Food Science of Animal Products

Review Article

Effects of enzymolysis on allergenicity and digestibility of food allergens

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Abstract: Food allergens are a significant concern for global public health. Enzymolysis, which includes enzymatic hydrolysis and enzymatic cross-linking, is known for its mild conditions, as well as its high efficiency and stability, making it a widely employed method to reduce the allergenicity of food proteins. A small group of eight foods-four of animal origin (milk, eggs, fish, and crustacean shellfish) and four of plant origin (tree nuts, peanuts, wheat, and soybeans) accounts for approximately 90% of all food allergies. However, there has been no comprehensive summary of how enzymolysis impacts the digestibility and allergenicity of these eight key foods, based on both *in vitro* and *in vivo* studies. This paper seeks to provide a systematic review of the influence of enzymolysis on food allergenicity, with a focus on molecular characteristics, epitopes, immunoglobulin (Ig) E/IgG binding capacity, and digestive stability during *in vitro* digestion. The potential immune regulation effects of enzymolysis products in cell and animal allergy models are also highlighted.

Keywords: enzymatic hydrolysis; enzymatic cross-linking; allergens; *in vitro* digestion

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1 Introduction

Food allergies present a global public health challenge. In developed nations, food allergens pose a substantial health risk, affecting 5%–10% of the population[1–2]. Approximately 90% of all food allergies are attributed to eight specific foods, including four of animal origin (milk, eggs, fish, and crustacean shellfish) and four of plant origin (tree nuts, peanuts, wheat, and soybeans), with proteins being the predominant allergens[2–3].

The allergenicity of food proteins refers to their potential to induce sensitization and anaphylactic reactions, which underscoring the importance of methods to mitigate this risk. Enzymatic hydrolysis is a mild process that may disrupt the original functional properties of proteins and has been recognized as an effective strategy to reduce protein allergenicity. Frequently utilized enzymes include hydrolytic enzymes (trypsin and papain), whereas other enzymes, such as tyrosinase (TYR), polyphenol oxidase (PO), laccase (Lac), and acyltransferase transglutaminase (TG), are primarily employed for protein cross-linking. The fundamental mechanisms can generally be divided into two types: 1) proteases break down allergens into peptides, amino acids, or other small molecules, resulting in changes to their spatial structure; 2) enzymes cause intermolecular or intramolecular cross-linking, which leads to alterations in protein conformation[4]. Overall, enzymatic hydrolysis is an effective approach for decreasing protein allergenicity. The reduction in allergenicity post-enzymatic treatment is likely due to the cleavage of allergenic epitopes, with the extent of reduction dependent on the degree of epitope destruction[2,5].

Upon identifying epitopes within these allergens, the immune system may trigger clinical symptoms. Most food allergies are categorized as immunoglobulin (Ig) E-mediated, which typically arise within minutes to hours and manifest in symptoms such as diarrhea and asthma. In extreme cases, these reactions may become life-threatening[6]. Allergies result from the recognition of food allergen epitopes by Igs, leading to an immune response. Consequently, allergenicity is often evaluated by the IgE-binding capacity to allergens, using techniques like Western blotting (WB) and enzyme-linked immunosorbent assay (ELISA), in addition to cell models (e.g., rat basophilic leukemia cells and basophil activation tests) and animal models[2,6]. Previous reviews have examined the effects of food processing on allergenicity[2,5,7], however, a detailed analysis of how enzymolysis, including both enzymatic hydrolysis and cross-linking, impacts the digestibility and allergenicity of the eight major allergenic foods-milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybeans-has not yet been comprehensively presented. Given the growing prevalence of food allergies and the need for strategies to reduce allergenicity in food products, a systematic review of enzymolysis is necessary. Such a review would provide crucial insights into how enzymatic treatments can modify allergenic proteins, improve digestive stability, and reduce immune responses. Additionally, by focusing on recent advancements in IgE/IgG binding capacity and findings from *in vitro* digestion, as well as cell and animal models, this review will offer a deeper understanding of the potential applications of enzymolysis in mitigating food allergies and inform future research and food industry practices.

2 Cellular immunity in food allergy

The majority of immune reactions in food allergies are mediated by IgE and are referred to as immediate type I hypersensitivity responses. Symptoms generally manifest within minutes and nearly always within an hour following consumption[8]. $CD4^+$ T cells, which play a role in immune responses to food allergies, can be classified into T helper type 1 (Th1) and Th2 subtypes according to the cytokines they secrete. A skewed Th2 response is a hallmark of food allergies (Figure 1). Th1 cells are mainly responsible for secreting interferon γ (IFN-γ) and interleukin (IL)-12, while Th2 cells primarily produce IL-4, IL-5, and IL-9. Clinically, individuals with food allergies display a Th2-dominant response, which is marked by elevated serum levels of IL-4, IL-5, and IL-13, along with reduced levels of the Th1 cytokine IFN-γ[5,9].

During sensitization, food allergens encounter dendritic cells (DCs) in the intestinal lamina propria. These DCs present antigens to naive $CD4^+$ T cells, leading to the differentiation and proliferation of Th2 cells. Th2 cells stimulate B cells to produce excessive IgE antibodies. IgE then binds to FcεRI receptors on mast cells or basophils. When allergens enter the bloodstream, they bind to IgE on these cells, initiating degranulation and the release of histamine and other inflammatory mediators, which result in the clinical symptoms associated with food allergies[10]. Regulatory T cells (Tregs), including $CD4^+CD25^+Foxp3^+$ Tregs and $IL\text{-}10^+$ Tregs, help manage the Th1/Th2 balance in cow's milk allergy by directly inhibiting the Th2 response[11–12]. However, children with food allergies often have fewer Tregs and impaired Treg function, which correlates with severe allergic reactions[13].

Furthermore, regulatory DCs (RegDCs; particularly $CD11c^+CD103^+$ DCs and immature DCs) and regulatory B cells (Bregs) are also involved in allergic immune responses. RegDCs and Bregs secrete suppressive cytokines, such as IL-10 and transforming growth factor β (TGF-β), facilitating the conversion of $CD4^+$ T cells into Tregs[11,14–17]. Food allergens reduce the number of $CD103^+$ DCs in allergic mice, while increasing the percent of $CD103^+$ DCs can alleviate allergic responses[16,18–19]. Bregs mitigate allergen-induced allergic responses and play a role in promoting immune tolerance in patients[20–21]. *In vitro* studies show that Bregs from human peripheral blood mononuclear cells enhance the percent of Tregs cells[22]. Thus, RegDCs and Bregs are crucial in food allergy development by regulating Treg cell differentiation and the Th1/Th2 balance.

3 *In vitro* gastrointestinal digestion models for allergenicity

Experimental models for food allergies provide accurate methods for allergen detection and evaluation and offer robust approaches for studying mechanisms. Common models include mast cells, basophils (as shown in Figure 1), and animal models such as BALB/c mice, C3H/HeJ mice, and BN rats[6]. Currently, the mechanism underlying food allergies is well-understood. Figure 1 illustrates this mechanism, highlighting how food allergies increase the permeability of intestinal epithelial cells to allergens, which in turn influences certain cytokine pathways and triggers a Th2-type inflammatory response. The interaction between the food matrix and processing methods during digestion in the gut is crucial in determining allergenicity[23]. Digestion can result in the disruption or exposure of conformational and linear epitopes in food proteins, thereby modifying their sensitizing and allergenic properties. This process is ongoing and dynamic within the human body (Figure 2), starting in the mouth. Once swallowed, food proteins rapidly pass through the esophagus and enter the stomach, where gastric acid denatures them. Protein digestion predominantly takes place in the duodenum, where pancreatic enzymes, including trypsin and chymotrypsin, collaborate with other enzymes secreted by the small intestine to break down food components. Over the past few decades, *in vitro* gastrointestinal digestion models, both static and dynamic, have been developed and widely utilized in the evaluation of potential protein allergenicity, including enzymatically cross-linked, processed, and recombinant proteins. The static INFOGEST protocol, which simulates protein digestion under physiological conditions in the human gastrointestinal tract,

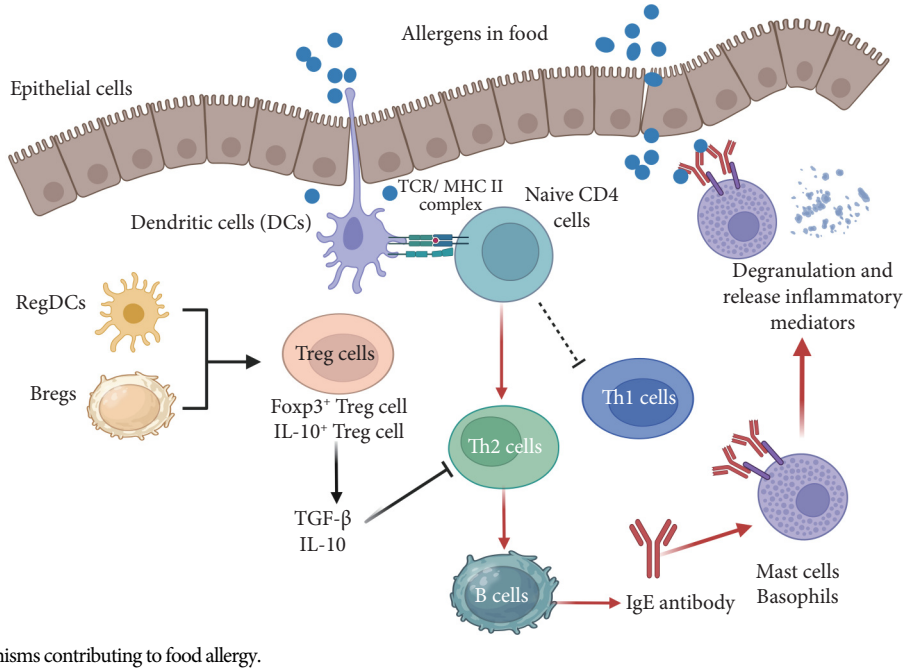

Figure 1 Immune mechanisms contributing to food allergy.

