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Research progress on the effects of thermal processing on different food allergen proteins

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Abstract. Food allergy is a global public health and food safety problem, and the removal or attenuation of allergenic proteins through food processing has historically been a focus in the field of food science. This paper introduces the effect of thermal processing technology on allergen protein sensitization of different foods (milk, peanuts, eggs, tree nuts, and aquatic products). Although processing cannot completely eliminate the allergen allergenic potential, the effects can be minimized by the selection of processing methods and processing parameters. It is hoped that this paper can provide a certain reference for the research and development of hypoallergenic or desensitized foods to ensure that desensitized foods can be obtained without affecting the nutritional value of foods to meet the normal dietary needs of people with food allergies. In addition, due to the vast territory of China, residents in different regions have different eating habits and lifestyles for people with food allergies, which makes it very difficult for people with food allergies to carry out dietary interventions. Therefore, one of the indispensable and important missions of the modern food industry is to solve the problem of dietary restrictions for people with food allergies, and the latest research results summarized in this article will help this.

Keywords: thermal processing, food processing,food allergy, allergenic protein.

1. Introduction

Food allergies have become a major challenge in the global public health and food safety sector, and in recent years, the focus on this issue has shown an alarming increase. The current national food safety standard lists eight allergenic substances, which are also proposed by the Codex Alimentarius Commission of FAO/WHO. Nearly 5 percent of adults and up to 8 percent of children suffer from food allergies, which have a serious negative impact on patients' daily lives.

The primary mission of the modern food industry is to provide consumers with safe and reliable food, however, reducing allergic reactions in food is an extremely challenging task. Therefore, under the premise of ensuring that the nutritional value of food is not affected, it is crucial to choose appropriate food processing methods to control food allergens, so as to obtain desensitized foods to meet the normal dietary needs of food-sensitive people. Although the presence of many components in food can trigger allergic reactions, protein is considered the most important and common food allergen. According to the findings, thermal processing can disrupt the secondary or tertiary structure of sensitized proteins in food

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by altering the interactions within and between protein molecules. Alteration of these structures has an impact on the binding capacity of sensitizing epitopes, particularly on the specific binding capacity of immunoglobulin E/immunoglobulin G(IgE/IgG). Eventually, it will lead to a fundamental change in the allergenicity of food allergens. Therefore, an in-depth exploration of the allergenic mechanism of heat treatment on different food allergens can help to explore safer and more effective treatment options, thereby reducing the risk of allergic reactions in allergic people. Therefore, the study on the relationship between thermal processing and the sensitization changes of food allergens has practical significance and plays a guiding role. Combined with literature reports, this paper expounds on the influence of thermal processing on different allergenic foods, hoping to provide some reference for the next use of thermal processing research and development to produce hypoallergenic or desensitized foods.

2. Food allergy

One or more specific food components in the human body, the body produces specific antibodies against food allergens, sensitizing the body. When exposed to these foods again, specific IgE leads to mast cells, basophils in the target organ site degranulation release histamine and other bioactive substances. Thus, allergic inflammation, physiological dysfunction and/or tissue damage are caused, which in turn triggers a series of clinical symptoms, and may even lead to serious life danger [1]. Its specific clinical manifestations include acute urticaria, angioedema, bronchospasm, anaphylactic shock, atopic dermatitis, and allergic gastrointestinal diseases. Food allergic reactions often cause certain damage to the human body, but not all allergic people have corresponding clinical manifestations, so it is very important to correctly judge whether there is a food allergic reaction.

3. Effects of thermal processing on different food allergens

As a commonly used food processing technology, thermal processing has been widely used in practical food processing and research to change protein structure through heat induction, including steaming, boiling, baking, frying, frying, microwave heating, pasteurization and high temperature and high pressure. Thermal denaturation exposes proteins to different functional groups. When heat energy is applied, proteins begin to slowly lose their natural structure, including the primary structure development of protein molecules, the loss of secondary and tertiary structures, and the formation of covalent and non-covalent interactions within and/or between molecules. The effect of thermal processing on allergens is mainly caused by the change of conformational epitopes of allergens, but the effect on linear epitopes is not obvious. As the heat applied increases, the changes become greater, and these changes can lead to the exposure, masking or destruction of conformational epitopes of food-sensitizing proteins, thereby affecting their sensitization.

