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The impact of processing on food allergens: a molecular dynamics perspective and research outlook

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Abstract

Food allergies are a significant global public health concern, affecting an estimated 3–8% of the population in Western nations. Although the structural and immunological basis of food allergens is increasingly well understood, the mechanisms by which processing modifies their allergenicity remain largely unresolved. This narrative review synthesizes current evidence on the effects of thermal and nonthermal processing treatments, such as high hydrostatic pressure, enzymatic hydrolysis, digestion, and chemical modification, on the structure and immunoglobulin E (IgE)-binding ability of food allergens. Fish allergens, primarily parvalbumin, were used as the primary case study throughout, given their high thermal stability, cross-reactivity, and the availability of molecular dynamics (MD) data. The review also examines how MD simulations have contributed to understanding these processing effects at the atomic scale, including conformational changes, epitope exposure, and digestibility under thermal stress. The synthesized evidence shows that, while processing can reduce allergenicity by disturbing epitopes or improving digestibility, it can also have the opposite effect by unmasking hidden epitopes or generating new ones, depending on the protein identity, processing conditions, and food matrix. A major gap identified is the limited application of long-term MD simulations under relevant stress conditions, which affects the interpretative value of existing studies. Combining MD simulation results with experimental validation offers a promising path for developing processing strategies for safer food products.

Keywords

cross-reactivity, epitope stability, fish allergy, food processing, IgE-binding, molecular dynamics simulation, parvalbumin, thermal treatment

Introduction

Allergens are substances that trigger undesirable immune reactions in sensitized individuals. While generally harmless to most people, they can provoke severe responses in those with hypersensitivity. In the

context of food allergy, the reaction is predominantly immunoglobulin E (IgE)-mediated: upon initial sensitization, allergen-specific IgE antibodies are produced and bind to high-affinity receptors on mast cells and basophils. Subsequent exposure triggers degranulation and the release of histamine and other mediators, resulting in symptoms ranging from mild discomfort to life-threatening anaphylaxis [1, 2].

Allergens are broadly categorized by their route of entry: food allergens (milk, eggs, peanuts, tree nuts, fish, shellfish, soy, and wheat), inhalant allergens (pollen, dust mites, and animal dander), contact allergens (nickel and certain preservatives), and injected allergens (insect venoms and medications) [1, 3–5]. Food allergens have received the most research attention because of their global prevalence and the severity of reactions they can provoke. Approximately 90% of food allergies are attributed to a defined group of proteins that share common structural traits, such as resistance to heat and digestion, compact folding, and the presence of sequential and conformational IgE-binding epitopes [1].

The allergenicity of a protein is governed by both intrinsic factors, including its three-dimensional structure, disulfide bond content, glycosylation, and resistance to proteolysis, and extrinsic factors, such as the host's genetic background, gut microbiota composition, and epithelial barrier integrity [6, 7]. Conformational stability is particularly relevant: proteins with more stable tertiary structures tend to resist denaturation and maintain their IgE-binding epitopes under physiological and processing conditions [8, 9]. In contrast, processing-induced structural changes can disrupt these epitopes, reduce allergenicity, or expose previously hidden regions, potentially increasing allergenicity.

In this review, special emphasis is placed on fish allergens—particularly parvalbumin—given the severity of fish allergy reactions, the exceptional thermal stability of parvalbumin across species, its well-characterized cross-reactivity, and the availability of molecular dynamics (MD) simulation data that make it a strong model system for computational allergen research. While the structural and allergenic properties of parvalbumin are well characterized in the experimental literature, the application of MD simulations to study processing-induced structural changes in fish allergens remains limited, making them a particularly timely and tractable system for computational investigation. Understanding how different processing treatments modify the structure and immunogenicity of fish allergens, and how MD simulations can inform and predict these changes, is the central aim of this review.

The remainder of this article is organized as follows: Food allergens covers major food allergens, relevant regulatory frameworks, and an overview of fish allergens. Overview of food processing treatments and their effect on allergenicity examines thermal and nonthermal processing treatments and their effects on allergenicity. MD simulations in allergen research discusses the MD simulation methodology and its application to allergenic proteins, including current limitations and future directions.

Literature search and methodology

This narrative review summarizes the current literature on the effects of food processing on allergen structure and immunogenicity, with a focus on MD simulation as an analytical tool. Literature searches were conducted using Scopus, PubMed, Web of Science, and Google Scholar, using search terms including "food allergens", "food processing", "allergenicity", "molecular dynamics simulation", "parvalbumin", "tropomyosin", "IgE-binding epitopes", and combinations thereof. Articles were selected based on relevance to the core topics of the review, with a preference for peer-reviewed studies published in the last decade, while important older works were included where appropriate. Non-English publications and grey literature were excluded. Studies were prioritized based on methodological rigor, direct relevance to processing-induced structural changes and IgE-binding outcomes, and experimental validation of findings. For MD simulation studies specifically, selection was guided by the availability of allergen-specific findings and whether results were validated against experimental data. Studies focused solely on food quality or sensory outcomes without allergenicity data, and those lacking sufficient methodological detail, were excluded.