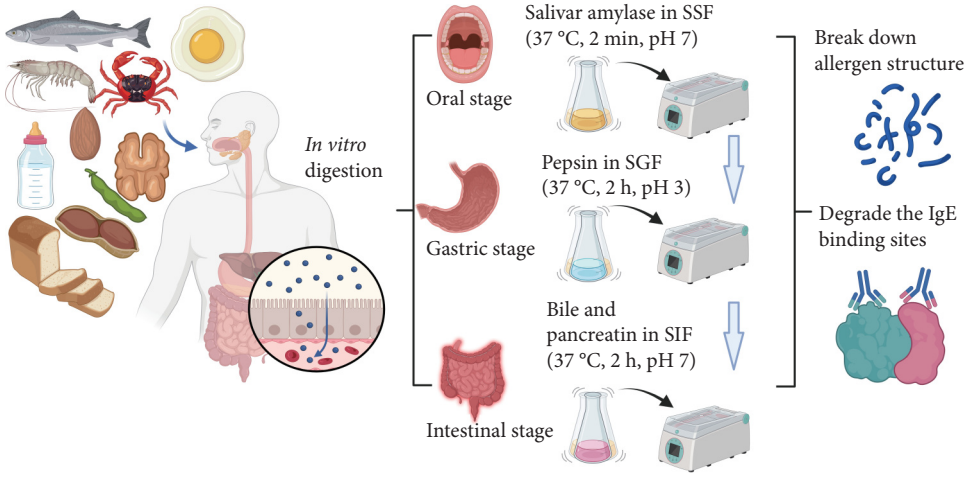

Figure 2 Representation of structural changes and allergenicity of major allergic foods during *in vitro* digestion. SSF, simulated salivary fluid; SGF, simulated gastric fluid; SIF, simulated intestinal fluid.

comprises three stages (oral, gastric, and intestinal)[24]. Dynamic simulated digestion models, on the other hand, consist of various devices designed to closely mimic the physiological conditions of the human digestive process[25]. The dynamic models replicate the transport of digested food, the ongoing secretion of digestive juices, fluctuations in enzyme concentrations, and pH changes over time[23]. Despite the advantages of dynamic models, static models remain popular due to their ease of use, affordability, and minimal equipment requirements. These *in vitro* gastrointestinal digestion models have proven effective in assessing the impact of food structure on allergen breakdown during digestion[26–27].

4 Aniamal protein

4.1 Eggs

4.1.1 Effects of enzymolysis on the allergenicity of egg allergens

Egg allergy is estimated to affect 1.8%–2.0% of children under five years old. The most significant allergenic proteins are located in the egg white, including ovalbumin (OVA), ovotransferrin (OVT), ovomucoid (OVM), and lysozyme (LZM)[28–29]. OVA is the predominant allergen of egg white, comprising 54%–58% of its total weight[30]. After hydrolysis by pepsin, neutral protease, and alkaline protease, the IgE-binding capacity of OVA was reduced by 90.1%, 70.0%, and 99.8%, respectively. In the case of OVM, hydrolysis by these enzymes resulted in reductions of 92.5%, 99.5%, and 99.4% in IgE-binding capacity, respectively[28]. Some peptides in OVA hydrolysates, including sequences 125–176, 188–198, 326–336, and 370–385, retained IgE-binding capacity, explaining the residual allergenicity. In pepsin hydrolysates of OVM, longer peptides, possibly linked by disulfide bonds, were identified, potentially enhancing IgE recognition[31]. In addition, enzymatic cross-linking can significantly alter the structural properties and allergenicity of egg allergens. OVA treatment with PO and caffeic acid (CA) reduced α-helix and β-structure, with increased random coil structure. This enzyme cross-linking reduced OVA's IgE binding by 38.6%, demonstrating that this treatment significantly alters OVA structure and reduces allergenicity[32]. Chemical grafting of ferulic acid (FA) on protein surface was applied to modulate the cross-linking of LZM and OVA, which enhanced protein functionality and reduced IgE binding[33]. The above research provide valuable insights into how enzymatic hydrolysis and enzymatic cross-linking can significantly alter the structural properties and allergenicity of OVA. Enzymatic hydrolysis can degrade proteins, achieving both higher bioactive peptides and desensitization effects. However, it often fails to completely destroy allergen epitopes[31]. Additionally, some hydrolysate fragments after protein degradation may cause undesirable effects such as bitter peptides or heightened allergenicity[34].

Reports have shown that combining enzymolysis with physical methods to pretreat proteins can often yield better results, such as altering the protein structure, creating more proteolytic sites, and facilitating faster protein breakdown[35]. Enzymatic cross-linking of OVA and OVT was improved by TG with heat[36]. Heat denaturation (100 °C for 5 min) assisted enzymatic cross-linking may provide a new cross-linking method to develop hypoallergenic foods with the reduced IgG and IgE binding capacities of OVA, as well as the decreased OVA-induced degranulation capacity of human peripheral blood basophilic leukemia cells (KU812)[37]. The combined application of enzymatic hydrolysis and thermal processing techniques can enhance the degradation of allergenic proteins by enzymatic hydrolysis. After heat treatment of egg white proteins (EWP) (95 °C water bath for 10 min), chymotrypsin and pepsin hydrolysis were performed for 3 h, resulting in egg white peptides. After ultrafiltration, products with molecular weights greater than 3 kDa (PHMW) and less than 3 kDa (PLMW) were obtained, and it was found that the allergenicity of the low molecular weight PLMW EWPs was significantly reduced after hydrolysis and ultrafiltration[38]. Moreover, different methods of enzymatic hydrolysis have different effects on the degradation of allergenic proteins. The enzymatic hydrolysis by immobilized enzymes differs from that of free enzymes, the degree of hydrolysis of EWP by free alkaline protease and immobilized enzymes was 25% and 20%, respectively[39]. After hydrolyzing EWP with immobilized enzymes, the IgG binding capacity of the hydrolysates decreased by 20%, while the IgG binding capacity of the hydrolysates in the free enzyme group decreased by 55%. The IgE binding ability of the hydrolysates was reduced by 20% in the immobilized enzyme group, compared to a 30% reduction in the free enzyme group[39]. These findings suggest that both proteases and free alkaline protease hydrolysis of EWPs can significantly decrease the IgG and IgE binding capacity of hydrolysates, but the

free enzyme treatment has a greater impact on IgG and IgE binding ability compared to immobilized enzymes. Additionally, the hydrolysates of free alkaline protease exhibited a blue shift phenomenon and significantly increased hydrophobicity, possibly due to the exposure of a large number of hydrophobic groups during hydrolysis. In contrast, the hydrolysates from immobilized enzymes showed a slight red shift in the fluorescence emission peak, and hydrophobicity slightly decreased. Compared to free alkaline protease, immobilized alkaline protease has better thermal stability and storage stability[39]. In another study, liquid EWPs were hydrolyzed using neutral protease and thermolysin in both free and immobilized forms, followed by simulated oral, gastric, and intestinal digestion of the hydrolysates. The bands of egg proteins (such as LZM, OVA, OVT, and OVM) in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis were weakened or disappeared after simulated digestion, and a new band (40.5 kDa) appeared in EWP hydrolysates with the reduced IgE binding capacity[28–29]. Compared with free enzymes, immobilized enzymes increased the surface hydrophobicity of the hydrolysates. During *in vitro* simulated digestion, EWP peptides were further degraded by digestive enzymes, resulting in digestion products with higher surface hydrophobicity. Liquid chromatography-mass spectrometry data showed that most EWP allergenic epitopes were degraded after enzymatic hydrolysis combined with *in vitro* digestion, but epitopes 38–49 and 357–366 remained intact, while OVM epitopes 1–10, 1–14, 1–20, 4–20, 11–20, 71–75, and 101–105 were preserved[29]. The above researches indicate that immobilized enzymes demonstrated greater effectiveness than free enzymes in inactivating IgE epitopes in EWP hydrolysates and their *in vitro* digestion products. Compared to *in vitro* digestion, the enzymatic treatment had a more significant effect on reducing immunoreactivity in these studies.

4.1.2 Alterations in allergenicity of enzymolysis-modified egg allergens during digestion in vitro and in vivo

Enzymatic hydrolysis products are favorable for gastrointestinal digestion, and their products in the digestive tract have low allergenicity. OVA was pretreated with PO and CA, and SDS-PAGE analysis revealed that both native and cross-linked OVA resisted simulated gastric digestion, with band intensity remaining unchanged for up to 60 min. However, unlike native OVA, cross-linked OVA in simulated intestinal fluid (SIF) rapidly degraded within 5 min, and the IgE binding capacity of the cross-linked products was reduced compared to that of native OVA[40]. EWP were hydrolyzed with pepsin and papain for 5 h, reducing IgE binding ability by 65% and 41%, respectively. After *in vitro* digestion, IgE binding continued to decrease in pepsin hydrolysates, but increased in papain hydrolysates[41]. The degree of hydrolysis (DH) of the papain hydrolysates significantly increased after simulated gastrointestinal digestion (from 80% to 87%), indicating that the papain hydrolysates might have aggregated through hydrophobic interactions and were disrupted during gastrointestinal digestion, exposing previously hidden IgE binding epitopes[41]. In addition, protein modifications during enzymatic hydrolysis can also alter the allergenicity of OVA in animal model. After treatment with *N*-acetylglucosaminidase, OVA was converted into *N*-OVA, which induced about a 60% reduction in serum IgE levels and histamine content in OVA sensitized mice[42]. LZM and OVA cross-linked with FA and Lac (generating FA-LZMs and FA-OVAs complexes) significantly increased the phenol content, proportion of dimers, oligomers, and polymers in the complexes, and reduced the IgE binding ability of LZM but not altering that of OVA[40]. The reduction in IgE binding capacity was positively correlated with the extent of enzymatic cross-linking, indicating that cross-linking influences protein allergenicity by modifying the integrity of IgE binding epitopes. Another study using the BALB/c mouse allergy model showed that cross-linked OVA induced milder allergic symptoms. Compared with the native OVA group, the cross-linked OVA significantly reduced the levels of allergen-specific antibodies (IgE, IgG, IgG1, and IgG2a) in the serum, and inhibited the production of IL-4, IL-5, IL-13, and IFN-γ in splenocyte induced by OVA[40]. The hydrolysate of OVA with pepsin also reduced the ability to trigger allergic symptoms in mice, reduced Th2 responses and increased Th1 responses[43]. EWP hydrolysate significant reduced both serum histamine and specific IgE in BALB/c mouse model of egg allergy, accompanied by a reduction of both IL-4 and IFN-γ production in splenocyte and increased *TGF-β* and *Foxp3* mRNA expression[44]. The pepsin hydrolysate of OVA reduced the sensitizing potential in a BALB/c mouse model of egg allergy, which was mediated through the induction of Treg cells and the upregulation of TGF-β, IL-10, IL-17, Foxp3, and retinoid-related orphan nuclear receptor γt (RORγt) in intestinal tissues[45]. As $Foxp3^+$ Tregs inhibit the Th2 response[11–12], dietary supplementation with enzymatically hydrolyzed egg white peptides induced a high percentage of $CD4^+Foxp3^+$ Treg cells and low IL-4 production in splenocytes, which alleviates allergic responses in a mouse model of egg white allergy[46]. These studies provide evidence that enzymolysis products within the digestive tract exhibit significantly lower allergenicity compared to their intact counterparts. Furthermore, they suggest that hydrolyzed allergens are more effective than whole allergens in preventing the development of allergies *in vivo*, enhancing long-term desensitization, and promoting intermolecular tolerance.

