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Major allergens in 8 types of allergenic foods and allergen detection techniques

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Yan Lu is responsible for writing articles; Chun-Ming Dong is responsible for collecting information. All authors have read and agreed to the final draft.

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Abbreviations

WHO, World Health Organization; ELISA, enzyme-linked immunosorbent assay; WB, Western blot; NC membrane, nitrocellulose membrane; C lines, control lines; T lines, detection lines; LC-MS/MS, liquid chromatography-tandem mass spectrometry; ICA, immunochromatography assay; QPCR, quantitative polymerase chain reaction; LAMP, loop-mediated isothermal amplification.

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Abstract

In recent years, the prevalence of allergens in food warning notices, both domestically and internationally, has become the second leading concern after microbial contamination. Among the various factors that threaten human health reported by the World Health Organization, food allergy ranks fourth, and food allergy has become a global security problem. As of now, no definitive treatment for food allergies exists, making the avoidance of allergen-containing foods the most effective prevention method. Consequently, labeling foods with allergen information serves as a crucial warning for allergic populations. Moreover, to enhance comprehension of food allergies, this article provides a brief overview of their definition and sensitization mechanisms. The main focus lies in highlighting the structure of primary allergens found in eight commonly allergenic foods and the resulting clinical symptoms they cause. Additionally, a summary of commonly employed allergen detection techniques is presented, with an analysis of their principles, advantages, and limitations. Looking ahead, the integration of diverse technological approaches to establish an efficient, accurate, and affordable allergen detection method remains a significant trend. This article has certain reference value for understanding the direction of food allergies.

Keywords: food allergy; allergen labeling; sensitized food; major allergens; detection technique

Background

Food allergies have emerged as a significant global public health issue. Over the past few decades, as living standards have improved, the consumption of seafood has risen steadily, leading to an increased prevalence of allergic diseases. Furthermore, the fast-paced lifestyle of people has driven the popularity of processed foods, including finished products, semi-finished products, convenience foods, and instant foods. Unfortunately, the use of various seasonings, preservatives, and other chemicals in food processing poses a threat as these substances are known allergens. Moreover, the expansion of dietary choices, particularly the introduction of genetically modified foods, has further contributed to the heightened risk of allergic diseases [1]. Regrettably, the incidence of allergen-related conditions is on the rise, and the symptoms associated with these allergies are becoming more diverse, complex, and severe [2].

Food allergy and food pollution are distinct concepts. Food pollution encompasses the entire process of food production, from planting and growing to harvesting, fishing, slaughtering, processing, storage, transportation, sales, and consumption. During this process, certain toxic and harmful substances may interfere, leading to a reduction in nutritional value and hygiene quality of the food or varying degrees of harm to human health. On the other hand, food allergy refers to an immune reaction that occurs when humans ingest or come into contact with foods containing specific allergens [3]. Unlike the toxic and harmful substances found in food pollution, food allergens are antigenic substances capable of selectively activating human cells, triggering specific antibody reactions, and causing allergic responses [4]. These food allergens are mostly proteins or glycoproteins with molecular weights ranging from 10 kd to 70 kd, constituting only a small portion of the total protein content in the food. However, even in small quantities, food allergens can lead to severe allergic reactions [5].

According to research in clinical allergy, the majority of food allergies fall under type I allergy, also known as immediate hypersensitivity. The sensitization mechanism for this type of allergy is depicted in Figure 1.

Upon initial exposure to an allergen, the body produces and releases IgE antibodies in response. These IgE antibodies remain present in the body but do not elicit an allergic reaction at that time. However, upon subsequent contact with the same allergen, it binds to the pre-existing IgE antibodies in the body. This binding triggers the high-affinity receptors on mast cells or basophils, leading to the activation of these cells. As a consequence of this activation, mast cells or basophils release histamine and other inflammatory substances into the bloodstream. These substances cause various allergic symptoms, posing a threat to human health [6].

Provisions of various countries on the labeling of food allergens

To prevent and minimize the adverse effects of allergens on consumers and enable them to avoid foods containing allergens, the most effective approach is to provide clear labeling on food packaging [7]. By indicating the presence of allergens in the product, individuals with allergies can make informed choices and avoid potential health risks.

However, due to variations in dietary preferences among different countries or regions and differences in people's adaptability to certain foods, the list of allergenic foods can vary. As a result, some countries and regions have established their own regulations and requirements for labeling food allergens, aligning them with the guidelines set forth by the Codex Alimentarius Commission and adapting them to their national conditions, as shown in Table 1 [8, 9].

Common food allergens

According to statistics, there are approximately 180 known food allergens that can trigger allergic reactions. As of 2016, the International Union for Immunization and the World Health Organization (WHO) have officially recognized and named 297 food allergens. Among these, 62 plant- derived foods contain 205 allergens, and 40 animal-derived foods contain 92 allergens. Notably, 90% of allergic reactions are caused by eggs, fish, milk, wheat. Eight types of food, including peanuts, soybeans, tree nuts, and crustaceans, are also known as the "eight major" allergenic foods [10, 11].

Crustacean aquatic products

Crustacean aquatic products, such as shrimp, lobster and crab, hold great significance in human nutrition due to their abundance of high-quality proteins, essential nutrients, polyunsaturated fatty acids, and vitamins. These delectable aquatic delights are highly favored by consumers for their delightful taste [12, 13]. Despite their nutritional benefits, it is crucial to be aware that even minute quantities of crustacean ingredients can lead to severe allergic reactions, and in some cases, these reactions can be life-threatening.

Globally, crustaceans are among the most prevalent triggers of food allergies, affecting both adults and children. In the Asia-Pacific region, they stand out as the primary cause of food allergies among children [14]. Unlike food allergies such as milk and eggs, most children can develop tolerance to milk and eggs as they grow older, but allergies to crustaceans, peanuts and tree nuts persist [15]. Crustacean allergy is particularly noteworthy in emergency departments, where it is considered the most frequent type of food allergy among patients older than 6 years of age [16, 17].

