little is known about the molecular allergens involved in the allergic reactions.

Diagnosis

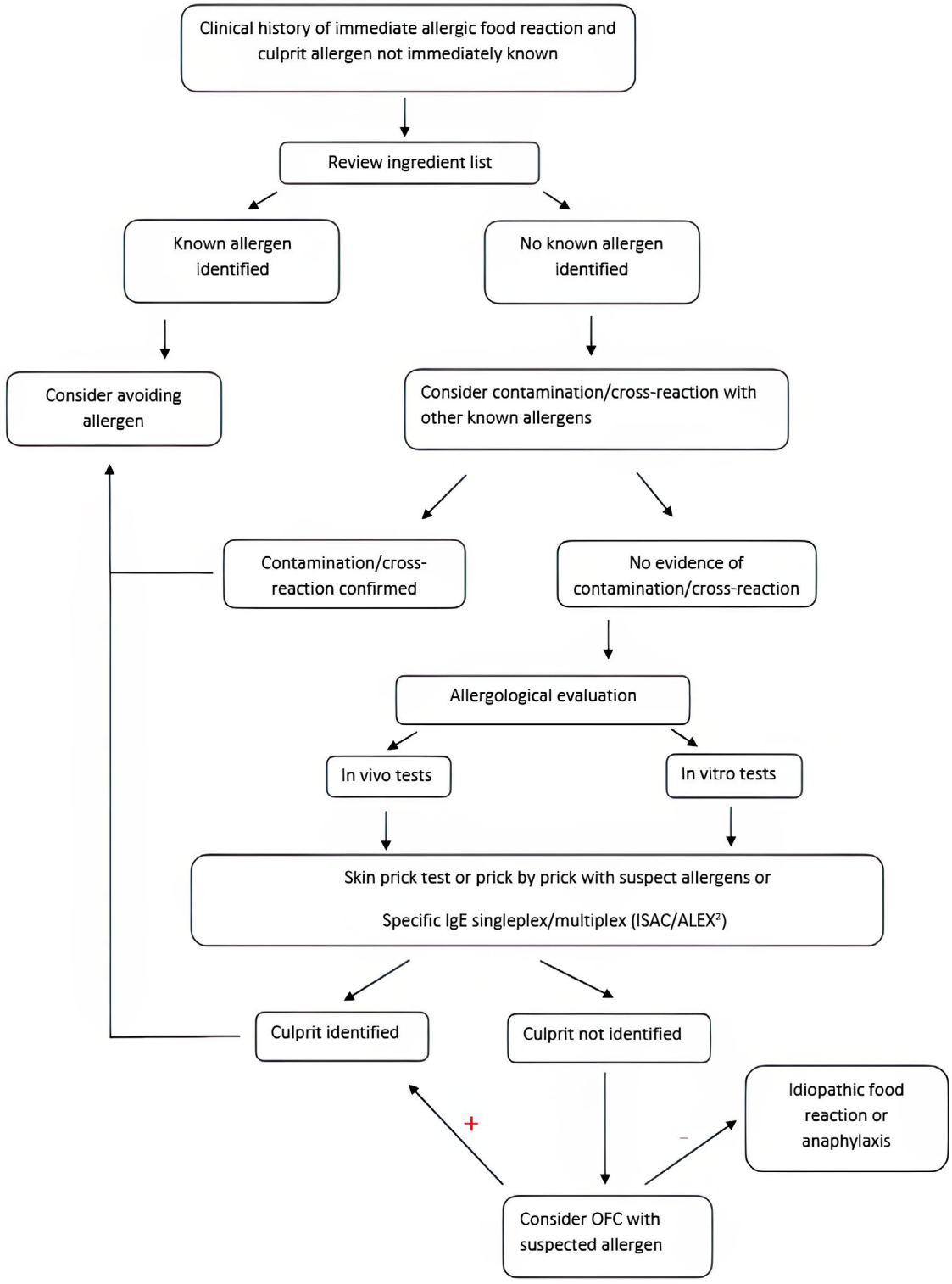
The identification of the culprit allergen is not always simple because most of these allergens are not actually subjected to man-