4.2 Crustacean shellfish

4.2.1 Effects of enzymolysis on the allergenicity of allergens in crustacean shellfish

Crustacean shellfish is a common trigger of food allergies and accounts for a significant proportion of emergency department visits related to severe food allergic reactions[47–48]. Tropomyosin (TM) is a major protein, making up nearly 20% of the total protein content in crustacean shellfish[4]. After treatment of TM with papain, bromelain, and ficin, SDS-PAGE showed that the intensity of the low molecular weight (< 35 kDa) protein bands in the papain hydrolysates was stronger than in those treated with ficin and bromelain, indicating that papain hydrolysates contained more small peptides. Previous studies have identified TM as the primary allergen in shrimp, accounting for 5%–7% of shrimp muscle. This heat-stable protein is resistant to denaturation by conventional food processing methods[47–48]. Although TM was enzymatically hydrolyzed, ELISA tests revealed that the IgE binding capacity of the hydrolysates did not decrease in these studies, which indicated that some epitopes retained their IgE binding capacity, and in certain cases, enzymatic hydrolysis might even increase IgE binding capacity by exposing more epitope regions[49]. In addition, the combined application of other processing technologies and enzymatic hydrolysis can enhance the degradation of allergenic proteins. The shrimp *Metapenaeus dobsoni*, treated with 0.5% papain and high-pressure steaming, showed reduced TM band

intensity on SDS-PAGE image. ELISA and WB revealed that this combined treatment significantly reduced IgE binding capacity compared to high-pressure steaming alone[50], indicating that the combined treatment caused greater disruption of allergenic epitopes. Enzymatic cross-linking reactions have been extensively studied in reducing the allergenicity of crustacean proteins. Blue crab TM was treated with horseradish peroxidase (CHP) and TYR at 37 °C for 1 h, reducing IgE binding by 63.5% and 34.5%, respectively. SDS-PAGE revealed new protein bands (45, 66 and 116 kDa), indicating enzyme-induced aggregation. The secondary structure of the proteins also changed, with decreases in α-helix, β-sheet, and β-turn contents in both CHP and CTC[51]. Shrimp TM treated with TYR and CA for 24 h showed reduced IgG and IgE binding by 37.19% and 49.41%, respectively. The changes in tertiary and secondary structures contributed to the reduced IgE binding[47–48]. Theses enzymatic cross-linking altered the secondary structure of the proteins, characterized by a reduction in α-helix content, an increase in β-turn, random coil, and β-sheet content, and a blue shift in the ultraviolet (UV) absorption peak, indicating changes in the tertiary structure of the protein[47–48]. TM from shrimp was treated with Lac + CA and TG, resulting in enhanced protein aggregation, significantly reduced IgG and IgE binding capacities, and altered spatial structure[52]. These studies suggest that enzymatic cross-linking may reduce TM allergenicity by altering its protein conformation. The β-turn structure may be directly related to allergenicity, while changes in α-helix content and UV absorption compared to fluorescence intensity better reflect the allergenic changes in TM[48,53].

4.2.2 Alterations in allergenicity of enzymolysis-modified allergens in crustacean shellfish during digestion in vitro and in vivo

The allergenicity of cross-linked products in the gastrointestinal digestion system varied. *In vitro* simulated digestion experiments found that TM cross-linked by horseradish peroxidase (HRP) was more easily degraded by pepsin than TM cross-linked by TYR[51]. After *in vitro* simulated digestion, the number of low molecular weight (16 and 32 kDa) bands increased for both natural TM (35 kDa) and the enzyme cross-linked products, but the high molecular weight proteins (> 180 kDa) in the enzyme cross-linked (TYR + CA) products were resistant to pepsin digestion. WB analysis showed that compared to natural TM, the IgE binding ability of the enzyme cross-linking products was completely disappeared[54]. Arginine kinase (AK) from blue crab underwent heat-induced polymerization with TYR cross-linking, reducing IgE binding by 99.5%. The cross-linked products showed greater resistance to gastrointestinal digestion. After pepsin digestion, IgG binding decreased, and after trypsin digestion, both IgG and IgE binding capacities were reduced[55]. TM from shrimp treated with Lac + CA or TG promoted TM degradation by pepsin, and the binding ability of the digestion products with IgG and IgE completely disappeared[53]. Importantly, animal/cell experiments also demonstrated that proteins treated with enzymatic cross-linking technology could alleviate allergic immune responses. The cross-linked products (CHP and CTC) from HRP and TYR significantly reduced β-hexosaminidase and histamine levels in rat basophilic leukemia cells (RBL-2H3). In allergic mice, CHP and CTC lowered serum IgE, IgG1, and histamine levels, increased Tregs, promoted DC maturation (increased major histocompatibility complex class II), and inhibited Th2 cytokine (IL-4 and IL-13) production in splenocytes and mesenteric lymphocytes[51]. TM from *M. ensis* shrimp was treated with TYR and CA, which significantly reduced β-hexosaminidase, histamine, tryptase, IL-4, and IL-13 levels in RBL-2H3 and KU812 cells. Animal experiments showed that the enzymatic cross-linking products reduced serum TM-specific IgE and IgG1 content, as well as histamine, mouse monocyte chemoattractant protein-1 (mMCP-1), IL-4, IL-5, and IL-13 levels in allergic mice, while induced high level of IL-10 and IFN-γ[54]. TM from shrimp, treated with TG or TYR, significantly reduced β-hexosaminidase, histamine, IL-8, and tumor necrosis factor-α secretion in laboratory of allergic diseases 2 mast cells. Animal experiments showed that the enzymatic cross-linked products reduced allergic symptoms, decreased histamine levels in the serum, significantly reduced IL-4 secretion in spleen cells, and increased the proportion of $CD4^+CD25^+$ Treg cells in the spleen[47]. AK cross-linked products significantly inhibited mast cell degranulation and reduced histamine secretion in RBL-2H3 cells[55]. TM from shrimp treated with Lac + CA or TG showed similar results in animal and cell experiments[52]. These studies indicate that enzymatic cross-linking treatment can significantly alleviate TM-induced allergic reactions through multiple mechanisms. By enhancing the digestibility of crustacean allergens, the treatment reduces their persistence in the gastrointestinal tract. Additionally, it modulates DC function, promoting the maturation of these antigen-presenting cells, which in turn enhances immune tolerance. The treatment also increases the presence of $Foxp3^+$ Tregs, which play a crucial role in maintaining immune homeostasis. Furthermore, enzymatic cross-linking helps balance Th1/Th2 immunity, reducing the overactive Th2 responses that are commonly associated with allergic reactions, thereby providing a more regulated immune response.

4.3 Fish

4.3.1 Effects of enzymolysis on the allergenicity of fish allergens

Fish allergy is a significant global health concern, with prevalence rates estimated to be as high as 7% in pediatric populations[56]. Parvalbumin (PV), the primary fish allergen found in muscle tissue, varies significantly between fish species, with content ranging from 0.5–2.0 mg/g of raw muscle, and this variation correlates with the differing allergenicity of fish[57]. Fish frame slurry from rainbow trout was pretreated with microwave heating at 90 °C for 5 min and hydrolyzed with 3% alkaline protease at 55 °C for 2–10 min. SDS-PAGE showed protein degradation to below 6.5 kDa, and ELISA revealed a 90% reduction in PV and a 93% decrease in antigenicity after treatment[58]. After treatment with pepsin, the antigenicity of TM from salmon and mullet gradually decreased, with the antigenicity of salmon and mullet TM completely disappearing after 8 and 60 min[59]. The combined application of other processing technologies and enzymatic hydrolysis can enhance the degradation of allergenic proteins. PV from common pandora and sardine was heat-treated (90 °C, 30–120 min) and then treated with pepsin. SDS-PAGE showed almost complete degradation after enzyme treatment alone, while heat combined with enzyme treatment left faint protein bands. IgE binding in common pandora PV was reduced by 22.9% with enzyme treatment and 50.5% with heat and enzyme. In sardine, IgE binding was reduced by 57.9% and 46.6%, respectively[60–61]. PV treated with TYR and CA showed enhanced high molecular weight protein bands (> 180 kDa) on SDS-PAGE. Circular dichroism revealed a looser secondary structure, with reduced α-helix content and more

random coils. Enzymatic cross-linking decreased fluorescence intensity, shifted the tertiary structure, and significantly reduced IgE binding[62]. Additionally, after 60 min of pepsin digestion, native PV (12 kDa) was hydrolyzed into 3–5 kDa bands, while cross-linked PV formed larger bands (> 12 kDa). WB showed that cross-linked PV lost IgG binding after 15 min, while native PV lost it after 60 min. During trypsin digestion, native PV remained stable for 240 min, while cross-linked PV showed similar digestion behavior in both pepsin and trypsin[53]. PV cross-linked with Lac and propyl gallate (PG) showed a shift in protein bands from 12–14 kDa to primarily 35 kDa, with higher molecular weight bands (about 100 kDa) emerging on SDS-PAGE. WB analysis revealed a 34.8% reduction in IgG binding capacity compared to native PV. Cross-linking decreased α-helix and β-turn content, increased β-sheet and random coil content, caused a slight blue shift in the UV absorption peak, and reduced fluorescence intensity, indicating tertiary structure changes[63]. Overall, enzymatic hydrolysis and cross-linking significantly reduce the allergenicity of fish proteins, particularly PV, the major fish allergens. Combining these methods with other processing techniques holds promise for creating safer fish-based products for allergic individuals. These approaches effectively degrade allergenic proteins, alter their secondary and tertiary structures, and reduce IgE and IgG binding capacities, thereby enhancing protein digestibility and decreasing allergenicity, showing great potential in mitigating fish allergies.

4.3.2 Alterations in allergenicity of enzymolysis-modified fish allergens during digestion in vitro and in vivo

In vitro simulated digestion showed that, compared to native PV, cross-linked PV by Lac and PG exhibited stronger resistance to pepsin digestion, with bands still present after 150 min of pepsin digestion. However, cross-linked PV was more easily digested by trypsin, and the IgG binding capacity completely disappeared after trypsin digestion[63]. RBL-2H3 cell assays showed that, compared to native PV, the levels of β-hexosaminidase, histamine, IL-4, and IL-13 in cross-linked PV were reduced by 35.6%, 66.9%, 56.3%, and 37.5%, respectively[63]. RBL-2H3 cell assays also showed that cross-linked PV by Tyr and CA significantly inhibit the degranulation, and the secretion of IL-4 and IL-13[53]. TG-catalyzed glycosylation of TM alleviated allergic symptoms in sensitized mice, resulting in a decreased percent of IL-4$^+$ Th2 cells, an increased percent of IFN-γ^+ Th1 cells and Treg cells. Additionally, serum levels of IgE and IgG1 antibodies were decreased in the treated mice[64]. These studies indicate that enzymatic cross-linking of allergens like PV enhances resistance to pepsin digestion and reduces IgG binding, while also lowering the release of allergic mediators such as histamine and IL-4. Additionally, TG-catalyzed glycosylation of TM alleviates allergic symptoms by modulating immune responses and reducing IgE levels, making enzymatic modification a promising strategy for reducing fish allergies.

