The detection and regulation advances of food allergens

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Abstract
Food allergy has become an important food quality and safety issue, posing a challenge to the food industry and affecting consumer health. On the one hand, from the perspective of food processing industry, the diversity of food raw material ingredients, exogenous additives, and processing forms make the presence of allergens in modern food processing more complex. In addition, due to the lack of allergen identification, effective detection and allergenicity evaluation systems, there are serious deficiencies in the current theories and techniques for food allergen screening and detection, tracking and prediction, intervention and control; On the other hand, from the perspective of public health, meeting consumers’ right to know whether there are raw materials containing food allergens in processed foods, and improving the credibility of government and people's satisfaction have become urgent matters; In addition, as people come into contact with more and more new borne novel foods, the probability of food allergy is also increasing. The food safety and health problems induced by increasingly complex, widespread and severe food allergy are difficult to avoid. In view of this, in response to the increasingly serious food allergy issues, this paper introduced the detection methods of food allergens, summarized the reduction and control techniques of food allergens, and elaborated hypoallergenic foods, which aims to provide the basis for preventing and controlling food allergy and ensuring the physical health of food allergy patients.

Keywords: Food allergen; Allergenicity; Detection; Non-thermal processing; Hypoallergenic food
**Background**

Food allergy refers to the allergic immune response induced by ingestion or contact food that containing allergens [1], which usually induces allergic reactions in skin, digestive system, respiratory system, and other tissues and organs. In severe cases, food allergy could lead to shock or even death in allergic patients [2-5]. According to the food allergy prevalence survey, in recent years, there has been an increasing prevalence of food allergy [4-5], which poses a threat to the health of patients with food allergy history. The Food and Agriculture Organization of United Nations (FAO) and the World Health Organization (WHO) have listed eight major categories of food allergens, namely crustaceans, fish, eggs, milk, peanuts, nuts, soybeans and wheat [6]. In 2021, sesame was listed as the 9th most common food allergen by the Food and Drug Administration (FDA) of United States. However, there is currently no medical cure to completely refrain from food allergy, the most effective way to avoid food allergy is still strictly not to consume food that contains allergens.

Food allergies are immune responses triggered by specific allergens in certain foods. The immune system of individuals with food allergies identifies these proteins as harmful, leading to the release of chemicals such as histamine, which cause allergic symptoms. The immunoglobulin E (IgE)-mediated food allergy is the most common type anaphylaxis. As to food allergy occurrence, the immune system produces IgE when firstly exposed to specific food allergens, then the IgE bind to the surface of mast cell and basophil. Upon re-exposure to the same or similar allergen, the allergens could bind to the IgE antibodies on mast cell and basophil, leading to their degranulation, triggering the release of histamine and other chemicals, inducing allergic reactions. Therefore, accurate diagnosis of food allergies is crucial for proper management and prevention of allergic reactions. Currently, skin prick tests, blood tests (serum IgE testing) and oral food challenges are commonly used to diagnose food allergies.

However, in the past decades, as consumers have more access to various foods, and new borne novel foods are being introduced to the market, it has become increasingly difficult for people with food allergy to eliminate foods that containing allergens. Therefore, reducing food allergens and developing hypoallergenic foods have important practical significance for the physical health of food allergic patients. However, the understanding of detection and reduction control techniques for food allergens is still limited. Therefore, this paper summarizes the main detection techniques for food allergens, elaborates the research progress in reducing and controlling food allergens, introduces the current status of hypoallergenic foods, and innovatively summarizes and prospects the new trends and future research directions in the field of food allergen, with the aim of preventing and controlling food allergy, which could provide a basis for ensuring the physical health of patients with food allergy.

**Current detection techniques for food allergens**

Currently, there are many foods contains allergens in the world, among which the main allergenic foods are crustaceans, fish, eggs, milk, peanuts, tree nuts, soybeans, wheat, and sesame, which could trigger over 90% of food allergy reactions [7-10]. Food allergens are the molecular basis for inducing food allergic reactions, therefore, the detection of food allergens has become the key for food allergy prevention and treatment.

The current detection of food allergens mainly includes the following techniques (Figure 1): (1) Immunoassay: Immunoocolloid gold technique, immune chromatography technique, enzyme linked immunosorbent assay (ELISA) technique, which are immunological techniques that use antibodies to detect the presence of specific proteins, including allergenic proteins. It involves the binding of antibodies to target proteins, followed by colorometric or fluorescent detection; (2) Non-immunoassay for allergens: Liquid chromatography and mass spectrometry, which is an analytical technique that measures the mass-to-charge ratio of ions. In allergen detection, it is used to identify and quantify proteins based on their unique mass spectra; (3) Nucleic acid test: Polymerase chain reaction (PCR), which is a molecular biology technique that amplifies specific DNA sequences. In the context of food allergens, it is used to detect and amplify the DNA of allergenic proteins.