3.1. Effect of thermal processing on the allergenicity of milk protein

In the process of processing and preserving milk and its products, thermal processing is a widely used process, and the key factor in dairy product quality lies in heating temperature and time. Normally, after thermal processing, the groups of proteins change, resulting in polymers that form heat-induced polymers while also hiding the epitopes of bovine milk protein inside the molecule to a certain extent, resulting in reduced sensitization of proteins. Thermal processing of milk will have a variety of effects on whey protein, internal forces of casein, casein monomers, casein micelles, the interaction between milk proteins, and amino acid components of proteins, resulting in changes in some physical and chemical properties of proteins in milk.

Thermal processing of milk proteins may result in structural changes and subsequently immunogenicity that are influenced by temperature, processing time, natural properties of proteins, and physicochemical factors in the environment such as pH. Changes in immunogenicity may have multiple effects on the expression of antigenicity and allergenicity, including but not limited to increasing, decreasing, and maintaining unchanged. There are many studies on the structural changes of food proteins caused by thermal processing, changing the structure of food proteins leads to disruption, masking, or exposure of conformational epitopes, with implications for immunogenicity.

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Thermal processing can alter the binding ability of α-lactoalbumin to β-lactoglobulin IgG and thus alter sensitization. Fei et al. treated α-lactoalbumin and β-lactoglobulin with different heating conditions, and detected the changes of the binding ability of their allergen IgG by indirect enzyme linked immunosorbent assay (ELISA) [2]. Studies have shown that in the temperature range of 60-75 ℃, the antigenicity of α-lactobumin is lower than that without heat treatment, and its binding to IgG is weakest at 60 ℃, with no significant change at 75 ℃, and its binding ability to IgG is higher at 80 ℃. At different temperatures, the effect of β-lactoglobulin IgG binding capacity is opposite to that of α-lactalbumin. After heat treatment, the binding of lactoglobulin to IgG is significantly enhanced and is consistently greater than that of untreated natural β-lactoglobulin. At 60-80 ℃, the antigenicity of β-lactoglobulin decreases with increasing temperature, and the antibody activity with β-lactoglobulin antibody is weakest at 80 ℃, but it is still higher than untreated lactoglobulin antibody.

Casein is the protein with the highest content in milk. Casein is very stable to thermal processing, and its sensitization is only partially reduced or has no change due to heating. Bloom et al. found that casein's immune reactivity would not be affected basically after it was heated at 95 ℃ for 60 minutes [3]. However, studies have shown that at a high temperature of 65-70 ℃, the sensitization of α-casein decreases, while the sensitization of β-casein increases significantly, so it can be concluded that allergen proteins exhibit different sensitivities under different heating conditions.

3.2. Influence of thermal processing on peanut protein allergenicity

Peanuts are not only a nutritious and delicious food, in addition, peanut protein is also one of the important natural active ingredients, with immunomodulatory effects and anti-inflammatory functions. According to statistics, in China, about 4% of food allergy sufferers are allergic to peanuts, and in the UK, about 1.0% of the population suffers from fatal food allergies, of which 0.5% of the population is allergic to nuts and peanuts. As peanuts are widely used as raw materials or ingredients in various processed foods, it poses a huge risk to food safety. Food allergens that can be identified by the serum of more than 50% of patients are considered to be the main allergens, of which Ara h 1, Ara h 2, Ara h 3 and Ara h 6 are identified as the main allergen proteins in peanuts. In the production process of peanuts, thermal processing technology is widely used, including but not limited to boiling, baking, frying and other methods. These thermal processing processes will cause a series of denaturation reactions of peanut protein, such as structural changes, such as unfolding, aggregation, and chemical modification, so the change of structure and allergenicity of peanut protein after thermal processing has attracted extensive research and attention.

Different allergens have different sensitivity to thermal processing. The study of Zhou et al. showed that the structure of Ara h 1 protein was little affected by boiling, but it was more sensitive to shell baking and frying [4]. In baking and frying, baking with a shell has the greatest effect on the structure of Ara h 1, followed by frying, and shelled baking has the least effect. There was no change in the cleavage site near the C-terminal α helix of Ara h 1 monomer before and after thermal processing, and thermal processing had little effect on the cleavage site. In Ara h 2 protein structure, baking in shell was the most sensitive method. Baking in shell, frying and boiling in shell had the same level of structural influence, while boiling in shell had the lowest level. The sensitivity of Ara h 3 protein structure was similar to that of boiling and hulling baking, and the best effect of Ara h 3 protein structure change was achieved in hulling baking and frying.