4.4 Milk

4.4.1 Effects of enzymolysis on the allergenicity of milk allergens

Milk serves as a crucial protein source for infants and young children. However, cow's milk is also among the most common allergenic foods responsible for food allergies in children. The α-lactalbumin (α-LA) and β-lactoglobulin (β-LG) are the key allergens in the whey fraction, comprising 5% and 10% of total milk proteins, respectively[65]. Milk treated with alkaline protease, protamex, and flavourzyme showed varying degrees of protein hydrolysis. SDS-PAGE revealed that flavourzyme left three protein bands, protamex left a low-intensity 9.0 kDa band, while alkaline protease fully hydrolyzed all proteins. Alkaline protease proved most effective, and all enzyme treatments significantly reduced the IgE binding capacity of milk proteins (casein and β-LG)[66]. Similarly, ELISA tests showed that the IgE binding capacity of milk treated with alcalase, protamex, flavourzyme, neutrase, and pepsin was significantly lower compared to untreated milk[67]. Whey protein treated with pepsin and papain showed the disappearance of β-LG bands on SDS-PAGE. The pepsin hydrolysate, filtered through a 10 kDa membrane, did not trigger immune responses[68]. Serine protease extracted from *Yarrowia lipolytica* yeast was used to treat whey protein and casein, and after 24 h of hydrolysis, the hydrolysis rates of whey protein concentrate (WPC)-80 and casein reached 34.2% and 31.1%, respectively. At this point, the IgE binding ability of the hydrolysates was also at its lowest[69]. After glycosylation treatment of β-LG with TG combined with glucosamine (GlcN), it was found that the structure of β-LG unfolded and aggregated, with a reduction in β-sheet and α-helix content, as well as an increase in intrinsic fluorescence and surface hydrophobicity. The glycosylated β-LG reduced IgE/IgG binding capacity by 56.3% and 52.7%, respectively[70]. Whey protein isolate was treated with a mixture of immobilized trypsin and chymotrypsin on agarose gel combined with glycosylation using 10 kDa dextran. SDS-PAGE showed that after enzymatic glycosylation, the band intensity of α-LA and β-LG decreased, and new low molecular weight bands were formed, indicating that α-LA and β-LG were hydrolyzed into small peptides. Compared to the untreated or non-glycosylated group, the specific IgE binding capacity was reduced by nearly 99% after enzymatic glycosylation[71]. Whey proteins were treated with TG combined with GlcN and oligochitosan, respectively. In the hydrolyzed products, the contents of α-helix and β-sheet were reduced, significantly decreasing the IgG binding capacity of β-LG and α-LA. Due to the larger molecular weight of oligochitosan, it had a more pronounced effect on decreasing the antigenicity of whey proteins compared to GlcN[72].

The combined application of other processing techniques with enzymatic hydrolysis can enhance the degradation of allergenic proteins in milk. β-LG heated in a water bath is resistant to pepsin, with 37% of β-LG remaining intact after heating. However, microwave heating increased the enzymatic hydrolysis of β-LG[73]. When high hydrostatic pressure (HHP) is combined with enzymatic hydrolysis, the DH increases with HHP changes, and α-LA and β-LG can be completely hydrolyzed at pressures exceeding 300 MPa. With longer treatment times (combined treatment), a large amount of free sulfhydryl groups is exposed, altering the structure of whey proteins, disrupting tertiary structures and allergenic epitopes, thereby changing their antigenic capacity. HP combined with bromelain reduced antigenicity compared to chymotrypsin[74]. Under normal pressure, β-LG can resist digestion by pepsin and usually requires 24 h for complete hydrolysis, whereas HP can accelerate the β-LG hydrolysis. β-LG was completely hydrolyzed after 30 min of pepsin treatment at 400 MPa. Feeding whey protein treated with HP and pepsin to whey protein-allergic mice did not cause changes in body temperature or allergic reactions[75]. β-LG exhibited resistance to pepsin digestion under standard pressure conditions; however, HP enhanced the hydrolysis of β-LG, leading to a reduction in its antigenicity[74]. Additionally, HP pretreatment (400 MPa, 37 °C, 30 min) combined with pepsin treatment (30 min) has been shown to achieve complete hydrolysis of β-LG, effectively mitigating allergic responses in mice[76]. Ultrasonic treatment combined with enzymes (alkaline protease + papain) can enhance the enzymatic hydrolysis of whey proteins, producing more small molecular

proteins (< 15 kDa)[77]. In conclusion, various enzymatic and processing treatments, including the use of proteases, glycosylation, HP, and ultrasonic methods, have been shown to effectively reduce the allergenicity of milk proteins, particularly β-LG and α-LA. These treatments degrade allergenic proteins, alter their structure, and reduce their IgE/IgG binding capacities. HP and glycosylation in combination with enzymatic hydrolysis, offer promising approaches to fully hydrolyze resistant milk proteins and significantly decrease antigenicity, making them valuable strategies for developing hypoallergenic milk products.

4.4.2 Alterations in allergenicity of enzymolysis-modified milk allergens during digestion in vitro and in vivo

Recent studies indicate that enzyme treatment can alleviate cow's milk-induced allergic reactions by enhancing the digestibility of milk, regulating antibody production and maintaining the Th1/Th2 immune balance. Milk hydrolysates alleviated allergic symptoms in milk protein-allergic mice and reduced total IgG1 levels in serum, with no significant differences between the alcalase, protamex, and flavourzyme treatment groups[67]. *In vitro* degranulation experiments using rat basophilic cells showed that the maximum degranulation value for hydrolyzed whey protein obtained by microwave treatment was 6.53%, compared to 45.97% for native whey protein isolate[73]. Alcalase-, protamex-, and flavourzyme-treated cow's milk reduced the allergic symptoms in mice and levels of total IgG1, as well as the allergic inflammation[67]. The percent of Th1 cells and the proportions of Foxp3$^+$ Tregs increased significantly[78]. Overall, enzyme treatments have shown promising potential in alleviating cow's milk-induced allergic reactions by improving milk digestibility, regulating antibody production, and maintaining Th1/Th2 immune balance. Studies in mice have demonstrated that milk hydrolysates can reduce allergic symptoms and serum IgE levels, while *in vitro* experiments have shown significant reductions in degranulation responses with hydrolyzed whey protein. However, research on the effectiveness of these treatments in digestion models remains limited, highlighting the need for further investigation in this area. The effects of enzymolysis on the allergenicity of allergic foods from animal products have summarized in Table 1.

Table 1 List of enzymolysis impacts the allergenicity of allergens in animal products.

Allergen	Enzymolysis conditions	Anti-allergic effects	Reference
OVA	Pepsin (172 U/mg), pH 1.5, 37 °C for 24 h; neutral protease (0.025 U/mg), pH 7.0, 50 °C for 1 h; alkaline protease (0.005 U/mg), pH 7.0, 50 °C for 1 h	Pepsin, neutral protease and alakline protease reduce IgE binding capacity by 90.1%, 70.0%, and 99.8%, respectively	[31]
	N-acetylglucosaminidase (0.25 U/mL), pH 6.0, 37 °C for 18 h	IgE binding capacity is reduced significantly	[42]
	PO (1 000 U/mL) and CA, pH 7.0, 50 °C for 8 h	IgE binding capacity is reduced significantly	[40]
	PO (1 000 U/mL) and CA in system ionic strength 50 mmol/L, pH 7.0, 50 °C for 8 h	IgE binding capacity is reduced by 38.6%	[32]
	FA (0.1–1 mmol/L) and Lac (0.024–0.200 U/mg), pH 6.5, 28 °C for 24 h	No significant effects on IgE binding capacity	[33]
LZM	Pepsin (172 U/mg), pH 1.5, 37 °C for 24 h; neutral protease (0.025 U/mg), pH 7.0, 50 °C for 1 h; alkaline protease (0.005 U/mg), pH 7.0, 50 °C for 1 h	Pepsin, neutral protease and alakline protease reduce IgE binding capacity by 53.9%, 99.3%, and 99.5%, respectively	[43]
	FA (0.1–1 mmol/L) and Lac (0.024–0.2 U/mg), pH 6.5, 28 °C for 24 h	IgE binding capacity is reduced by 51%	[33]
Ovomucin	Pepsin (172 U/mg), pH 1.5, 37 °C for 24 h; neutral protease (0.025 U/mg), pH 7.0, 50 °C for 1 h; alkaline protease (0.005 U/mg), pH 7.0, 50 °C for 1 h	Pepsin, neutral protease and alakline protease reduce IgE binding capacity by 92.5%, 99.5%, and 99.4%, respectively	[43]
AK	Heat aggregation (52 °C) for 30 min followed by TYR 37 °C for 8 h	IgE binding capacity is reduced by 99.5%	[55]
TM	Papain (3%, 5%), pH 9.0, 4 °C for 3–16 h; bromelain (3%, 5%), pH 9.0, 4 °C for 3–16 h; ficin (4%, 8%), pH 7.5, 4 °C for 3–16 h	Allergen is hydrolyzed by enzymes, but IgE binding capacity is not significantly reduced	[49]
	Papain (0.5%) for 30 min followed by high-pressure treatment (0.1 MPa, 121 °C) for 30 min	IgE binding capacity is not significantly reduced	[50]
	Pepsin (30 μg/mL), pH 2.0, 37 °C for 1 h; heat treatment (90 °C) for 30–120 min followed by pepsin (30 μg/mL), pH 2.0, 37°C for 1 h	IgE binding capacity is reduced by 57.9% and 46.6%, respectively	[61]
	Horseradish peroxidase, 37 °C for 1 h; glutaminase (1–100 U/mg), 37 °C	IgE binding capacity is reduced by 63.5% and 34.5%, respectively	[51]
	Glutaminase (1–100 U/mg), 37 °C	The allergenicity is reduced significantly	[48]
	Glutaminase (1 U/mg), 37 °C for 24 h; TYR (800 U/mL), 37 °C for 24 h	IgE binding capacity is reduced by 84.2% and 39.6%, respectively	[47]
	TYR and CA, 37 °C for 24 h	IgE binding capacity and IgG binding capacity is not significantly reduced	[54]
	Lac (200 U/g) and CA, pH 7.4, 37 °C for 12 h; glutaminase (200 U/g), pH 7.4, 37 °C for 12 h	IgE binding capacity and IgG binding capacity is significantly reduced	[52]
	TYR and CA, pH 8.0, 37 °C for 24 h	IgE binding capacity and IgG binding capacity reduced by 49.41% and 37.19%, respectively	[79]

Table 1 (*Continued*)