Although the above detection methods for food allergens have certain sensitivity and accuracy, each commonly used allergen detection method has certain shortcomings: (1) Enzyme linked immunosorbent assay (ELISA) technique is currently the most widely used and common allergen immune detection method due to its high accuracy. However, during detection, ELISA requires multiple antibodies, has the shortcomings of possible false positive/negative test results due to matrix effects, and unsuitable for detecting the genetically modified food allergens; (2) Liquid chromatography and mass spectrometry have the advantages of fast detection speed, high sensitivity and low detection limits. However, the shortcomings of ELISA lie in the suitability for detecting the already-known food allergens, and require standard allergen as reference for quantitative detection. The accuracy of detecting unknown allergens and novel allergens is not high, and precise detection needs to be combined with immune detection techniques; (3) The PCR method has the characteristics of high sensitivity, good stability, fast detection speed, etc., while the PCR needs to be observed by ultraviolet light after agarose gel electrophoresis and ethidium bromide staining, or by sodium polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining, the detection process is relatively cumbersome, and it is easy to bring pollution and false positive detection results. In addition to the commonly used allergen detection techniques, novel detection techniques for food allergens are also being developed, such as the loop mediated isothermal amplification (LAMP) and biochip detection technique, to accurately detect the hidden food allergens in processed foods, reduce the risk of mixing other food components containing allergens, and decrease the risk of food allergy among consumers.

Currently, the research on food allergen detection technology is still in its early stages, and a single commonly used detection method cannot meet the diverse requirements of actual food detection processes. Investigating multi-channel and high-throughput detection technologies for simultaneous detection of multiple food allergens has become a research hotspot in the field of food allergen detection. Thus, integrating multiple allergen detection methods and constructing detection methods with lower detection costs, simpler and more efficient detection methods has also become a trend and focus of current food research. In addition, with the rapid development of genetic modification and genetically modified foods, the introduction of exogenous gene products will also bring novel unknown allergens into food. Therefore, it is necessary to continuously optimize and improve allergen detection methods, and develop new high-throughput, high sensitivity, and fast food allergen detection technologies.

**Reduction and control techniques for food allergens**

Food allergy has become an important food safety issue. As reported, food processing could affect the allergenicity of food allergens. Although current food processing methods cannot completely destroy the ability of allergenic foods to induce food allergy, while by adjusting specific technical and parameters during processing, the allergenicity of food allergens could be minimized. Therefore, one of the important tasks of food industry is to reduce allergens using appropriate food processing approaches without significantly reducing the nutritional components in food, providing consumers safe food with low or no allergenicity, with meeting the normal dietary needs of allergen susceptible population.

Currently, the effects of thermal treatment on reducing the allergenicity of food allergens are not very ideal, which is due to the molecular structure of allergens is very stable and has heat resistance, resulting in low reduction effects of thermal treatment on the
allergenicity. In recent years, the application of novel non-thermal processing techniques to reduce allergens and obtain foods with low or no allergenicity has become a research hotspot. Previous investigations have shown that non-thermal processing could disrupt the molecular structure and allergenic epitopes of allergen proteins, thereby reducing the allergenicity of food allergens. At present, the non-thermal processing that could reduce the allergenicity mainly includes: high pressure treatment, high voltage pulse electric field, irradiation technique and high intensity ultrasound [11–13].

High pressure treatment is a novel non-thermal processing developed in recent years. Previous research has found that high pressure treatment could affect the non-covalent interactions of allergens. By inducing (1) allergen elimination during extraction, (2) allergen aggregation, masking and destroying IgE-binding epitopes, (3) allergen unfolding, more digestion sites got exposed for protease, improved digestibility, high pressure treatment could reduce the allergenicity of food allergens (Figure 2). Yao et al. [14] found that high pressure treatment of wheat allergens could induce allergen aggregation, enhance the hydrophobic interactions between wheat allergens, promote the formation of disulfide bonds, thus reduce the wheat allergens. Moreover, when wheat was treated at 400 MPa for 20 minutes, its allergenicity could be reduced by up to 72.2%.

High voltage pulse electric fields have the characteristics of uniform transmission, short treatment time, low energy consumption, low heat generation, and little impacts on food nutritional and flavor properties. Pulse electric fields could affect the electrostatic interactions and charged groups between allergen protein groups, affect the electric field distribution, electrostatic interactions and the hydrophobicity of amino acid residues on the primary structure of allergen, therefore change the molecular structure of allergens, which in turn affects the allergenicity of allergen. Tu et al. [15] found that using a 25 kV/cm pulsed electric field to treat milk β-Lactoglobulin for 210 μs, the allergenicity of β-lactoglobulin got decreased to 42% before treatment. Yang et al. [16] found that with the increase of pulse electric field intensity and time, the allergenicity of ovalbumin allergens gradually increased at first and then got weakened. This was because the molecular structures of ovalbumin allergens got unfolded under low pulse electric field intensity conditions, and the exposure of epitopes led to stronger allergenicity. Subsequently, the exposed epitopes were destroyed by the pulse electric field, leading to the reduced allergenicity.