datory labelling in food products and many diagnostic tools are not available in common practice. However, a careful history and review of ingredient lists with the understanding of possible cross-reactions/contaminations with other foods is recommended in successfully identifying the culprit allergen. Prick by prick and/or commercial skin prick test together with the research of currently available specific commercial IgE (both in singleplex or multiplex methods) could be used to detect IgE sensitization to these potential allergens even if the oral food challenge (OFC) with the culprit agent remain the gold standard for the diagnosis (**figure 1**). Concerning the laboratory diagnostics, various specific IgE extracts for herbs and spices (*e.g.,* coriander, fennel, parsley, cumin, black and green pepper, paprika, garlic, onion, *etc*.) both ImmunoCAP and multiplex methods (ImmunoCAP ISAC or ALEX² microarrays) are available, although no specific molecular allergens are actually included. The diagnostic tools currently available for the investigation of legume allergies are mainly based on the priority legumes (*i.e.,* peanuts and soybeans) and little or nothing on the non-priority ones (*e.g.,* peas, lentils, chickpeas, beans, *etc*.) and again only specific IgE extracts without molecular allergens. Similarly, few seeds extracts are actually available (*e.g.,* pumpkin seeds, sunflower seeds, quinoa seeds, flaxseed, poppy seeds, hemp seeds and pine nuts) and from a molecular point of view, only the lipid transfer protein of hemp seeds (Can s 3) and the 2S albumin of poppy seed (Pap s 2S) (both only in the ALEX² microarray) can be currently assessed. Fewer diagnostic extracts are available for additives (*i.e.,* guar gum, carob gum, arabic gum, bovine gelatin and carmine red), wheat substitutes (*i.e.,* buckwheat, oat and millet) and other hidden allergens (*i.e.,* psyillum or ispaghula). Regarding buckwheat allergy, only the 2S albumin of *Fagopyrum esculentum* (Fag e 2) can be currently assessed but only with multiplex methods (ImmunoCAP ISAC or ALEX²) (**table II**).

Discussion

The progressive change in eating habits, together with the development of the food industry, the adoption of new technologies and globalization phenomena, have contributed to a great variability and availability of all sorts of foods. In this contest, a growing minority of people may become allergic to other lesser-known allergenic substances which are not always adequately reported in the current food allergen lists. A large variety of food reactions caused by hidden allergens has been reported in literature and, in various cases, severe systemic reactions up to anaphylaxis have been described. Sensitization to new vegetable allergens (*i.e.,* herbs and spices, legumes, seeds) and food substitutes are constantly increasing in the general population. In the European Anaphylaxis Registry during the period of 2007 to 2020 in the subgroup of "other tree nuts", pine nuts were the elicitors in over 19% of cases after almond and pistachio, while in the group of seeds, sunflower and pumpkin seeds were involved in 18% and 12% of cases, respectively (118). In children and adolescents, data on anaphylaxis taken from the European registry during the period of 2007 to 2015 have shown that among the subgroup of vegetables, celery was the main elicitor in 42% of cases, but other vegetables (*i.e.,* carrot, lettuce, tomato and cabbage) were the elicitors in over 58% of cases. In the subgroup of cereals, with the exclusion of wheat which was the leading elicitor in the 62% of cases, other cereals (*i.e.,* buckwheat, barley, sweet corn, rye, rice and spelt) were involved in over 38% of cases and in the legumes subgroup, pea and other legumes (*i.e.,* bean, chickpea, lupine and lentil) were the elicitors in over 12% of cases. The main elicitors remain peanuts and soy (88% of cases). Among the tree nuts subgroup, pine nuts were involved in over 5% of anaphylaxis and in the spices subgroup, sesame remained the main elicitor even if other spices (*i.e.,* curry, poppy, pepper, mustard, sunflower and pumpkin seed) were involved in above 44% of anaphylaxis (119). These data show how the ever-increasing impact of these allergens in terms of public health should not be underestimated. More difficult to estimate is the real prevalence of additives adverse reactions because symptoms are prone to subjectivity and markers of reactivity are not available (71). In addition, only in a few case reports or case series an IgE mediated mechanism was shown, so that it is difficult to estimate their real prevalence and the impact on public health. However, their reporting on product labels could avoid potentially serious and unwanted adverse reactions. Up to now, little is known about some hidden allergens from a molecular point of view, and this is true especially for various spices, some seeds (*e.g.,* Chia seeds, flaxseeds and quinoa seeds), additives (*e.g.,* pectin, gelatin, inulin and food dyes) and wheat substitutes (*e.g.,* oat, millet and amaranth) and this knowledge gap should be filled in the future. At the same time, for those allergens where a molecular profile is more defined such as some legumes, other seeds (*e.g.,* pumpkin, sunflower, hemp, poppy seeds and pine nuts) and buckwheat, there currently is a lack of diagnostic tools capable of identifying their molecular allergens and diagnoses are based on skin prick test or prick by prick with extracts or using commercial specific IgE for whole extracts. Future researches in this regard will be necessary to implement diagnostic possibilities at our disposal. We acknowledge the limitations of this review compared to a scoping or a systematic review, since we have included multiple study designs (*e.g.,* case reports, case series, retrospective and prospective studies, retrospective series and narrative reviews) with a lack of assessment of the quality and evidence of the included studies. Moreover, our review include a literature search that was limited to only one electronic database (*i.e.,* PubMed/Medline) and it is possible that we have not considered all available data or documentations actually present in literature. Future studies based on a larger amount of data with a more stringent and systematic use of the inclusion or exclusion criteria of the published

Figure 1 *- Diagnostic algorithm approach to evaluate allergic food reactions caused by a hidden allergen.*



OFC: oral food challenge.