Allergen	Enzymolysis conditions	Anti-allergic effects	Reference
	Alcalase (10 000 U/g), 55 °C for 1 h; protamex (10 000 U/g), 50 °C for 2 h; flavourzyme (6 000 U/g), 50 °C for 1 h	IgE binding capacity is reduced significantly	[66]
	Serine protease extracted from *Yarrowia lipolytica* yeast (10%), pH 8.0, 37 °C	IgE binding capacity is significantly reduced	[69]
	Immobilized trypsin and chymotrypsin mixture agarose gel, pH 9.0, 50 °C for 1–4 h followed by dextran, pH 6.5, 62 °C for 24 h for glycosylation treatment	IgE binding capacity is reduced significantly	[71]
	Microwave heating (37–70 °C) for 0–30 min followed by pepsin treatment for some time	Maximum degranulation of rat basophilic cells is reduced by 39.44%	[73]
	Pepsin (3%), pH 3.0, 37 °C for 4 h followed by ultrafiltration (10 kDa) treatment; papain (5%), pH 6.2, 55 °C for 4 h followed by ultrafiltration (10 kDa) treatment	IgE binding capacity is significantly reduced	[68]
β-LG	Ultrasound-ionic liquid coupling (300 W) for 15 min followed by alkaline protease (1 000 U/g) and papain (1 000 U/g) reaction at optimal temperature	Antigenicity is reduced by 88.01% and 88.46%, respectively	[77]
	High pressure (100–400 MPa) with *α*-chymotrypsin (10%), pH 7.5, 37 °C; high pressure (100–400 MPa) with bromelain (10%), pH 6.5, 45 °C	The allergenicity is reduced significantly	[74]
	High pressure (400 MPa) with pepsin (172 U/mg), pH 1.5, 37 °C for 30 min	Allergic responses, histamine and mMCP-1 levels in the serum of allergic mice are significantly reduced	[45]
	TG (10 U/mg) and GlcN, pH 7.5, 37 °C for 6 h for glycosylation treatment	IgE binding capacity and IgG binding capacity reduced by 56.3% and 52.7%, respectively	[70]
	TG (10 U/mg) and GlcN, pH 7.5, 37 °C for 4 h	The allergenicity is reduced significantly	[72]
	Alcalase (10 000 U/g), 55 °C for 1 h; protamex (10 000 U/g), 50 °C for 2 h; flavourzyme (6 000 U/g), 50 °C for 1 h	IgG binding capacity reduced by 10.27% and 5.86% by alcalase and protamex, respectively, while increased by 7.75% by flavourzyme	[66]
	Serine protease extracted from *Yarrowia lipolytica* yeast (10%) pH 8.0, 37 °C	IgE binding capacity is significantly reduced	[69]
	Immobilized trypsin and chymotrypsin mixture agarose gel, pH 9.0, 50 °C for 1–4 h followed by dextran, pH 6.5, 62 °C for 24 h for glycosylation treatment	IgE binding capacity is reduced by 99%	[71]
α-LA	TG (10 U/mg) and GlcN, pH 7.5, 37 °C for 4 h	IgG binding capacity is reduced significantly	[72]
	Ultrasound-ionic liquid coupling (300 W) for 15 min followed by alkaline protease (1 000 U/g) and papain (1 000 U/g) reaction at optimal temperature for some time	IgG binding capacity is reduced by 82.82% and 81.87%, respectively	[77]
	High pressure (100–400 MPa) with *α*-chymotrypsin (10%), pH 7.5, 37 °C for some time; high pressure (100–400 MPa) with bromelain (10%), pH 6.5, 45 °C for some time	IgE binding capacity is reduced significantly	[74]
	Alcalase, protamex, flavourzyme, neutrase, pepsin (2 000–10 000 U/g), reaction for 2 h	IgG binding capacity is reduced by 38.36%, 34.22%, 44.07%, 31.27%, and 38.95%, respectively	[67]
Casein	Alcalase (10 000 U/g), 55 °C for 1 h; protamex (10 000 U/g), 50 °C for 2 h; flavourzyme (6 000 U/g), 50 °C for 1 h	IgE binding capacity is reduced significantly	[78]
	Pepsin (30 μg/mL), pH 2.0, 37 °C for 1 h; heat treatment (90 °C) for 30–120 min followed by pepsin (30 μg/mL), pH 2.0, 37 °C for 1 h	IgE binding capacity is reduced significantly	[60–61]
	Microwave (90 °C, 800 W) for 5 min followed by alkaline protease (3%), 55 °C for 2–10 min	IgG binding capacity is reduced by 93%	[58]
PV	TYR (600 U/g) and CA, pH 6.5, 37 °C for 12 h	IgG binding capacity is significantly reduced	[62]
	TYR (600 U/g) and CA, pH 6.5, 37 °C for 12 h	*β*-Hexosaminidase, histamine, IL-4, and IL-13 levels in RBL-2H3 cell are significantly reduced	[53]
	Lac (100 U/g) and PG, pH 7.4, 37 °C for 8 h	*β*-Hexosaminidase, histamine, IL-4, and IL-13 levels in RBL-2H3 cell are significantly reduced	[63]

5 Plant protein

5.1 Soybean

5.1.1 Effects of enzymolysis on the allergenicity of soybean allergens

Soybean is extensively utilized in various food products and is well-known as a source of high-quality proteins. However, as one of the eight major food allergens, the prevalence of soybean allergy is rising globally, affecting approximately 0.3% of the general population and significantly impacting patients' quality of life[80]. Different proteases have distinct hydrolytic specificities, and the type of enzyme and hydrolysis conditions used can lead to varying degrees of hydrolysis and reduction in antigenicity and allergenicity. SDS-PAGE analysis of soy protein isolate (SPI) hydrolyzed by

alkaline protease and pepsin showed the complete disappearance of medium molecular weight allergenic proteins (67–72, 63, 47 kDa), while small molecular weight bands (29–33, 22 kDa) remained. This indicates that the main allergens, β-conglycinin and glycinin, were effectively degraded[81]. In addition, after treatment with flavourzyme and neutral protease, the SPI hydrolysates showed an increase in smaller molecular weight bands (31, 26, 20, 15 kDa) compared to untreated SPI, and the IgG binding capacity of the hydrolysates was significantly reduced by 64.46%[82–83], suggesting that the epitopes of SPI were likely disrupted by enzymatic hydrolysis, thereby reducing the antigenicity of β-conglycinin. SPI treated with alkaline protease and TG showed reduced protein bands for β-conglycinin and glycinin, especially in the acidic subunits, indicating hydrolysis of allergens in SPI[84]. Enzymatic cross-linking altered the fluorescence intensity and caused a red shift in the UV absorption peak of the protein, indicating changes in the protein's tertiary structure. ELISA results showed that, compared to the untreated control (SPI), the IgE binding capacity after enzymatic cross-linking was reduced by 44%–71%[84]. Proteases like alkaline protease, pepsin, flavourzyme, and neutral protease play significant roles in breaking down allergenic proteins and altering their structures. However, while these treatments reduce allergenicity by 44%–71%, the reduction is still limited. To further enhance allergenicity reduction, enzyme treatments should be combined with other food processing technologies for more effective results.

The combination of enzymatic hydrolysis with other processing techniques has shown a stronger effect in reducing the allergenicity of soy. When SPI was treated with high pressure (50 °C, 15 min, 100–600 MPa) and flavourzyme, SDS-PAGE showed a significant reduction in the major soy allergen Gly m5, with an increase in low molecular weight bands. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) and ELISA confirmed the hydrolysis of Gly m5 into smaller fragments, resulting in a 99.5% reduction in IgG binding capacity[85]. Additionally, enzymatic hydrolysis further enhanced the effect of fermentation in altering the antigenic epitopes or conformations in soybean meal (SM), reducing its IgG binding capacity. SM was fermented with various microbes and treated with alkaline protease, showing strong protein degradation in SDS-PAGE, and high molecular weight protein bands (78 and 72 kDa) disappeared, medium molecular weight bands decreased, and low molecular weight bands (< 10 kDa) increased. ELISA revealed fermentation significantly reduced IgG binding of glycinin and β-conglycinin, with enzymatic treatment further reducing their binding[86]. Alkaline protease (18.3 kDa) extracted from *Bacillus subtilis* was used to hydrolyze β-conglycinin, and it was found that the IgG binding abiltiy of β-conglycinin was reduced, showing an inverse relationship between DH and IgG binding ability[87]. The combined use of heat and enzymatic treatments in tofu production reduces soy protein allergenicity. After heating soy milk and treating it with microbial transglutaminase (MTG), SDS-PAGE showed significant reduction or disappearance of allergenic protein bands (β-conglycinin and glycinin), though protein aggregation led to high molecular weight bands. Structural changes, including increased α-helix and β-sheet content, decreased random coil content, and increased UV absorption, further reduced the allergenicity of soy proteins in tofu[88]. All these studies indicate that combining enzymatic hydrolysis with other processing techniques significantly reduces soy protein allergenicity. For example, high-pressure and enzymatic treatments degrade major allergens like Gly m5, while fermentation with enzymatic hydrolysis further reduces IgG binding of glycinin and β-conglycinin in SM. Additionally, heat and enzymatic treatments in tofu production lower allergenicity by altering protein structures and reducing allergenic protein bands.

5.1.2 Alterations in allergenicity of enzymolysis-modified soybean allergens during digestion in vitro and in vivo

Bioinformatics analysis revealed that gastrointestinal-resistant fragments of the allergenic proteins β-conglycinin and glycinin in soyben retained linear epitopes in their primary structure, potentially triggering an immune response when exposed to the intestinal mucosa. Additionally, these resistant peptides showed structural homology with epitope sequences from other legume species, posing a potential risk of cross-reactions for a broader group of allergic individuals[89]. MTG cross-linked process promoted the gastrointestinal digestion of soymilk protein, and reduced the IgE-binding capacity of tofu digestion products[88]. It was found that compared to natural soy proteins, the tofu processing significantly decreased production of IL-4, β-hexosaminidase, and histamine in KU812 cells, and the IgE binding ability of the digestion products after *in vitro* gastrointestinal digestion of tofu was reduced by about 55%[88]. Animal experiments showed that tofu proteins regulated the Th1/Th2 balance in allergic mice. Compared to natural soy proteins, tofu protein feeding significantly reduced the levels of IL-4, IL-5, and IL-13 in the spleen cells of mice by 81.6%, 83.8%, and 100%, respectively, and significantly promoted the levels of IFN-γ and IL-10, along with an enhanced percent of $Foxp3^+$ Treg cells[90]. Similarly, soybean powder hydrolysates decreased allergy symptom scores and regulated the imbalance of Th1/Th2 cells in BALB/c mice, with decreasing specific serum IgE and IgG1 binding ability, and suppressed mast cell degranulation rate[91]. These findings indicate that tofu processing and soybean protein hydrolysates reduce allergenicity by regulating the immune response. Exposure to soybean protein hydrolysates induces a shift toward a Th1 response, lowering allergenicity and balancing the Th1/Th2 response. However, research on the effectiveness of these treatments in digestion models remains limited.