Food irradiation treatment is an approach that utilizes ionizing radiation to treat food. In recent years, research on using food irradiation to desensitize allergenic foods has attracted more attention. Radiation treatment could lead to the breakdown or oxidation of allergen proteins, and at the same time, allergen proteins would undergo depolymerization or cross-linking reactions, resulting in the molecular spatial structure changes of allergen. These changes induced the destruction of conformational and linear epitopes, leading to the decreased allergenicity. Luo et al. [17] utilized 10 kGy of 60Co-γ irradiation to desensitize peanut allergens, after treatment, the IgE-binding capacity of peanut Ara h 2 allergen almost completely disappeared. This could be due to after irradiation treatment, water molecules produce hydroxyl radicals, which induce the cross-linking or cleavage of allergen epitope peptides by reacting with free radicals [18], leading to decreased allergenicity.

During the high intensity ultrasound processing, there will be ultrasonic cavitation effects. Allergens could absorb the energy of ultrasound, thereby damaging and degrading the molecular structures of allergen proteins [19]. Wang et al. [20] found that using 400 W ultrasound to treat kiwifruit allergens for 16 minutes could reduce the content of kiwifruit allergens to half. Zhang et al. [21] used high intensity ultrasound at 20 kHz (100-800 W) to treat the tropomyosin allergen of Exopalaemon modestus for 15 minutes, as founded, high intensity ultrasound treatment could reduce the allergenicity of shrimp tropomyosin, and after treating tropomyosin at 20 kHz, 800 W for 15 minutes, the IgE-binding capacity of tropomyosin got disappeared. As shown before, non-thermal processing technique has different reducing effects on the allergenicity of food allergens. In summary, non-thermal food processing techniques (e.g. high pressure processing, high voltage pulsed electric field, irradiation treatment, high intensity ultrasound, etc.) could disrupt the allergenic epitopes of food allergens by altering their molecular structures, thereby reducing the allergenicity. However, it’s essential to note that the effectiveness of these non-thermal processing techniques in reducing allergenicity may vary depending on the specific allergen and the food matrix. Additionally, thorough validation and testing are required to ensure that these methods achieve the desired reduction in allergenicity without compromising the safety and quality of the food product. Regulatory considerations and consumer acceptance are also important factors when implementing non-thermal processing techniques in the food industry.

Hypoallergenic foods

Hypoallergenic foods refer to foods that are unlikely to induce allergic reactions. Hypoallergenic foods are foods developed for consumers with food allergy, and have significantly lower allergenicity compared to the natural food allergens. At present, hypoallergenic food mainly includes hypoallergenic infant formula milk powder, hypoallergenic rice noodles, gluten free foods, etc. It mainly desensitizes allergens through enzyme hydrolysis, fermentation, physical methods and other techniques, and reduces the allergenicity by destroying linear epitopes and conformational epitopes that could combine with IgE.

Currently, infants and children are the high-risk cohort for food allergy. The research and development of hypoallergenic infant foods is at a rapid development stage. At present, the main hypoallergenic products include infant formula milk powder, hypoallergenic rice noodles and other complementary foods. Specifically, the hypoallergenic infant formula milk powder on the market is mainly achieved by enzymatic hydrolysis of milk protein to destroy the epitopes. According to the different hydrolysis degree of milk protein, hypoallergenic infant formula milk powder could be divided into amino acid formula, partially hydrolyzed formula, and deeply hydrolyzed formula. Among them, the amino acid formula is mainly composed of free amino acids, suitable for severe diarrhea induced by milk allergen protein. The partially hydrolyzed formula is usually recommended for primary intervention in high-risk infants and young children, while the deeply hydrolyzed formula is currently the most common product on the market, suitable for most milk allergy infants and young children [22–23].

Figure 1 The current detection techniques for food allergens.
Infant rice noodle is a common complementary food for infants and young children. At present, the removal of rice allergens is mainly achieved through physical methods, biotechnology and enzymatic methods. Among them, ultrasonic wave combined with soaking treatment of rice could remove 90% of allergen proteins. In addition, enzyme hydrolysis could degrade rice allergen protein and destroy allergen epitopes to reduce the allergenicity, then use the allergen-free rice to prepare hypoallergenic infant rice noodle products.