Different thermal processes reduce the degree of destruction of the linear epitope of Ara h 1 allergen, thereby enhancing the potential allergenicity of Ara 1, and this trend is, from high to low: shell baking≥ frying>hulled baking> boiled > fresh peanuts. Shelled boiling did not significantly affect the degree of destruction of Ara h2 linear epitopes, and hulled boiling, baking and frying all enhanced the degree of destruction of Ara h2 linear epitopes. In addition, the degree of linear epitope destruction of Ara h 3 before and after different thermal processing remains basically unchanged [4].

3.3. Effect of thermal processing on egg protein allergenicity

Eggs are rich in high-quality protein, carbohydrates, fats and trace elements, with rich nutritional value

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and relatively low price, widely used by various people, deeply loved by people, and widely used in bakery products, meat products, biscuits and other food industries. In recent years, with the improvement of living standards, consumers have put forward higher requirements for food safety, and eggs as a natural food are more and more favored. However, eggs as a food have the highest risk of triggering allergies in children. It is generally considered that the allergenic potential of protein in egg white is greater than that of protein in egg yolk, and ovalbumin accounts for more than half of the amount of egg white protein by weight, and in egg white, there are four main allergens, oogua (OVM), ovalbumin (OVA), ovotransferrin (OVT), and lysozyme (LYS). These allergens can cause varying degrees of allergic reactions in the body. The allergenicity of the allergen has a lot to do with its molecular structure, in the most commonly used processing method, thermal processing can affect the structure of the allergen protein, this method can make the structure of the allergen protein change, so that its epitope is exposed or hidden, so that its allergenicity changes. [5].

For example, according to the study of Liu et al., The structure and allergenicity of OVTs are significantly affected during heating due to their typical α-helical and β-folded structures and insufficient resistance to high temperatures [6]. When the heating temperature was greater than 80 ℃, the secondary structure would be destroyed, and with the extension of heating time and the rise of temperature, the tertiary structure would gradually unfold, the structure would become loose, the color groups would be exposed, the surface hydrophobicity would increase, and the sensitization would decrease significantly [7].

As a thermally unstable protein, the spatial structure of OVA undergoes many degrees of change after thermal processing. During the thermal processing of OVA molecules, the unfolded part of the OVA molecule presents hydrophobic residues and tends to polymerize, while the epitope is exposed to the molecule, and the interaction between the two causes the antigenicity to change continuously with the change of heating temperature and time. When the heating temperature exceeds 80 ℃, the OVA molecule is fully stretched, and the exposed sulfhydryl group promotes the formation of disulfide bonds between molecules, thereby promoting the interaction between protein molecules, producing a large number of polymers, but also significantly reducing their antigenicity.

The stable domain of the OVM is formed by the ligation of three disulfide bonds, which makes it relatively stable in thermal processing and trypsinization. In addition, egg whites after OVM removal are less allergenic compared to whole egg whites, so OVM is the most allergenic protein in egg whites. According to studies, after thermal processing, the allergenicity of OVM gradually decreased [8]. In addition, heat-induced OVM conformational changes can affect the differentiation activation and cytokine release of CD4+ cells, resulting in a decrease in their allergenicity [9].

Egg Lys is a globular protein that is thermally unstable and studies have found that thermal processing reduces the content of α-helical and β-folded structures of Lys, increases the content of β-turn and random curls, and changes the ordered structure of Lys to disorder. With the increase in heating time and temperature, the hydrophobic groups of proteins are exposed, and their surface hydrophobicity increases.

3.4. Influence of thermal processing on nut allergenicity

3.4.1. Influence of thermal processing on the allergenicity of walnut. Walnuts can be eaten raw, fried, pressed for oil and confectionery, as well as used as a major ingredient in cakes and cookies and other baked products, but they are also a large source of nut allergies. At present, many studies have confirmed that walnut allergy is mainly a fast-onset allergic reaction mediated by immunoglobulin (Ig) E, while walnut allergens found in the serum of patients with walnut allergy include Jug r 1, Jug r 2, Jug r 3 and Jug r 4. Jug r 1 is considered the main allergen protein in walnut.

Jiang et al. studied the changes in walnut protein extractable mass concentration, serum IgE binding capacity and rabbit polyclonal antibody IgG binding ability in walnut allergy patients, and analyzed the effects of dry heat, humid heat and high temperature and high pressure treatment on walnut protein antigenicity and sensitization. They found that dry thermal processing had no significant effect on the

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IgG binding ability of walnut protein [10]. The binding ability of walnut protein IgG was significantly decreased by 40% and 80% under humid heat and high temperature and high pressure treatment, respectively. Microwave treatment improved the IgG binding ability of walnut protein to some extent. It was proved that moist heat and high temperature and high pressure treatment significantly reduced the IgG binding ability of walnut protein, and effectively reduced the antigenicity of walnut protein. Cabanillas et al. compared the effects of different heat treatments on the binding capacity ofJug r 4 immunoglobulin E (IgE), and found that humid heat and high temperature and high pressure treatment significantly reduced the binding capacity of Jug r 4 IgE and effectively reduced the antigenicity of Jug r 4 [11].