5.2 Peanuts

5.2.1 Effects of enzymolysis on the allergenicity of peanut allergens

The primary allergens in peanuts, including arachis hypogaea (Ara) h1, Ara h2, Ara h3, and Ara h6, are detectable in 90% of serum samples from allergic patients, with Ara h1 being the most prevalent among total peanut proteins[92]. Alkaline protease, pepsin, flavourzyme, and neutral protease are commonly used to hydrolyze peanut proteins, with flavourzyme achieving the highest DH (26.5%) after 60 min. SDS-PAGE showed large proteins were broken into small peptides (< 10 kDa), significantly reducing IgE binding, though Ara h2 remained more resistant to hydrolysis than Ara h1[93]. When peanut proteins were hydrolyzed with alkaline protease alone, the levels of Ara h1, Ara h2, and Ara h3 were reduced, along with a significant decrease in IgE binding capacity[94]. Alkaline protease (1%) effectively hydrolyzed Ara h1 and Ara h2, but had a weaker effect on Ara h6[95]. Treatment with flavourzyme for 30 min led to an increase in the IgE binding capacity of Ara h1, Ara h2, and Ara h3. After 30 min of hydrolysis, the content of Ara h1 decreased, while the bands for Ara h2 and Ara h3 disappeared, indicating that Ara h1 is resistant to flavourzyme hydrolysis[94]. Bromelain (0.04%)

effectively reduced the levels of Ara h1, Ara h2, and Ara h6 by 99.8%, 78.2%, and 42.4%, respectively. At a concentration of 0.16%, the levels of Ara h1, Ara h2, and Ara h6 were reduced by 100%, 82.4%, and 71.8%, respectively, and the IgE binding ability of the hydrolysates was significantly reduced[95]. This study also found that Ara h6 was more resistant to hydrolysis by bromelain, neutral protease, and papain[95]. Treatment with α-chymotrypsin and trypsin significantly degraded the peanut allergens Ara h1 and Ara h2, with Ara h1 being completely hydrolyzed and the content of Ara h2 reduced by 96.73%[96]. Overall, various proteases, including alkaline protease, pepsin, flavourzyme, bromelain, and α-chymotrypsin, have been shown to effectively hydrolyze peanut allergens, such as Ara h1, Ara h2, and Ara h3, significantly reducing their IgE binding capacity. While Ara h1 is generally more susceptible to enzymatic hydrolysis, Ara h2 and Ara h6 exhibit greater resistance, with Ara h6 being particularly challenging to degrade. These findings highlight the potential of enzyme treatments to reduce peanut allergenicity, though the effectiveness varies among specific peanut allergens.

The combination of other processing techniques with enzymatic hydrolysis can enhance the degradation of allergenic proteins. Boiling for 5 min improved α-chymotrypsin efficiency, with allergen degradation positively correlated with enzyme concentration. At high concentrations (0.15%), Ara h1 and Ara h2 were completely hydrolyzed in blanched peanut flour[96]. Boiling not only inactivates bacteria and other microorganisms but also loosens and softens the protein structure, promoting enzyme penetration[96]. Roasting peanuts is widely used in enzymatic hydrolysis studies. Following 3 h of treatment with a 3.5% alkaline protease solution, the Ara h2 content in raw peanuts was found to be higher than in roasted peanuts. This suggests that Ara h2 in raw peanuts is more resistant to alkaline protease hydrolysis, necessitating longer hydrolysis times or higher enzyme concentrations for complete elimination[97]. SDS-PAGE and WB analysis of raw peanuts showed that the Ara h1 band disappeared after alkaline protease treatment, and the intensity of the Ara h2 and Ara h6 bands was significantly reduced[97]. Roasted peanut flour was hydrolyzed with papain, ficin, and bromelain, followed by TG cross-linking. Ficin was most effective in reducing IgE binding, and increasing enzyme concentration further decreased IgE binding capacity. While TG enhanced protein hydrolysis, it had little impact on the IgE binding capacity of the hydrolysates[98]. SDS-PAGE analysis of defatted roasted peanut flour hydrolyzed with 3.5% alkaline protease showed the disappearance or weakening of larger proteins like Ara h1, Ara h2, and Ara h3 after 1 h, with an increase in 10–15 kDa bands. WB analysis confirmed that enzymatic hydrolysis significantly reduced the allergenicity of Ara h1, Ara h2, Ara h3, and Ara h6, though 5–15 kDa peptides retained some allergenicity[99]. Additionally, ultrasonic treatment (50 Hz) enhanced the degradation of Ara h2 by trypsin and chymotrypsin, although it had little effect on reducing Ara h1. The combination of ultrasonic treatment with trypsin was more effective than with chymotrypsin[100]. HP treatment (300 and 500 MPa) combined with PO significantly reduced Ara h1 and Ara h2 band intensity compared to PO alone, with greater pressure enhancing the effect. WB and ELISA tests confirmed that this combination significantly reduced the IgE binding capacity of Ara h1 and Ara h2, with the best results at higher pressures[101]. Thus, combining enzymatic hydrolysis with other processing techniques such as boiling, roasting, ultrasonic treatment, and HP treatment enhances the degradation of peanut allergens like Ara h1, Ara h2, and Ara h6. These methods improve enzyme efficiency, reduce allergenic protein content, and lower IgE binding capacity. However, the effectiveness varies depending on the allergen and the specific treatment used, with techniques like HP and ultrasonic treatment showing promise in further reducing peanut allergenicity.

5.2.2 Alterations in allergenicity of enzymolysis-modified peanut allergens during digestion in vitro and in vivo

Peanut allergens Ara h1 and Ara h3 were rapidly degraded by pepsin, while Ara h2 and Ara h6 showed resistance to pepsin digestion, likely due to their disulfide bridges maintaining structural integrity. Trypsin digestion of Ara h2 also resulted in large residual peptides, highlighting the digestion resistance of these particular allergens[102]. The digestion stability of major peanut allergens, assessed using an *in vitro* model simulating gastrointestinal digestion, revealed complete degradation of Ara h1. However, immunogenic fragments of Ara h2, Ara h6, and Ara h3 survived hydrolysis, with stable IgE-binding peptides primarily derived from Ara h3, as confirmed by MS/MS analysis[103]. *In vitro* simulated digestion models showed that the band intensity of roasted peanut digestion products was significantly weaker than that of raw peanuts, with only 21 kDa bands and 14 kDa or smaller bands remaining, indicating that roasted peanuts are more easily degraded by gastrointestinal enzymes. Testing with serum from peanut-allergic patients showed that the IgE binding ability of the digestive products from roasted peanuts was significantly decreased[104]. Roasted and boiled peanut proteins, including Ara h1 and Ara h3, were rapidly digested and barely detectable by gels or antibodies. However, digestion did not eliminate their allergenic potential, as Ara h3 isoforms were still identified after 120 min of digestion by mass spectrometry[105]. PO was used to modify Ara h2, and the apparent diameter increased after cross-linking, suggesting polymerization both between and within molecules. Cross-linked Ara h2 showed a significantly lower IgE binding capacity compared to untreated Ara h2, reducing its allergenicity. After gastric and intestinal digestion, the IgE binding capacity of cross-linked Ara h2 was further reduced by about 38%, and it degraded more effectively under simulated gastric digestion conditions[106]. Enzymatically cross-linked processing increased the bioavailability of the major allergen Ara h2 but did not significantly alter the allergenic or tolerizing properties of peanuts[107]. Recently, it is found that the allergic responses to peanuts in BALB/c mice was decreased by peanut hydrolysates, with decreased level of IgE and IgG, and low degranulation degree of mast cell and basophils[92]. These findings indicate that various processing and modification techniques, such as roasting, enzymatic hydrolysis, and cross-linking, have shown potential in reducing peanut allergenicity by enhancing protein breakdown and lowering immune responses. However, the number of studies investigating digestion models *in vivo* is still limited, indicating a need for further research to fully understand the effectiveness of these approaches in reducing allergenic responses.

5.3 Tree nuts

5.3.1 Effects of enzymolysis on the allergenicity of allergens in tree nuts

In Europe, peanut and tree nuts are among the leading causes of allergic reactions, and recent years have seen an increase in tree nut consumption[108]. After treatment with aspergillopepsin (an acidic protease), the band intensities of *Anacardium occidentale* allergens Ana o1 (50 kDa) and Ana o2 (20 and 32 kDa) in cashew extract were reduced by more than 95% compared to the natural cashew

extract, while the intensity and number of 10 kDa bands significantly increased. WB analysis showed that the IgE binding capacity of cashew protein was significantly reduced after enzyme treatment[109]. Additionally, when almond extract was heated (100 °C) and then treated with pepsin, its IgE binding ability was significantly reduced, and SDS-PAGE showed that all protein bands disappeared after the combined heat and enzyme treatment[110]. Pistachios and cashews subjected to natural, boiling, and high-temperature high-pressure treatments showed similar protein band patterns, but the band intensity in samples treated with high-temperature HP (138 °C, 256 kPa) was significantly reduced, with new bands appearing, indicating protein aggregation or degradation[111]. Enzyme treatment significantly reduced the large protein bands and IgE binding capacity in pistachios and cashews. However, mass spectrometry revealed that allergen fragments like pistachio allergens Pis v1, Pis v2, and Ana o3 remained, indicating strong stability[111]. Similar results were observed in tree nuts like hazelnut, pistachio, and cashew after processing with heat, pressure, and enzymatic digestion[108]. In conclusion, enzyme treatments, along with heat and pressure processing, have shown significant potential in reducing the allergenicity of tree nuts like cashews, almonds, and pistachios by decreasing protein band intensity and IgE binding capacity. However, some allergen fragments, such as Pis v1, Pis v2, and Ana o3, remain stable even after processing, indicating that while allergenicity is reduced, complete elimination of allergenic proteins remains a challenge. These findings suggest that combined processing techniques can be effective, but further optimization is needed to fully mitigate allergenic risks in tree nuts.