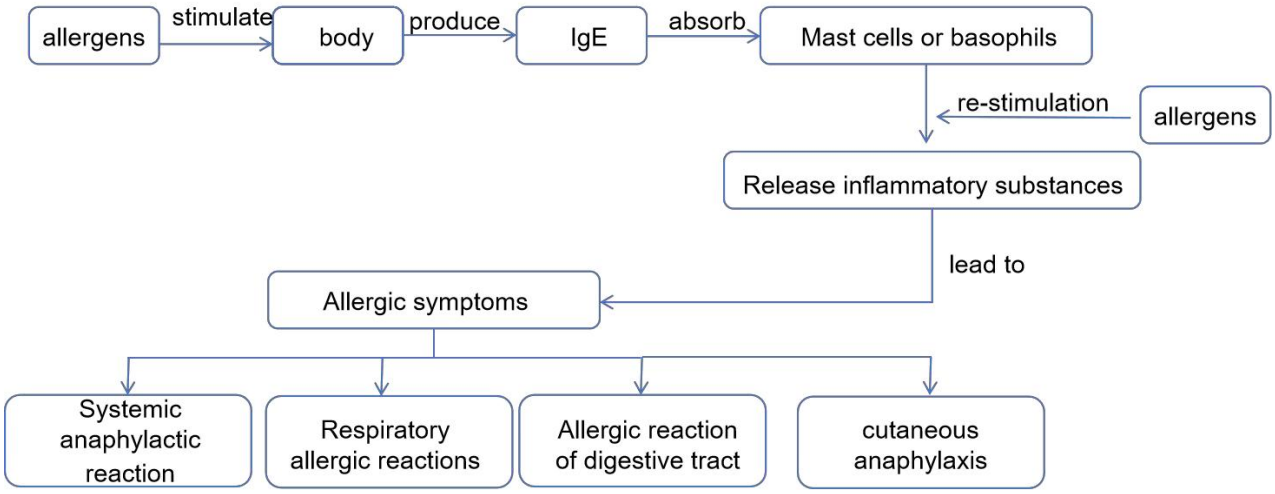

Figure 1 Sensitization mechanism

Table 1 Allergen labeling regulations

Country	Requirement	Source	Allergen
Codex Alimentarius Commission	Coerciveness	Codex General Standard for the Identification of Prepackaged Food (8th Edition, 2010)	Cereals and their products containing gluten, crustaceans and their products, eggs and their products, fish and their products, milk and their products, tree nuts and their products, peanuts and soybeans and their products, sulfite with a concentration exceeding 10 mg/kg, etc.
European Union	Coerciveness	New Food Labeling Act (EU) No. 1169/2011	Cereals and their products containing gluten, crustaceans and their products, eggs and their products, fish and their products, milk and its products, tree nuts and their products, peanuts and soybeans and their products, sulfite with a concentration exceeding 10 mg/kg, celery and its products, mustard and its products, sesame and its products, lupine and its products, mollusks and their products, etc.
The United States of America	Coerciveness	Food Allergy Safety, Treatment, Education, and Research Act (referred to as the FASTER Act)	Milk, egg food, fish food, crustaceans, aquatic products, tree nuts, wheat, peanuts, soybeans and other eight categories
Japan	Coerciveness	Food hygiene law	Eggs, milk, wheat, buckwheat, peanuts, shrimp, lobster, and crabs
Australia and New Zealand	Mandatory/suggested	Food Standards Code of Australia and New Zealand	Cereals made of gluten or its products, shells and their products, eggs and their products, fish and their products, milk and its products, peanuts, soybeans, and their products, sulfates with a concentration of more than 10 mg/kg, nuts, sesame seeds, and their products and royal jelly
Canada	Coerciveness	Food Allergen Labeling Scheme	Peanuts, eggs, milk, nuts, wheat, soybeans, sesame seeds, seafood (fish, crustaceans, and shellfish), mustard, sulfite, etc.
South Africa	Coerciveness	Food, Cosmetics, and Disinfectants Act No. 642 of July 20, 2007: On the Labeling and Advertising of Food in the 1972 Act (54 1972)	Milk, egg food, fish food, crustaceans, aquatic products, tree nuts, wheat, peanuts, soybeans, etc.
South Korea	Coerciveness	Food Labeling Standards (Notice 2003-27)	Egg products, dairy products, buckwheat, peanuts, soybeans, wheat, mackerel, crabs, pork, peaches and tomatoes
China	Recommendability	Allergenic Ingredients in Prepackaged Foods (GB/T 23779–2009): General Rules on Labeling of Pre-Packaged Food	Cereals containing gluten, crustaceans, fish, eggs, peanuts, soybeans, milk, nuts, and processed products containing these eight kinds of foods
Hong Kong, China	Coerciveness	Food and Drugs (Composition and Labeling) Regulations	Gluten grains, crustaceans, eggs, fish, peanuts, soybeans, milk, nuts, sulfites, etc.

Crustacean food allergies can trigger various allergic symptoms in affected individuals. These symptoms may manifest as skin reactions, including redness, itching, rashes, hives, and angioedema. Additionally, gastrointestinal reactions are also common, leading to symptoms such as nausea, vomiting, abdominal pain and spasms [18]. In recent years, domestically and internationally scholars has shed light on the distribution of allergens in crustacean aquatic products. They have found that most of the allergens are primarily present in the edible muscle parts of these creatures, which play a crucial role in muscle movement and energy metabolism [18]. Advancements in identification technology have facilitated the identification of specific allergens found in crustacean aquatic products. Table 2 highlights several allergens that have been of significant concern in the context of crustacean food allergies [18–21].

Egg

Eggs are highly nutritious, containing valuable proteins, carbohydrates, fats, and essential trace elements. They offer a rich nutritional profile at a relatively affordable price, making them popular among a wide range of consumers [22]. However, despite their nutritional benefits, eggs are also one of the most prevalent causes of food allergies, particularly in children. Allergy tests have shown a positive rate of 35% in children and 12% in adults.

The clinical symptoms of egg allergy encompass a variety of manifestations. Skin changes, such as rash, papules, angioedema (swelling of deeper skin layers) and congestion, are commonly observed. In children, unexplained bouts of crying are often linked to egg allergy. Additional symptoms include abdominal pain, nausea, vomiting, sudden fatigue, flatulence, and insomnia. In some cases, individuals with egg allergies may even exhibit cold-like symptoms. Prolonged exposure to egg allergens may also lead to respiratory allergic diseases. In severe cases, egg allergy can trigger asthma attacks and anaphylactic shock, a life-threatening condition [23].