3.4.2. Influence of thermal processing on the allergenicity of cashew nuts. In recent years, cashew nut allergy has been included in the category of severe allergy. Thermal processing is widely used in nut processing. Thermal processing applied to protein structure can lead to allergenic changes and can even be used to prevent cashew allergy and reduce allergic reactions in allergic people.

According to the study of Wang et al., compared with raw cashews, the soluble protein content of cashews roasted for 8min showed no significant change, while the insoluble protein content was significantly reduced, and the sensitization of each gram of protein was significantly enhanced [12]. Soluble protein content decreased significantly and insoluble protein content did not change significantly in cashews roasted for 12 min, and sensitization of protein per gram increased significantly. The soluble protein content decreased significantly and the insoluble protein content increased significantly in cashews after roasting for 16 min. There was no significant change in the sensitization of protein per gram. In fried cashews, soluble protein content decreased significantly, insoluble protein content increased significantly, and sensitization was enhanced per gram of protein.

3.5. Influence of thermal processing on the allergenicity of aquatic products

Aquatic products are popular among people because of their delicious taste and rich nutrients, while fish, shrimp and other aquatic products are common allergens. Fish allergens include Parvalbumin, fish vitellogenin and Collagen, while Tropomyosin is the main allergen of crustaceans and mollusks, Ovalbumin and Arginine kinase. At present, in addition to controlling the intake of allergenic substances in food, the molecular structure of allergic substances in food is adjusted by thermal processing, so as to achieve the purpose of reducing allergic substances in food. Thermal denaturation can lead to the decomposition or partial degradation of some major components in aquatic products, and will destroy the hydrogen bonding between some protein molecules, reducing its ability to cause allergic reactions. After high temperature treatment, the secondary and tertiary structure changes in the primary and secondary structures of seafood plasmomyos, which in turn causes allergic reactions.

Liu et al. analyzed IgE binding activity of digestive products before and after thermal processing of tropomyosin (TM), the major allergen of Litopenaeus vannamei, using enzyme-linked immunosorption (ELISA), and found that steaming and boiling treatment could significantly reduce IgE binding ability of TM protein and reduce sensitization [13]. Zheng et al. used SDS-PAGE electrophoresis, western blotting analysis and ELISA to conduct qualitative and quantitative tests on the immune activity of allergens [14]. They conducted boiling, steaming and high-temperature pressure treatment on the meat of Penaeus doufuensis, respectively, and found that the sensitization of major allergens under high-temperature pressure treatment decreased by 78% after 5 min. After boiling for 30 min, the sensitization was reduced by 97% and almost disappeared. In conclusion, the application of high temperature pressure in thermal processing can significantly reduce the sensitization of shrimp allergens.

However, there are still many studies showing that thermal processing has little effect on the sensitization of aquatic products allergens. Yu et al. found that ordinary steaming and other methods had no significant effect on the sensitization of crab meat allergenic proteins [15]. In addition, some studies have shown that thermal processing may increase the allergenic sensitization of aquatic products. Larly et al. found that the IgE binding activity of the allergen extracted from the flowertail shrimp increased after boiling for 5-25 min [16].

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4. Conclusion

In this paper, the characteristics of different food allergen proteins are comprehensively studied, and the influence of thermal processing technology on their allergenicity is discussed. At present, thermal processing methods have been widely used in a variety of foods. As thermal processing progresses, the structure of proteins and epitopes of antigens change, which affects the allergenicity of different food allergens, and this technology is simple, safe, efficient and relatively mature. Thermal processing, as a relatively mature and inexpensive processing technique, is simple and convenient to operate. At present, although processing cannot completely eliminate the allergenic potential of allergens, as long as the appropriate processing methods and parameters are selected, it is still expected to develop individually tolerated desensitized foods at the industrial level for special allergic people. However, the research field has gradually realized that there is a lack of evaluation methods to determine the impact of thermal processing on the allergenic potential of allergic proteins. Therefore, future research should pay more attention to in vitro experiments combined with in vivo experiments, and establish animal model tests, oral tests, skin prick tests and cytokines analysis to further verify the safety and efficacy of food processing technologies in reducing or eliminating the allergens, so as to meet the urgent needs of special allergic people for food safety.

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