5.3.2 Alterations in allergenicity of enzymolysis-modified allergens in tree nuts during digestion in vitro and in vivo

Enzymatic extraction altered the sequential and conformational characteristics of almond proteins, disrupting allergen proteins and epitopes through proteolysis. This process reduced almond protein immunoreactivity by 75% and increased *in vitro* digestibility from 79.1% to 88.5%, while reducing IgE and IgG reactivities in human sera[112]. Almond and pine nut protein extracts treated with pepsin and pancreatin showed complete degradation of the almond allergen amandin, with SDS-PAGE and WB analyses confirming the elimination of its immunoreactivity (IgE and IgG binding capacity). However, after treatment with pancreatin, a 30 kDa band remained stable, and bands of 38–41 and 45–50 kDa still exhibited immunoreactivity[113]. After pepsin treatment, pine nut proteins displayed low molecular weight bands (10–15 kDa) that retained immunoreactivity, while pancreatin treatment left the 28 and 55 kDa proteins still immunoreactive. However, combined treatment with pepsin and pancreatin completely degraded the pine nut proteins, eliminating their immunoreactivity[113]. Cashew protein extract treated with 1% pepsin resulted in smaller protein bands (3–6 kDa). In a cashew allergy mouse model, the allergic response to the hydrolysate was significantly reduced, with no significant effect on IL-4, IL-13, IL-5, and IFN-γ levels secreted by splenocytes[114]. These studies suggest enzymatic extraction and digestion treatments, such as with pepsin and pancreatin, significantly alter the structural and immunoreactive properties of nut proteins like almond, pine nut, and cashew. These treatments reduce allergen immunoreactivity, as seen with the complete degradation of key allergenic proteins in almonds and pine nuts, and significantly reduced allergic responses in cashew models. The enzyme treatment can alleviate allergic reactions by balancing the Th1/Th2 immune response, although its effect on promoting the proportion of $CD4^+CD25^+$ Treg cells has not yet been reported.

5.4 Wheat

5.4.1 Effects of enzymolysis on the allergenicity of wheat allergens

Wheat has become an important part of the human diet as society has developed due to its nutrient-rich. However, wheat allergy is a significant global public health issue, not everyone can tolerate wheat products. Wheat is commonly processed for food or cosmetics through various physical, chemical, or biological methods, which can affect allergens in different ways, not always achieving the desired allergenicity reduction[115]. Enzymatic modification has been shown to improve wheat protein's solubility, gelation, water absorption, foaming, and thermoplasticity[116]. Enzymolysis reduces aggregated protein content, free thiol groups, and IgA immunodetection, particularly for high molecular weight subunits, and also effectively hydrolyzes gliadin and glutenin in wheat flour[117–118]. Wheat flour proteins range from 6.9 to 101 kDa in size, with many allergens falling within the 14–98 kDa range[119]. When wheat flour was treated with alkaline protease and papain, SDS-PAGE analysis showed that most wheat proteins were degraded into proteins smaller than 6.9 kDa, indicating significant degradation of wheat proteins by enzyme treatment. ELISA results showed that the IgE binding capacity of enzyme-treated wheat flour was significantly reduced compared to untreated wheat flour[120]. Crude flour was treated with pepsin, trypsin, and protease to develop biologically modified gluten flour, resulting in lower protein band intensity and allergen spectrum compared to the control group[120]. Enzymolysis with prolyl endopeptidase and TG also reduced the immunoreactivity of wheat proteins[121]. Gluten treated with alkaline protease and papain showed altered secondary and tertiary structures, with reduced β-sheet and β-turn content. Fluorescence intensity decreased, UV absorption increased, and SDS-PAGE revealed significantly weaker protein bands (28–55 kDa) and more low molecular weight bands compared to native gliadin[120]. These findings suggest that enzymatic modification plays a crucial role in reducing the allergenicity of wheat proteins by altering their structural and immunological properties. Treatments with enzymes such as alkaline protease, papain, pepsin, and trypsin have shown significant degradation of wheat proteins, particularly those in the allergenic range, and effectively reduce IgE binding capacity. These modifications also improve the functional properties of wheat proteins, making enzymolysis a promising approach for developing hypoallergenic wheat products while maintaining their nutritional value.

5.4.2 Alterations in allergenicity of enzymolysis-modified wheat allergens during digestion in vitro and in vivo

In vitro simulated digestion showed that the protein band intensity of native gliadin significantly decreased after 10 min to 2 h of gastric digestion, generating new small molecular fragments. During intestinal digestion, the band intensity of gliadin decreased significantly until it disappeared completely within 10 min to 2 h. Compared to native gliadin, enzyme-treated gliadin significantly reduced total IgE levels (by 60%–80%), gliadin-specific IgE levels (by 40%–50%), IFN-γ levels (by 40%–60%), IL-4 levels (by about 15%), and histamine levels (by 60%–70%) in the serum of gliadin-allergic mice, with alkaline protease treatment showing the most significant reduction. Additionally, enzyme-treated gliadin increased the levels of TGF-β and the transcription factor Foxp3 in

the spleen tissue of allergic mice, significantly reducing RORγt levels, indicating that enzyme treatment can alleviate allergic reactions in mice by regulating the balance between Th17 and Treg cells[122]. After 4 h of treatment with pepsin at 37 °C, wheat gliadin was converted to pepsin-digested gliadin (P-GLI), and after 4 h of treatment with trypsin at 37 °C and pH 8.0, it was deamidated at 80 °C for 3 h using 0.1 mol/L hydrochloric acid, resulting in HCl-peptic-tryptic gliadin (HPT-GLI). ELISA results showed that compared to native gliadin, the IgE binding capacity of HPT-GLI was reduced by about 60%, and P-GLI by about 50%. Animal experiments showed no significant differences in the levels of mMCP-1, histamine, IL-4, and IL-5 between native gliadin and P-GLI, although the IgE and IgG levels of P-GLI were slightly lower than those of native gliadin[123]. Since the interaction of food proteins with DCs is crucial for allergic sensitization, enzymatic hydrolysis of gliadins altered their physicochemical properties, including molecular weight, hydrophobicity, and peptide size. These changes enhanced protein uptake and intracellular degradation by DCs, leading to modified immune stimulation[124]. Although natural gluten and its hydrolysates can cause severe allergic symptoms[125], most studies suggest that enzyme treatment can alleviate allergic reactions by improving allergen digestibility in wheat and balancing the Th1/Th2 immune response. However, its effect on promoting $CD4^+CD25^+$ Treg cells has not yet been reported. The effects of enzymatic hydrolysis on the allergenicity of plant-based foods are summarized in Table 2.

Table 2 List of enzymolysis impacts the allergenicity of allergens in plant products.

Allergen	Enzymolysis conditions	Anti-allergic effects	Reference
β-Conglycinin	Alkaline protease (0.5%), pH 8.0, 50 °C for 120 min; papain (0.5%), pH 7.0, 80 °C for 120 min; pepsin (0.5%), pH 2.0, 50 °C for 120 min	IgE binding capacity is reduced significantly	[82]
	Alkaline protease extracted from *B. subtilis*, pH 8.5, 55 °C for 40 min	IgE binding capacity is reduced significantly	[86]
	Flavourzyme (1 500 U/g), pH 6.5, 60 °C for 15 min, followed by neutral protease (2 000 U/g), pH 7.0, 55 °C for 15 min	IgE binding capacity is reduced significantly	[81]
	Alkaline protease, pH 8.0, 50 °C hydrolyzed to different degrees (2.5%, 5.0%, 10.0%) followed by TG (5 or 10 U/g), 37 °C for 4 h	IgE binding capacity is reduced significantly	[83]
	Fermented separately by *Aspergillus oryzae*, *B. subtilis*, *Lactobacillus plantarum*, *L. casei*, and *B. licheniformis* for 24 h, then treated with alkaline protease (5%), pH 8.0, 50 °C for 15 min	IgG binding capacity reduced by 78.32%–81.38%	[85]
	Heat treatment (75 °C for 10 min, then 95 °C for 5 min) followed by MTG (5 U/mg), pH 6.0, 50 °C for 3 h	IL-4, β-HEX, and histamine production in KU812 cell are significantly reduced	[87]
	Heat treatment (75 °C for 10 min, then 95 °C for 5 min) followed by MTG (5 U/mg), pH 6.0, 50 °C for 3 h	Allergen-specific IgE, mMCP-1, and histamine levels in allergic mouse serum significantly reduced	[89]
	High-pressure treatment (100–600 MPa, 50 °C, 15 min) and flavourzyme (0.5%), pH 8.0, 50 °C for 15 min	IgG binding capacity reduced by 99.5%	[84]
Glycinin	Alkaline protease (0.5%), pH 8.0, 50 °C for 120 min; papain (0.5%), pH 7.0, 80 °C for 120 min; pepsin (0.5%), pH 2.0, 50 °C for 120 min	IgE binding capacity is reduced significantly	[82]
	Alkaline protease, pH 8.0, 50 °C hydrolyzed to different degrees (2.5%, 5.0%, 10.0%) followed by TG (5 or 10 U/g), 37 °C for 4 h	IgE binding capacity is reduced by 44%–71%	[83]
	Fermented separately by *A. oryzae*, *B. subtilis*, *L. plantarum*, *L. casei*, and *B. licheniformis* for 24 h, then treated with alkaline protease (5%), pH 8.0, 50 °C for 15 min	IgG binding capacity is reduced by 86.29%–96.40%	[85]
	Heat treatment (75 °C for 5 min, then 95 °C for 10 min) followed by MTG (5 U/mg), pH 6.0, 50 °C for 3 h	Specific IgE, mMCP-1, and histamine levels in splenocytes are significantly reduced	[89]
	Heat treatment (75 °C for 5 min, then 95 °C for 10 min) followed by MTG (5 U/mg), pH 6.0, 50 °C for 3 h	IL-4, β-HEX, and histamine production in KU812 cell are significantly reduced	[87]
Ara h1	Alkaline protease (3.5%), pH 8.0, 40 °C	Allergen content and IgE binding capacity significantly are reduced	[96]
	Alkaline protease (1%–3.5%), pH 6.77, 50 °C for 2 h; papain (0.1%–0.5%), pH 6.77, 48 °C for 2 h; neutral protease (0.2%–1.6%), pH 6.77, 50 °C for 2 h; bromelain (0.02%–0.2%), pH 6.77, 45 °C for 2 h	Allergen content is reduced by 93%–100%, IgE binding capacity is significantly reduced	[94]
	Papain (0–400 AzU/g), 50 °C for 2 h, followed by TG (5 U/g), 37 °C crosslinking for 2 h; ficin (0–400 AzU/g), 50 °C for 2 h, followed by TG (5 U/g), 37 °C crosslinking for 2 h; bromelain (0–400 AzU/g), 50 °C for 2 h, followed by TG (5 U/g), 37 °C crosslinking for 2 h	Allergen content and IgE binding capacity are significantly reduced	[97]
	Alkaline protease (3.5%), pH 8.0, 40 °C for 3 h	Allergen content and IgE binding capacity are significantly reduced	[96]

Table 2 (*Continued*)