Egg allergens primarily reside in the egg white, which contains a total of 24 different proteins, and some minor proteins. Among these

proteins, four have been identified as the key triggers of allergic reactions when they bind with human serum IgE. These four protein components are ovomucoid, ovalbumin, ovotransferrin, and lysozyme, constituting approximately 11%, 54%, 12% and 3.5% of the total protein content in egg white, respectively [23, 24]. As shown in Table 3 [23–26].

Table 2 Main allergens in crustacean aquatic products

Allergen	Molecular mass/kDa	Protein species	Isoelectric point	Protein structure	Sensitization frequency
Tropomyosin	34–38	A protein in a fine myofilament that binds to muscle proteins	4.2	284 amino acid residues, two alpha-helices intertwined	72–98%
Arginine kinase	38–45	Phosphagen kinase	6.0	It consists of 359 amino acid residues, and the N-terminal region of α -helix and the C-terminal region of α -helix are wound into an unsymmetrical structure	10–51%
Sarcoplasmic calcium binding protein	About 20	EF hand calcium-binding protein	About 4.7	It contains 194 amino acids and contains the Ca^{2+} binding domain with helix-loop-helix characteristics	29–50%
Myosin light chain	About 20	Binding protein-like protein family	4.23	There are 153 amino acid residues, and the tertiary structure is composed of 8 α-helices tightly wound	19–55%

Table 3 Four allergens in egg white

Allergen	Molecular mass /kDa	Protein species	Isoelectric point	Protein structure	Characteristic
Ovomucoid (OVM, Gal D 1)	28	Phosphogl-ycoprotein	–	A glycosylated protein containing 186 amino acids has three independent tandem homologous domains (Gal D 1.1, 1.2 and 1.3), which are arranged in tandem in space, with nine intramolecular disulfide bonds and 20–35% carbohydrates.	The molecular structure is relatively stable, the antigenicity is the strongest, and it has thermal stability and digestive enzyme stability, which leads to some allergic patients' intolerance to cooked egg liquid.
Ovalbumin (OVA, Gal D 2)	44.5	Phosphogl-ycoprotein	4.5	It consists of 385 amino acid residues, which are wrapped around each other and folded to form a spherical structure with high secondary structure, most of which are alpha-helical and β-folded.	Egg albumin is the most abundant protein in egg whites and is intolerant of heat and digestive enzymes. It also has strong sensitization.
Ovotransferrin (OVT, Gal D 3)	77	Iron ion binding glycoprote-in	6.5	It consists of 686 amino acid residues, and its N-terminal and C-terminal domains contain sites for binding Fe^{3+}, 15 disulfide bonds, and 2.6% glycosyl components, respectively.	Ovtransferrin has antibacterial activity, immunomodulation, and antioxidant properties.
Lysozyme (LYS, Gal D 4)	14.3	Basic globulin	10.7	It contains four disulfide bonds and consists of 129 amino acid residues.	Lysozyme is chemically stable under acidic conditions, but its thermal stability is poor under alkaline conditions. It can be stored for a long time at dry room temperature and can be used as an insecticide and preservative in food processing.

Milk

Milk is a liquid food known for its complete nutritional composition and high nutritional value. It contains essential components such as proteins, fats, sugars, amino acids, calcium, phosphorus, iron and various trace elements. Additionally, milk is abundant in vitamins, enzymes, and antibodies, making it easily digestible, absorbable and utilizable by the human body [27]. Despite its nutritional benefits, the prevalence of milk allergy has been on the rise in recent years, paralleling the increasing consumption of milk and dairy products in the population. In children, the incidence of milk allergy has been reported to range from 1.2% to 17%. Notably, among the eight major food allergens identified by the Food and Agriculture Organization of the United Nations, milk ranks first.

Milk allergy can manifest through a range of clinical symptoms affecting different systems in the body. Commonly observed skin symptoms include eczema, urticaria (hives), acute itching and specific dermatitis. Digestive tract symptoms may include nausea, vomiting, abdominal pain, diarrhea and dry stool. In more severe cases, milk allergy can also impact the respiratory and cardiovascular systems. It is essential to note that severe milk allergies can result in systemic reactions, including anaphylactic shock, which poses a life-threatening risk [24].

The richness of protein in milk indicates that theoretically, any protein present in natural milk has the potential to be allergenic. However, it is generally understood that there are three primary allergens in milk, which are α-lactalbumin and β-lactoglobulin found in whey protein, as well as casein. The casein group consists of four subtypes: α-S1 casein, α-S2 casein, β-casein, and κ-casein [28]. As shown in Table 4 [24, 29, 30].

Peanut

As a very important cash crop, peanut has rich nutritional value. They boast a remarkable protein content of approximately 26%, which is twice that of wheat. Moreover, the protein present in peanuts is easily absorbed and utilized by the human body, enhancing its nutritional value [31]. Peanuts are widely cultivated worldwide, offering abundant yields, and find extensive usage in various processed foods. However, peanuts are also a common group of allergenic food [32].

Peanut allergy primarily manifests in the gastrointestinal tract, followed by the skin and respiratory system. Almost all patients with peanut allergy, nearly 100%, will experience allergic symptoms such as erythema (redness) around the mouth, swollen lips, nausea, and vomiting. These symptoms are a significant concern for individuals with peanut allergies, and at present, there is no definitive cure [33].

As of now, the WHO's allergen naming data (http://www.allergen.org) includes 18 known peanut allergens. Among these allergens, the primary allergenic proteins found in peanuts are Ara h 1 and Ara h 3, which belong to the cupin superfamily, and Ara h 2 and Ara h 6, which belong to the 2S albumin family [34]. Studies have demonstrated that Ara h 2 and Ara h 6 exhibit greater resistance to high temperatures and proteases compared to Ara h 1 and Ara h 3. This enhanced resilience may be attributed to the presence of more disulfide bonds in Ara h 2 and Ara h 6, which contribute to the persistence of allergic reactions even under adverse conditions. As shown in Table 5 [24].