Table II *- In vivo and in vitro diagnostic tools available for the diagnosis of hidden allergens except the European declared allergens celery, mustard, peanut, soybean, lupin and sesame.*

Class of allergens	*In vivo* tests	*In vitro* tests		
	Skin prick tests/prick by prick	Specific IgE singleplex (ImmunoCAP)	Specific IgE multiplex microarrays	
			ISAC	ALEX²
Herbs and spices	SPT and PBP for single herb or spice	Anise, basil, bay leaf, black pepper, cumin, chili pepper, clove, coriander, dill, fennel, garlic, ginger, green pepper, lovage, mace, marjoram, mint, onion, oregano, paprika, parsley, tarragon, thyme, vanilla, *etc*. No commercial specific IgE for saffron, pink peppercorns and sumac	//	Anise, cumin, garlic, onion, oregano, paprika
Legumes	SPT and PBP for single legume	Pea, green and white bean, chickpea, fenugreek, lentil, lupin seed, carob	//	Chickpea, white bean, lentil, pea
Seeds	PBP for specific seed	Pumpkin seed, sunflower seed, flaxseed, hemp seed, quinoa seed, poppy seed, pine nut	//	Pumpkin seed, sunflower seed, poppy seed (both extract and Pap 2S albumin), hemp seed (both extract and Can s 3), quinoa
Additives				
Thickeners	PBP with pectin and gelatin containing foods, guar gum, carob gum, arabic gum and tragacanth gum	Bovine gelatin, guar, carob and arabic gum No commercial specific IgE for pectin, fish gelatin and tragacanth gum	//	//
Food dyes	PBP with fresh carmine and annatto	Carmine red No commercial specific IgE for annatto	//	//
Prebiotics	PBP with inulin containing foods	No commercial IgE available	//	//
Food substitutes				
Wheat substitutes	PBP or SPT with buckwheat, oat, millet and amaranth	Buckwheat (*Fagopyrum esculentum),* oat and millet No commercial specific IgE for amaranth	Buckwheat (Fag e 2)	Buckwheat (both extract and Fag e 2), oat and millet
Meat substitutes	PBP with Quorn	No commercial specific IgE available	//	//
Complementary and alternative medicine	PBP with fresh psyllium	Ispaghula	//	//

SPT: skin prick test; PBP: prick by prick.

studies could provide broader and more detailed information on this complex topic.

Conclusions

Hidden allergens are potential causes of misdiagnosed allergic reactions or idiopathic anaphylaxis. During the last decade, the growing trend of wheat or meat exclusion with the adoption of plant-based diets or new sustainable foods is contributing to the exposure to new emerging allergens. Food allergies are on the rise, with an increasing prevalence worldwide and the main allergens involved in anaphylactic reactions in adults are predominantly of plant origin, mainly legumes and nuts (120). In this review, we tried to summarize the clinical implications and the molecular characteristics of several potential hidden allergens: our findings showed how there is a need to expand our knowledge, especially on vegetables allergens (*i.e.,* herbs and spices, legumes and seeds), additives and food substitutes, both from a molecular point of view and from potential cross-reactivity. Moreover, it will be important to take into consideration other potential allergens used in the context of complementary and alternative medicine. The European Union declaration is currently mandatory for a few plant derived allergens: cereals containing glutens, peanuts, soybeans, tree nuts, celery, mustard, sesame seeds and lupin. At present, no declarations for other cereals (*e.g.,* buckwheat, oat, millet, quinoa, rice), legumes (*e.g.,* peas, lentils, chickpeas, beans), vegetables (*e.g.,* garlic, onions, fennel, parsley, cumin, carrot, tomatoes) and several seeds (*e.g.,* pumpkin, sunflower, flaxseed, poppy, hemp seeds), potentially causing severe allergic reactions, are yet proposed. Curiously, among additives none for the allergens for which an IgE-mediated mechanism has been demonstrated (*e.g.,* carmine red, guar gum, gelatin, pectin) are subject to mandatory declarations and only sulphites, for which the hypersensitivity mechanism still remains unclear and debated, are reported. Therefore, the need to cover more allergens groups re-evaluating the inclusion of same debated allergens, would suggest a revision of the current list of priority food allergens in order to avoid the onset of unpredictable and life-threatening allergic reactions.

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Conflict of interests

The authors declare that they have no conflict of interests.

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