Another common hypoallergenic food on the market is gluten free foods. As the name suggests, gluten free food refers to food that does not contain gluten. At present, there is no globally recognized standard for gluten free food, and the standards for gluten free food vary from country to country. The FDA requires food with a gluten content below 20 mg/kg to be labeled as ‘gluten free’. The EU, Canada, Argentina, and the International Codex Alimentarius Commission standards have the same limit requirements for ‘gluten free’ as the FDA, while Australia and New Zealand have more stricter regulations, require that gluten be 'not detected' in ‘gluten free’ products. China has currently established testing standards and methods for gluten in food, while has not yet formulated and promulgated standards for identifying gluten free foods.

Except for infant foods and gluten-free foods, hypoallergenic foods also include hypoallergenic formulas for adults, hypoallergenic dog and cat foods. Hypoallergenic foods are an important research direction for controlling food allergy. With the continuous innovation and development of food processing approaches, as well as the emergence of new processing techniques, more and more hypoallergenic foods will be introduced to the market in the future, which could benefit patients suffering with food allergy.

**Conclusion and future outlook**

In recent years, the incidence prevalence of food allergy is increasing year by year, which seriously endangers the health and quality of life for food allergic patients, which has brought about life and economic burden to allergic patients and their families. With the rapid development of food industry, potentially allergenic raw food materials (e.g. soy protein, milk powder, egg powder, etc.) are increasingly used in modern food processing. Therefore, avoiding food allergy through diet has become increasingly difficult for patients with food allergy.

Currently, there is no cure for food allergies, and the primary treatment is strict avoidance of the allergenic foods. Therefore, detection and control of food allergens are crucial in ensuring the health and safety of people with food allergies. Food allergies occur when the immune system reacts to proteins in certain foods, leading to a range of symptoms that can vary from mild to severe, including life-threatening anaphylaxis. Therefore, detection and control of food allergens could contribute to the well-being of individuals with food allergies. (1) Accurate labeling: Proper labeling of food products is essential for individuals with food allergies. Food manufacturers are required to clearly list all ingredients, including common allergens, on product labels. Detection of allergens in the manufacturing process helps ensure accurate labeling, enabling individuals to make informed choices about the foods they consume; (2) Allergen testing: Food manufacturers use various testing methods to detect the presence of allergens in their products. These tests may include enzyme-linked immunosorbent assays (ELISA) and polymerase chain reaction (PCR) techniques. Regular testing helps identify and eliminate cross-contamination risks during the production process; (3) Cross-contamination prevention: Cross-contamination occurs when allergenic proteins from one food item are unintentionally transferred to another. Proper sanitation practices and equipment segregation in food processing facilities are critical to preventing cross-contamination. Detection methods assist in identifying areas where cross-contact is more likely to occur, allowing for targeted control measures; (4) Supplier verification: Food manufacturers should verify the allergen status of ingredients received from suppliers. This involves obtaining assurances from suppliers regarding allergen control practices and conducting periodic audits to ensure compliance with safety standards; (5) Education and training: Food handlers, including restaurant staff and kitchen personnel, need to be educated about food allergies and trained in proper food handling procedures. This includes understanding the importance of preventing cross-contamination and being aware of the signs and symptoms of an allergic reaction; (6) Allergen-free menus: Restaurants and food service establishments can play a significant role in ensuring the safety of individuals with food allergies by offering allergen-free menu options. Proper training of kitchen staff, clear communication with customers, and diligent ingredient sourcing are essential components of allergen control in these settings; (7) Consumer awareness: Individuals with food allergies need to be vigilant about reading food labels and communicating their dietary needs to restaurant staff. Increased awareness among consumers helps create a more supportive environment and encourages food manufacturers to prioritize allergen control.

In response to the current situation of food allergy, it is necessary to conduct in-depth research on the following issues: (1) The incidence prevalence of food allergy in different countries and their main allergenic foods are not clear, and the investigation of food allergy in different countries is still lacking. Therefore, it is urgent to conduct a worldwide investigation of food allergy prevalence, so as to accurately
grasp the actual worldwide situation of food allergy, which could provide basic data support for better prevention and treatment of food allergy; (2) In order to ensure the physical health and quality of life for people susceptible to food allergy, it is necessary to establish detection techniques and allergen analysis methods for different food allergens, build new methods for comprehensive evaluating the in vitro and in vivo allergenicity, develop new ideas for efficient reducing allergens, and promote the development of hypoallergenic foods; (3) Considering the role of food derived anti-allergic active substances in alleviating allergic reactions and the anti-allergic molecular mechanisms, further exploration is needed to investigate the effectiveness of anti-allergic active substances in alleviating food allergy. Therefore, further in-depth research is needed to provide theoretical basis and technical support for preventing and controlling food allergy, and ensuring the physical health of food allergic patients.

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