Nut

Nuts are a valuable source of nutrition, containing unsaturated fatty acids like oleic acid and linoleic acid, along with protein, vitamins, minerals, and dietary fiber [35]. Research has demonstrated that nuts play a crucial role in regulating blood lipids, preventing and treating coronary heart disease, enhancing brain memory, reducing cancer risk, and guarding against heart, nerve, and lung diseases [36]. Despite their numerous health benefits, nuts also fall among the eight major categories of allergic foods.

Table 4 Main allergens in milk

Allergen		Molecular mass /kDa	Protein species	Protein structure	Characteristic
Casein	α-S1casein α-S2casein β-casein κ-casein	5.7–3.5	Calcium binding protein	It consists of four independent proteins.	Of the four isoforms, α-S1 casein is the most sensitizing, with nearly 70% disordered structure and only a few secondary structures.
α-lactalbumin		14	Lysozyme family protein	A two-piece structure containing α-single ring and a 310-helix larger subdomain.	It is a calcium-binding protein involved in lactose synthesis that has high stability and protects its interior from solvent damage.
β-lactoglobulin		18	Lipid transporter	It is composed of subunits of two units connected by non-covalent bonds and mainly exists in the form of a dimer.	It is a member of the lipid transfer protein family, acting as the transporter of lipids, hormones, steroids, etc.

Table 5 Main allergens in peanuts

Allergen	Molecular mass /kDa	Protein species	Characteristic
Ara h 1	63.5	7S globulin	The secondary structure contains β -angle, and the quaternary structure is a trimer complex formed by three monomers.
Ara h 2	17–20	2S albumin	A monomeric protein.
Ara h 3	57	11S globulin	The N-terminal and C-terminal domains of monomer form contain two ciupin folds (consisting of two parallel β -corners, random crimp, and three α -helices).
Ara h 6	15	2S albumin	It consists of two chains connected by disulfide bonds: the N-terminal chain is about 5 kDa, and the C-terminal chain is about 9 kDa.

A nut allergy can lead to various symptoms, including rash, swollen throat, nausea, and vomiting. In severe cases, life-threatening symptoms such as acute asthma and anaphylactic shock may occur [37]. Various types of nuts are available, such as almonds, cashews, walnuts, hazelnuts, pistachios and Brazil nuts. Table 6 provides an overview of the main allergens associated with each type of nut [37].

Fish

Fish serves as a crucial source of animal protein nutrition, and as its market and consumer base continue to expand, the potential for sensitization issues cannot be overlooked. Generally speaking, individuals allergic to fish often experience allergic reactions not only to a single type of fish but also to various types of fish. This is particularly significant in regions where fish consumption is popular, as the prevalence of fish allergies tends to be relatively high [38].

The symptoms of fish allergy encompass a wide range of manifestations. Allergic reactions to fish may lead to blushing, hives (urticaria), nausea, vomiting, diarrhea, temperature reversal (feeling cold or hot suddenly), and even neurological symptoms such as blurred vision. In some cases, cardiovascular symptoms may arise, including decreased blood pressure and heart block [39].

Parvalbumin is the primary allergenic protein found in fish. It is a calcium-binding protein that plays a crucial role in intracellular calcium ion exchange and is involved in the physiological process of muscle relaxation. With a molecular weight of approximately 10–13 kDa and an acidic isoelectric point (pH 3.9–5.5), parvalbumin possesses distinct characteristics such as high water solubility, heat resistance and resistance to enzymatic degradation. It is widely distributed among the sarcoplasmic proteins of fish, amphibians and vertebrates [40]. Aside from parvalbumin, due to the vast variety of fish products available, there might be other potential fish allergens yet to be identified.

Wheat

Wheat is one of the three major food crops in the world, and wheat and its products are also the main food source for many countries, but it is also one of the eight allergic foods identified by the Food and Agriculture Organization of the United Nations, ranking fifth [41].

Wheat allergy is actually a type I hypersensitivity reaction triggered by specific wheat sensitizing proteins. This allergy can lead to various clinical conditions, including celiac disease, specific dermatitis, wheat-dependent exercise-induced allergy symptoms, Baker's asthma, recurrent urticaria and rhinitis. The specific clinical manifestations are itchy skin, redness, nasal congestion, runny nose, asthma, diarrhea, dyspnea, etc., leading to insomnia, lethargy, loss of appetite, thereby reducing people's quality of life. Although in most cases wheat allergy causes a mild reaction, in some cases it can be life-threatening [42].

As of May 12, 2020, the International Union of Immunological Societies Allergen Naming Branch of the WHO has approved 15 food-borne wheat allergens. As shown in Table 7 [43].

Among them, Tri a 14 is the main allergen causing Baker's asthma and rhinitis; Tri a 19 is the main allergic protein that causes wheat exercise-induced allergy [44]. Some studies have shown that thioredoxin can alleviate allergic reactions, so whether Tri a 25 is an allergen remains controversial; Tri a 36 is the main protein that causes food allergy, and studies have shown that recombinant Tri a 36 has a higher sensitivity than Tri a 19 in the diagnosis of wheat food allergy [45].

Table 7 Food-borne wheat allergens

Allergen	Protein species
Tri a 12	Inhibitory protein
Tri a 14	Nonspecific lipid transfer protein 1
Tri a 17	β-amylase
Tri a 18	Wheat germ agglutinin isolectin I
Tri a 19	ω5-gliadin from wheat
Tri a 20	γ-gliadin from wheat
Tri a 25	Thioredoxin
Tri a 26	High molecular weight glutenin
Tri a 36	Low molecular weight glutenin Glu B3-23
Tri a 37	α-purothionine
Tri a 41	Mitochondrial NFKB-1 ubiquitin ligase activator
Tri a 42	CDNA hypothetical protein
Tri a 43	CDNA hypothetical protein
Tri a 44	Endosperm transfer cell-specific PR60 precursor
Tri a 45	Elongation factor 1