Allergen	Enzymolysis conditions	Anti-allergic effects	Reference
Ara h2	PO (1 600 U/mL), pH 7.0 for 80 min	IgE binding capacity is reduced significantly	[105]
	Alkaline protease (1%–3.5%), pH 6.77, 50 °C for 2 h; papain (0.1%–0.5%), pH 6.77, 48 °C for 2 h; neutral protease (0.2%–1.6%), pH 6.77, 50 °C for 2 h; bromelain (0.02%–0.2%), pH 6.77, 45 °C for 2 h	Allergen content is reduced by 53%–100%, IgE binding capacity is significantly reduced	[94]
	Papain (0–400 AzU/g), 50 °C for 2 h, followed by TG (5 U/g), 37 °C crosslinking for 2 h; ficin (0–400 AzU/g), 50 °C for 2 h, followed by TG (5 U/g), 37 °C crosslinking for 2 h; bromelain (0–400 AzU/g), 50 °C for 2 h, followed by TG (5 U/g), 37 °C crosslinking for 2 h	Allergen content and IgE binding capacity are significantly reduced	[97]
	Alkaline protease (3.5%), pH 8.0, 40 °C for 3 h	Allergen content and IgE binding capacity are significantly reduced	[96]
Ara h6	Alkaline protease (1%–3.5%), pH 6.77, 50 °C for 2 h; papain (0.1%–0.5%), pH 6.77, 48 °C for 2 h; neutral protease (0.2%–1.6%), pH 6.77, 50 °C for 2 h; bromelain (0.02%–0.2%), pH 6.77, 45 °C for 2 h	Allergen content is reduced by 2%–99%, IgE binding capacity is significantly reduced	[94]
	Alkaline protease (3.5%), pH 8.0, 40 °C	Allergen content and IgE binding capacity of Ara h1, Ara h2, Ara h3, and Ara h6 are significantly reduced	[96]
Peanut protein	Heat treatment (150 °C for 15 min) followed by *in vitro* digestion, finally small intestine brush border membrane enzyme (1.02 mU/μL), pH 7.2, 37 °C for 4 h	IgE binding capacity is reduced significantly	[103]
	2% and 10% flavourzyme + 5% alkaline protease, 60 and 55 °C, 80 and 60 min	Mouse allergic responses, the degranulation degree of mast cell and basophils, the secretion of specific antibodies (IgE and IgG), and the binding ability of peanut protein with peanut-specific IgE antibodies are decreased	[91]
	Pepsin (3 000 U/mg), pH 2.0, 37 °C for 30 min	Allergic symptoms in mice are significantly reduced	[113]
	Aspergillopepsin (76S APU/mL), pH 1.2, 37 °C	IgE binding capacity is reduced significantly	[109]
	Boiling (100 °C) for 30 or 60 min followed by ultrasound (40 kHz), protease P "Amano" 3SD (1 mg/mL), pH 7.0, 55 °C for 2 h; high pressure (121 °C, 120 kPa) for 15 or 30 min followed by ultrasound (40 kHz), protease P "Amano" 3SD (1 mg/mL), pH 7.0, 55 °C for 2 h; high pressure (138 °C, 256 kPa) for 15 or 30 min followed by ultrasound (40 kHz), protease P "Amano" 3SD (1 mg/mL), pH 7.0, 55 °C for 2 h	IgE binding capacity is reduced by 19.8%–73.4%	[111]
Cashew protein	Boiling (100 °C) for 30 or 60 min followed by ultrasound (40 kHz), protease P "Amano" 3SD (1 mg/mL), pH 7.0, 55 °C for 2 h; high pressure (121 °C, 120 kPa) for 15 or 30 min followed by ultrasound (40 kHz), protease P "Amano" 3SD (1 mg/mL), pH 7.0, 55 °C for 2 h; high pressure (138 °C, 256 kPa) for 15 or 30 min followed by ultrasound (40 kHz), protease P "Amano" 3SD (1 mg/mL), pH 7.0, 55 °C for 2 h	IgE binding capacity of allergens 7S globulin (Ana o1), 2S albumin (Ana o3), PR-10 (Bet v1 homolog), and lipid transfer protein (LTP) (Cor a8) are reduced significantly	[108]
	Pepsin (2 546 U/mL), pH 1.2, 37 °C	IgE binding capacity is reduced by 91%	[110]
	Boiling (100 °C) for 30 or 60 min followed by ultrasound (40 kHz), protease P "Amano" 3SD (1 mg/mL), pH 7.0, 55 °C for 2 h; high pressure (121 °C, 120 kPa) for 15 or 30 min followed by ultrasound (40 kHz), protease P "Amano" 3SD (1 mg/mL), pH 7.0, 55 °C for 2 h; high pressure (138 °C, 256 kPa) for 15 or 30 min followed by ultrasound (40 kHz), protease P "Amano" 3SD (1 mg/mL), pH 7.0, 55 °C for 2 h	IgE binding capacity is reduced by 19.2%–25.7%	[111]
Pistachio protein	Boiling (100 °C) for 30 or 60 min followed by ultrasound (40 kHz), protease P "Amano" 3SD (1 mg/mL), pH 7.0, 55 °C for 2 h; high pressure (121 °C, 120 kPa) for 15 or 30 min followed by ultrasound (40 kHz), protease P "Amano" 3SD (1 mg/mL), pH 7.0, 55 °C for 2 h; high pressure (138 °C, 256 kPa) for 15 or 30 min followed by ultrasound (40 kHz), protease P "Amano" 3SD (1 mg/mL), pH 7.0, 55 °C for 2 h	IgE binding capacity of allergens 7S globulin (Ana o1), 2S albumin (Ana o3), PR-10 (Bet v1 homolog), and LTP (Cor a8) are reduced significantly	[108]
Hazelnuts protein	Alkaline protease (200 U/mg), pH 9.0, 65 °C for 1 h; papain (200 U/mg), pH 7.0, 65 °C for 1 h	Total IgE, IFN-γ, IL-4, and histamine in allergic mouse serum are significantly reduced	[122]
Wheat protein	0.06% alkaline protease, pH 8.0, 53 °C for 62 min, followed by 0.07% papain, pH 7.0, 48 °C for 32 min	IgE binding capacity is reduced by approximately 75%	[120]
	Pepsin (1%), 37 °C for 4 h, followed by trypsin (1%), pH 8.0, 37 °C for 4 h	IgE binding capacity is reduced by 50%–60%	[123]

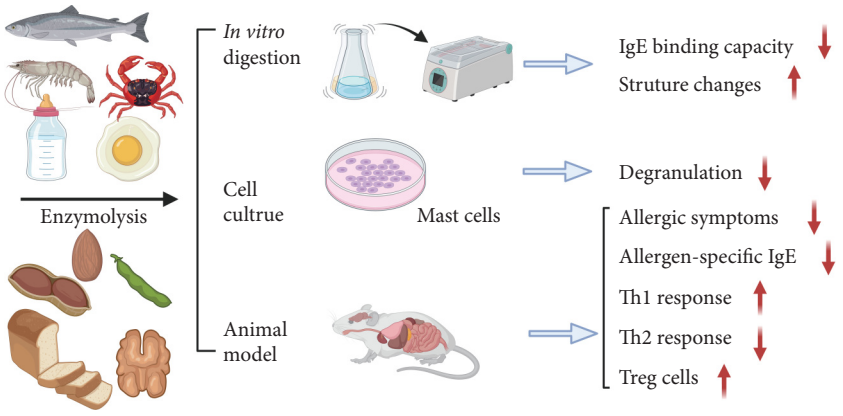

Figure 3 The effects of enzymolysis on the digestibility and allergenicity of allergic foods.

6 The application of enzymolysis in food products

IgE-mediated food allergy, the most common type (5%–10% prevalence), causes symptoms ranging from mild itching, stomach pain, and rash to severe anaphylaxis, and is diagnosed by the presence of specific IgE antibodies to suspected allergen[126]. In addition to providing symptom relief, hydrolysates may play a proactive role in modulating the immune system by engaging in various mechanisms. These mechanisms could include altering immune cell responses, reducing inflammation, and promoting the development of immune tolerance. Through these processes, enzymolysis (such as hydrolysates) may not only alleviate symptoms but also contribute to the long-term management of allergic reactions or immune-related conditions. This immunomodulatory potential highlights their value beyond simple symptom management, suggesting a broader therapeutic role in immune system regulation[127]. The effects of enzymolysis on the allergenicity of allergic foods from plant or animal products have been summarized in Figure 3.

Among enzymolysis protein products, infant formula with hydrolyzed protein is the most popular and important, designed to promote digestion and reduce the risk of milk allergies in babies[65]. The use of hydrolyzed protein, which avoids intact allergenic peptides, is hypothesized to lower the risk of allergic sensitization and conditions such as cow's milk allergy and atopic eczema. reastmilk substitutes like infant formula are widely used in Europe but carry health risks compared to breastmilk. To reduce these risks, manufacturers often replace intact cow's milk protein with hydrolyzed protein in formula[65,128]. Hydrolyzed formula has been recommended for allergy prevention for around 30 years. Recent reviews and meta-analyses have evaluated the safety and efficacy of various hydrolyzed formulas in treating cow's milk allergy, but the results remain controversial due to differences in the sample populations of children and varying treatment durations[127,129]. Numerous studies have shown that infants fed hydrolyzed formula have a lower risk of developing allergies compared to those fed non-hydrolyzed formula[130–131]. Extensively hydrolyzed formula (eHF) plays a well-established role in managing formula-fed infants and young children with gastrointestinal conditions such as malabsorption[129]. Current guidelines recommend cow's milk protein-based eHF, from whey or casein, as the first choice for treating cow's milk allergy, with amino acid formulas reserved for more severe cases. Rice hydrolyzed formulas (rHF) and soy hydrolyzed formulas (sHF) are also available, with both eHF and rHF well tolerated by most children with cow's milk allergies, without growth or adverse effect concerns[127,132]. In clinical practice, the choice of hypoallergenic formula should consider the infant's age, symptom severity, frequency, persistence, immune phenotype, growth pattern, formula cost, and proven tolerance and efficacy[127].

Hydrolyzed protein is a versatile ingredient used in various food products, from infant formula to sports supplements, offering benefits like improved digestibility, reduced allergenicity, enhanced flavor, and increased solubility. It is found in medical nutrition, sports products for muscle recovery, and as a flavor enhancer in seasonings. With rising demand for easily digestible, hypoallergenic proteins, hydrolyzed protein is becoming more common in the food industry.

7 Conclusion

Food allergies represent a significant global health concern. Enzymatic hydrolysis holds considerable promise for the industrial production of hypoallergenic, high-quality hydrolyzed proteins. This paper assesses the effectiveness of enzymatic hydrolysis in reducing the allergenicity of food allergens, and the effects of enzymolysis on the digestibility and allergenicity of allergic foods from plant or animal products have been summarized. Current research primarily employs highly specific digestive enzymes, which may promote the reduction in allergenicity, as indicated by decreased IgE/IgG-binding capacity. However, the underlying mechanisms influencing allergenicity require further investigation in animal or cell models, as well as in human studies.

Author contributions

Jing Yang: Conceptualization, supervision, funding acquisition, writing-review & editing. **Shuling Zhou:** Methodology, investigation, writing-original draft. **Yan Chen:** Investigation, writing-original draft. **Jiajia Song:** Writing-review & editing. **Jiawang Jin:** Writing-original draft. **Ruiping Gao:** Funding acquisition.

Conflict of interests

The authors declare that they have no financial conflicts of interest that could be perceived as influencing the research presented in this paper.

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