Table 6 Main allergens in nuts

Allergen		Molecular mass /kDa	Protein family	Protein species
Walnut	Jug r 1	15–16	Alcohol soluble protein	2S albumin
	Jug r 2	44	Cupin	7S globulin
	Jug r 4	58.1	Cupin	11S globulin
Hazel	Cor a 9	40	Cupin	11S globulin
	Cor a 11	48	Cupin	7S globulin
Cashew	Ana o 1	50	Cupin	7s-like globulin
	Ana o 2	55	Cupin	11S-like globulin
	Ana o 3	14	Alcohol soluble protein	2S albumin
Almond	Pru du 3	9	Alcohol soluble protein	Nonspecific lipid transfer protein type 1
	Pru du 4	14	profilins	Actin binding protein
	Pru du 5	10	PR-10	60S acidic ribosomal protein P2
	Pru du 6	360	Cupin	11S globulin
Pistachio	Pis v 1	7	Alcohol soluble protein	2S albumin
	Pis v 2	32	Cupin	11S globulin subunit
Brazil nuts	Ber e 1	9	Alcohol soluble protein	2S sulfur-rich storage protein
	Ber e 2	29	Cupin	11S globulin

Soybeans

Soybean, a legume plant, being rich in protein and various essential amino acids required by the human body. It has been consumed by humans as food for thousands of years and is commonly processed into a variety of products, such as soy sauce and soy milk. Soy products also find extensive use in the food industry as thickeners, emulsifiers, and protein fillers due to their versatile properties [46]. At the same time, soybean is also one of the eight recognized food allergens in the world, and soybean is a priority allergen in some countries [46, 47].

Soybean allergy is a significant health concern, with statistics indicating an incidence rate ranging from 0.2% to 0.4% in the general population. In children, the allergy rate can be as high as 13%, and with the increasing consumption of soy foods and by-products, the prevalence of soy allergy is on the rise. The symptoms of soybean allergy can include nausea, vomiting, erythema around the mouth, swelling of the lips and throat, oral ulcers, and various gastrointestinal issues. These symptoms not only impact the quality of life for individuals with soy allergies but can also pose a serious threat to their lives [48].

Currently, the World Allergen Database contains 43 known soybean allergens. However, the majority of allergic reactions are triggered by four main allergenic proteins: β-conglycinin, glycinin, Gly m Bd 30K, and Gly m Bd 28K. As shown in Table 8 [49].

Allergen detection technology

It is known that most food allergens are predominantly proteins or peptides with molecular weights ranging from 10kd to 70kd. Therefore, technologies specifically designed for protein detection play a crucial role in detecting allergens in food products. Additionally, many sensitized foods contain various allergens in different quantities. As a marker for potentially allergic food products or ingredients, specific DNA fragments can also be targeted for detection. Consequently, food allergen detection technology can be broadly categorized into two groups. As science and technology continue to advance, new allergen detection techniques are being developed, as depicted in Figure 2. It is worth noting that these detection techniques not only serve the purpose of allergen detection but can also be applied to other areas, such as detecting biological pollutants in food contamination.

Detection technology based on protein level

Enzyme-linked immunosorbent assay (ELISA). ELISA, also known as enzyme labeling method, is a commonly used immunoassay technique developed on the basis of immunoenzymology. The principle is to organically combine the specificity of antigen-antibody immune reactions with the efficient catalytic action of enzymes, label antibodies with enzymes or perform antigen-antibody reactions, and reflect the content of antigens or antibodies in the test sample based on the color depth of the substrate after enzyme action. ELISA can detect both antigens and antibodies, and according to different methods of fixing antigens, ELISA can be divided into direct and indirect methods. Indirect methods include sandwich method and competitive method [50, 51]. The schematic diagram of different ELISA principles is shown in Figure 3.

Ji Liang et al. developed a highly sensitive ELISA and tested 37 kinds of fish with important commercial value, among which 28 kinds of fish (76%) can be detected by ELISA, which significantly improved the detection of non-immunoreactive or weakly immunoreactive fish that could not be detected by immunochemistry before [52]. In addition, the protein recovery rate of this ELISA can reach 84.8–122.1%. ELISA has many advantages, such as strong specificity, high sensitivity, simple operation, and the ability to detect samples in large quantities. However, ELISA also has some limitations, such as the difficulty in preparing antibodies, the high selectivity of reagents, the inability to conduct multi-residue detection, and the proneness to false-positive results in detection [53–57].

Table 8 Major allergens in soybean

Allergen	Molecular mass /kDa	Protein species	Protein structure
β-conglycinin	210	7S globulin	It is a trimer composed of α' subunit, α subunit and β subunit through hydrophobic interaction and electrostatic interaction.
Glycinin	360	11S globulin	It is a hexamer composed of five subunits, including G1, G2, G3, G4 and G5.
Gly m Bd 30K	34	7S globulin	A monomeric protein consisting of 379 amino acid residues.
Gly m Bd 28K	26	7S globulin	It consists of 473 amino acid residues.

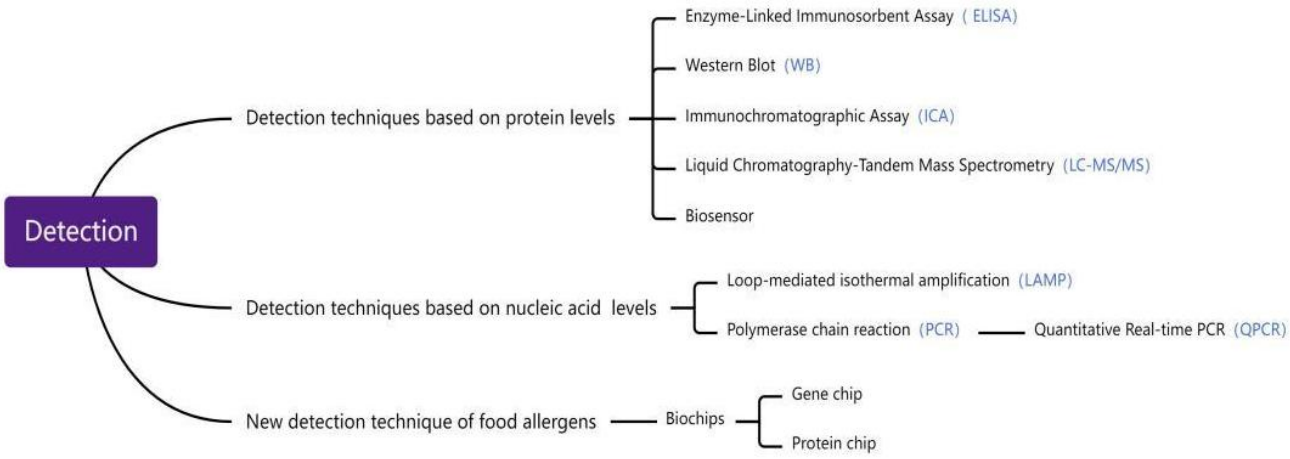

Figure 2 Food allergen detection technology

Western blot (WB). WB is an advanced immunobiochemical technology that has evolved from gel electrophoresis and solid phase immunoassay. This method combines the high resolution capability of gel electrophoresis with the high specificity and sensitivity of immunoassays. Its underlying principle is to detect specific proteins in complex samples through the specific binding of antigens and antibodies. WB can be used for qualitative and semi-quantitative detection of antigens [58]. One of the significant advantages of WB is its ability to effectively avoid false-negative results caused by the presence of protein inhibitors in the sample. However, this method has a drawback in that it takes a relatively long time to analyze the samples. Additionally, the complexity of the food matrix can impact the accuracy of the analysis [59]. The specific operation steps of conventional WB: 1. collect cells for culture to prepare samples; 2. load the prepared samples onto a separate channel of the gel; 3. use SDS-PAGE for separation; 4. transfer the protein samples on the gel to the PVDE membrane; 5. seal and wash the PVDE membrane; 6. use the primary antibody as the "probe", use the secondary antibody for "color development" and then detect and analyze the samples [60]. As shown in Figure 4.

Immunochromatography assay (ICA). ICA is an extended application based on the principle of ELISA. The detection platform is composed of a sample pad, a bonding pad, a nitrocellulose membrane (NC membrane) and a water-absorbing pad, which are spliced and combined by PVC plates with certain hardness. Control lines (C lines) and detection lines (T lines) are solidified on the NC membrane, in which the color development of the C line indicates that the detection is effective, and the color development of the T line indicates positive or negative. The detection results can be directly identified by vision based on the color development bands and color depth on the NC membrane, so as to carry out qualitative or semi-quantitative analysis [61]. Kun-Mei Ji et al. used colloidal gold immunochromatography to detect 124 kinds of imported and exported foods in China [62]. The results showed that 68 kinds of food samples labeled with peanuts were positive for Ara h 1, while 54 kinds of food samples labeled without peanuts were negative for Ara h 1, but 2 kinds were positive for Ara h 1, which indicated that the labels of most manufacturers were accurate. Compared with ELISA, ICA has the advantages of simple operation and short detection time, and high-throughput detection can be carried out by adding T lines, but its accuracy is low [46]. The principle diagram of ICA detection is shown in Figure 5.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS). LC-MS/MS is a powerful analytical technology used to separate complex samples through liquid chromatography and then qualitatively detect them using a mass spectrometer. The detection principle is to ionize the sample through the ion source into different charged particles, and then use the accelerating electric field to make the charged particles enter the mass analyzer. Then, under the double action of the electric field and magnetic field, the ions with different plasmic/nuclear ratio (m/z) are separated and the molecular weight is

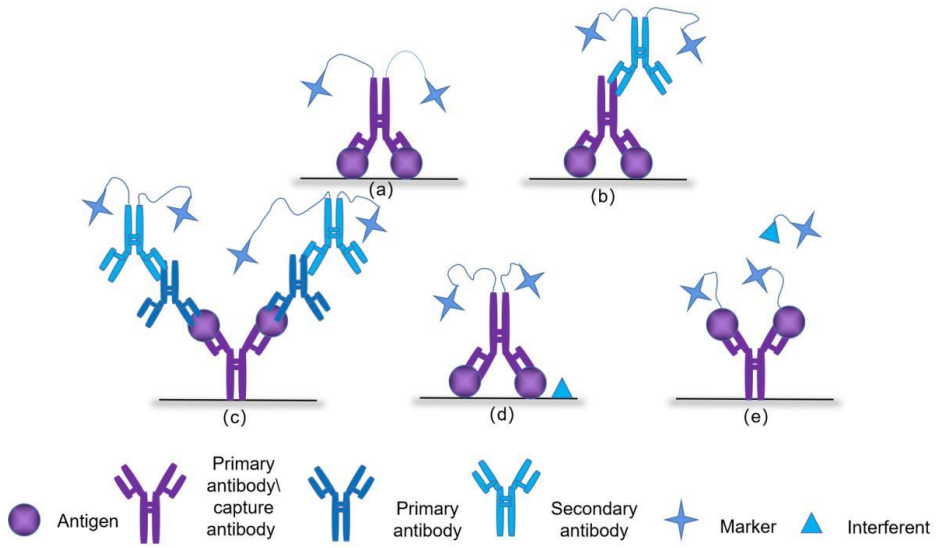

Figure 3 Schematic diagram of different ELISA. (a) direct ELISA; (b) indirect ELISA; (c) sandwich ELISA; (d) competitive ELISA with labeled antibody; (e) competitive ELISA with labeled antigen. ELISA, enzyme-linked immunosorbent assay.

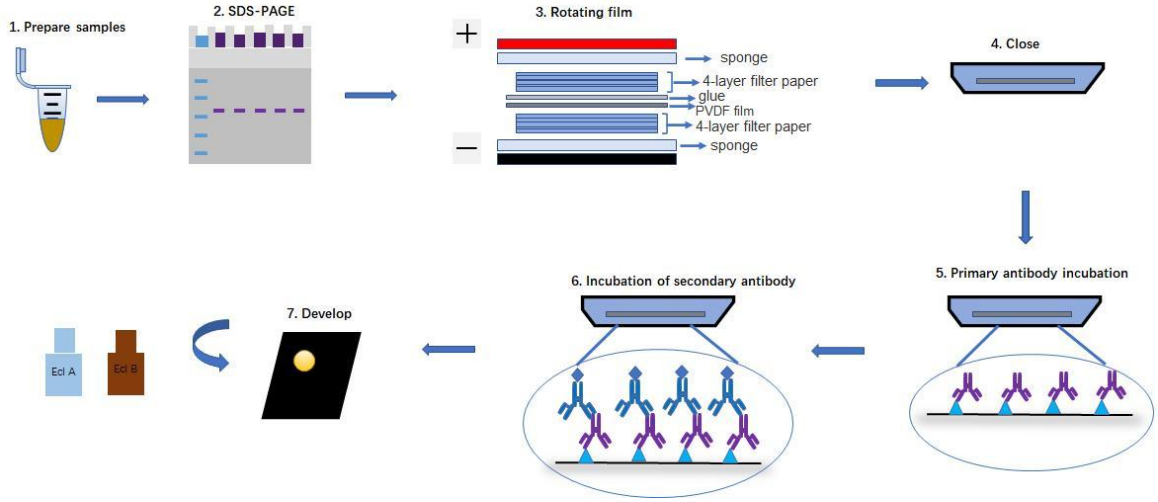

Figure 4 Schematic diagram of WB. WB, Western blot.

calculated. Finally, the protein or peptide is analyzed and identified by computer [50, 59]. Akira Torii et al. used LC-MS/MS to detect walnuts and almonds in processed foods, and the results showed that: The detection limit of walnut 2S albumin peptide GEEMEEMVQSAR was 0.22 ± 0.02 μg/g, and the detection limit of almond 11S globulin peptide GNLDFVQPPR was 0.08 ± 0.02 μg/g [60]. The linear relationship between these peptides was good (R^2 > 0.999). The concentration of protein in the sample solution ranged from 0.1 to 50 μg/mL, and the recoveries were 90.4 to 101.5%. Figure 6 shows the schematic diagram of the detection principle of LC-MS/MS. Although the instruments and equipment required by LC-MS/MS are expensive and require professional operation, LC-MS/MS has the advantages of fast, high specificity and high throughput, which can overcome the drawbacks of low throughput and cross interference existing in immunological methods, and also overcome the shortcomings of PCR technology that cannot directly detect sensitized proteins, and has good development potential [61].

Biosensor. Biosensors are mainly composed of biometric components and signal conversion components. After specific combination of target analytes and recognition components, physical and chemical signals generated are converted into light and electrical signals that can be detected to achieve detection purposes [62]. Its detection principle is shown in Figure 7.

Sun et al. utilized nanoparticles to enhance the sensing signal and combined it with a surface plasmon resonance sensor to detect legume lectin [63]. The achieved detection limit in their study was an impressive 0.023 μg/mL, demonstrating the high sensitivity and performance of biosensors. As a modern and emerging detection technology means, biosensors are not only accurate and fast, but also simple and easy to operate, with relatively strong sensitivity and specificity, which can effectively improve the quality of food detection work and effectively guarantee food safety.

Detection Technology Based on Nucleic Acid Levels

Quantitative polymerase chain reaction (QPCR). PCR is a nucleic acid synthesis technology that massively amplifies a small amount of DNA fragments of the target gene in an initial sample in vitro [64]. QPCR is a real-time quantitative method developed on the basis of PCR. Its principle is that when PCR amplification, a pair of primers are added at the same time, a specific fluorescent probe is added. The probe is an oligonucleotide, and the two ends are labeled with a reporter fluorophore and a quench fluorophore. When the probe is intact, the fluorescence signal emitted by the reported group is absorbed by the quenched group. At first, the probe binds to any single strand of DNA. During PCR amplification, the 5'-3' end exonuclidenase activity of taq enzyme degrades the probe enzyme, separating the reporter fluorophores from the quench fluorophores, so that the fluorescence monitoring system can receive the fluorescence signal, that is, for each amplified DNA strand, a fluorescence molecule is formed, and the accumulation of fluorescence signal is completely synchronized with the formation of PCR products. QPCR has achieved a breakthrough from qualitative to quantitative PCR, and can realize the qualitative and quantitative analysis of allergens in a variety of complex foods and the identification of species. However, QPCR is prone to contamination of samples and false positive results in the detection process, resulting in detection errors [24].

Loop-mediated isothermal amplification (LAMP). LAMP is a DNA amplification method that was first reported by Notomit et al. in 2000 [65]. It has since gained popularity as a highly specific, rapid, and efficient technique for amplifying DNA. Based on 6–8 different DNA sequences of target genes, 4–6 specific primers were developed and designed to perform large amounts of amplification under the action of chain replacement DNA polymerase at a constant temperature of 60–65 °C, and specific and significant amplification of target genes could be achieved in a short time [66]. Compared to ordinary nucleic

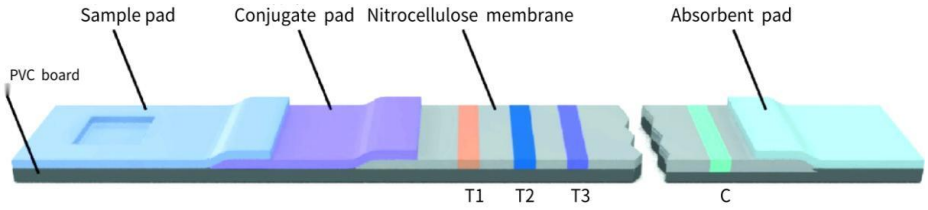

Figure 5 Schematic diagram of ICA. ICA, immunochromatography assay.

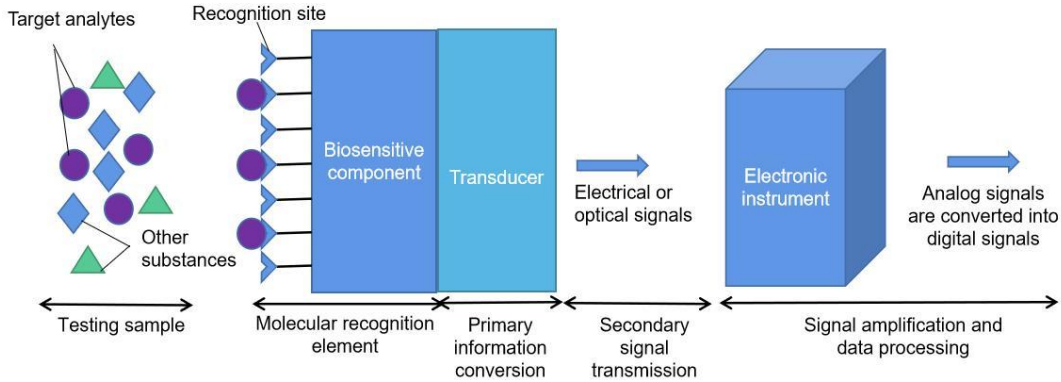

Figure 6 schematic diagram of the detection principle of LC-MS/MS. LC-MS/MS, liquid chromatography-tandem mass spectrometry.

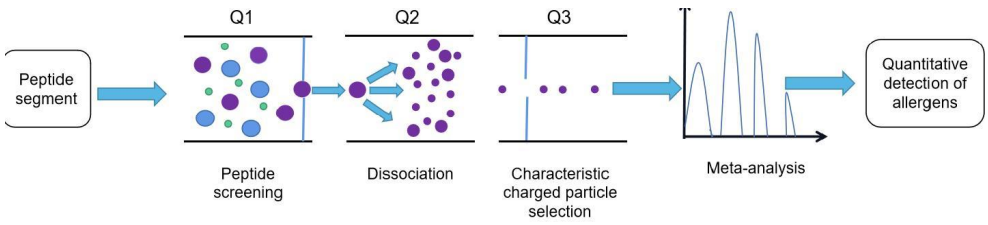

Figure 7 Schematic diagram of the composition and working principle of a biosensor

acid amplification technology, LAMP greatly reduces the requirements for experimental equipment, eliminating the need for expensive experimental equipment similar to thermal circulators, optimizing the experimental process, reducing detection time, and allowing the detection results to be determined directly by visual observation of whether white turbidity or green fluorescence is generated, making it suitable for rapid on-site detection of food allergens. Despite the great difficulty of primer design, LAMP still has broad development prospects in the field of food safety detection [24].

New food allergen detection technology

Biochips. As a new and effective detection tool, biochip technology has been gradually applied to the detection of food allergens in recent years [67]. Biochips mainly include gene chips and protein chips, in which gene chips are hybridized with labeled sample molecules after fixing a large number of probe molecules on the support, and the number and sequence information of sample molecules are obtained by detecting the hybridization signal strength of each probe molecule. Protein chip is to spot protein molecules on the solid substrate surface in a pre-designed array, and then add protein molecules with special labels that are specifically bound to it, and conduct qualitative and quantitative analysis of target proteins according to specific signal display [68]. Yi-Qun Zhang prepared a three-dimensional chip carrier with good fixed effect based on the three-dimensional chip carrier, and built a test technology for allergens in seafood [69]. In addition, in order to distinguish allergenic proteins from non-allergenic proteins and improve the accuracy and efficiency of food allergen detection, Lu et al. A method was developed to distinguish allergens from non-allergenic homologues, and the potential sensitization of the query sequence was quantitatively estimated [70]. This technique can characterize known allergens in protein families, providing technical support and methodological basis for the future determination of allergens in food based on this technique, and has good application value.

Advantages and disadvantages of detection technology

In summary, each detection technology has certain advantages and limitations. Now, the advantages and disadvantages of the detection technologies discussed in the article are summarized (as shown in Table 9). It is found that no single technology can combine all the advantages. For the analysis of some complex samples, more than one technology may be required for comprehensive detection.

Table 9 Advantages and disadvantages of detection technology

Detection techniques		Advantages	Disadvantages
Detection techniques based on protein levels	ELISA	Strong specificity, high sensitivity, simple operation, and capable of detecting samples in large quantities.	It is difficult to prepare antibodies, high selectivity to reagents, unable to carry out multi-residue detection, and prone to false positive results in detection.
	WB	WB has both high resolution of gel electrophoresis and high specificity and sensitivity of immunoassay, which can effectively avoid the phenomenon of false negative results due to the presence of protein inhibitors.	It takes a long time to analyze samples; The complexity of the food matrix will also affect the accuracy of analysis.
	ICA	Compared with ELISA, ICA has simple operation and short detection time, and can be used for high-throughput detection by adding detection lines (T-lines).	Low accuracy.
	LC-MS/MS	It has the advantages of rapidity, high specificity and high throughput, and can overcome the shortcomings of low throughput and cross interference in immunological methods and the shortcomings that PCR can not directly detect sensitized proteins.	Instruments and equipment are expensive and need professional operation.
	Biosensor	High speed, high accuracy and simple operation.	Requires specialized instruments and high requirements for sample preprocessing.
Detection techniques based on nucleic acid levels	QPCR	QPCR has achieved a breakthrough in PCR from qualitative to quantitative , enabling qualitative and quantitative analysis of allergens in a variety complex foods, as well as species identification.	Prone to sample contamination and false positive results, resulting in detection errors.
	LAMP	The requirements for experimental equipment have been reduced, the experimental process has been optimized, the detection time has been shortened, and the detection results can be directly determined by observing with the naked eye whether white turbidity or green fluorescence is generated.	Primers are difficult to design.
New detection technology	Biochips	It can simultaneously detect multiple allergens in a single sample; Implement high-throughput detection of allergens in multiple samples;The amount of samples required for detection is small, and the detection process takes a short time.	The instability of protein chips and the uncertainty of gene chips.

ELISA, enzyme-linked immunosorbent assay; WB, Western blot; ICA, immunochromatography assay ; LC-MS/MS, liquid chromatography-tandem mass spectrometry; QPCR, quantitative polymerase chain reaction; LAMP, loop-mediated isothermal amplification.

Conclusion

Currently, there is no specific treatment for food allergies worldwide, and the incidence of allergic diseases continues to rise. Society's growing concern about food safety issues has driven the continuous improvement and advancement of food allergy regulations and related detection technology, which plays a crucial role in preventing food allergies.

Among the detection technologies currently used, ELISA based on protein levels and real-time quantitative PCR based on nucleic acid levels are the most widely utilized. However, it is important to note that the detection results can be easily influenced by food processing techniques and the composition of food substrates. Additionally, food is susceptible to cross-contamination during processing, packaging, and transportation, making accurate detection of hidden allergens in complex food substrates extremely important.

Considering the rapid development of the food industry, it holds great significance to develop convenient, high-quality, and accurate allergen detection technology that builds upon the existing methods. This will not only aid in safeguarding food safety but also help in mitigating the occurrence of food allergies.

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