Food allergens

Although this review focuses on protein-based food allergens, it is worth noting that other compounds can trigger adverse immune or toxic reactions in sensitive individuals. For instance, mycotoxins are fungal metabolites that can contaminate food and feed and have been associated with immunomodulatory effects, although they operate through different mechanisms than IgE-mediated allergy; therefore, they are outside the scope of this review. Similarly, non-protein allergens, such as certain food additives and haptens, exist but represent a comparatively minor contributors to the global food allergy burden. The present review is focused on protein allergens, in which the link between processing technology and MD simulation is currently best described in the literature and of clear clinical interest.

Typical food allergens consist of various proteins that provoke immune reactions in sensitive individuals. These allergens are generally noted for their structural stability and resilience to breakdown under physiological and processing conditions in the food industry. Primary food allergens are mainly water-soluble glycoproteins with molecular weights between 10,000 and 70,000 Da (Daltons). The molecular weights of some allergens are listed in Table 1, which was based on Bøgh and Madsen [10]. Their ability to withstand heat, acid, and proteolytic enzymes greatly enhances their potential to cause allergies [1, 2]. This stability enables them to maintain their characteristics despite the use of different food-processing techniques. Proteins found in common allergenic foods, such as milk, eggs, peanuts, tree nuts, fish, shellfish, soy, and wheat, have been extensively investigated.

Table 1. Molecular weight of some allergenic proteins of plant and animal origin.

Source	Allergen	Size (kDa)	Epitope type
Peanut	Ara h 1 (7S globulin)	64	Both
	Ara h 2 (2S albumin)	17	Both
	Ara h 3 (11S globulin)	60	Linear
	Ara h 6 (2S albumin)	15	Both
Soy	Gly m 1 (hydrophobic protein)	7	Conformational
	Gly m 5 (7S globulin) α-subunit	67	Both
	Gly m 5 (7S globulin) β-subunit	48	Both
	Gly m 6 (11S globulin)	60	Linear
	STI (Trypsin-inhibitor)	19	Conformational
Black gram	Uncharacterized protein	28	Not characterized
Hazelnut	Cor a 1 (PR-10)	17.5	Conformational
Brazil nut	Ber e 1 (2S albumin)	9 + 4	Conformational
Sesame seed	Ses i 1 (2S albumin)	9 + 4	Conformational
Sunflower seed	SFA-8 (2S albumin)	12.5	Conformational
Rice	RAP (trypsin inhibitor)	~16	Not characterized
Yellow mustard	Sin a 1 (2S albumin)	9 + 4	Conformational
Oriental mustard	Bra j 1 (2S albumin)	9 + 4	Conformational
Potato	Sola t 1 (Patatin)	43	Conformational
Celery	Api g 1 (PR-10)	15	Conformational
	Api g 2 (ns-LTP)	10	Conformational
Peach	Pru p 3 (ns-LTP)	10	Conformational
Grape	Vit v 1 (ns-LTP)	9	Conformational
Cherry	Pru av 1 (PR-10)	9	Conformational
	Pru av 3 (ns-LTP)	10	Conformational
	Pru av 4 (profilin)	15	Conformational
Apple	Mal d 1 (PR-10)	17.7	Conformational
	Mal d 2 (TLP)	31	Conformational
Melon	Cuc m 2 (profilin)	14	Conformational

Table 1. Molecular weight of some allergenic proteins of plant and animal origin. (*continued*)

Source	Allergen	Size (kDa)	Epitope type
Kiwi	Act c 1 (papain)	27.4	Conformational
	Act c 2 (TLP)	24	Conformational
Avocado	Pers a 1 (chitinase)	32	Conformational
Cow's milk	Bos d 4 (ALA)	14.2	Both
	Bos d 5 (BLG)	18.3	Both
	Bos d 6 (BSA)	37	Conformational
	Bos d 7 (IgG)	160	Not characterized
	Bos d 8 (β-casein)	24	Linear
Hen's egg	Gal d 1 (OVM)	28	Linear
	Gal d 2 (OVA)	44	Both
	Gal d 3 (OT)	78	Conformational
	Gal d 4 (HEL)	14	Both
Fish	Parvalbumin, cod	12	Conformational
	Lep w 1, whiff parvalbumin	12	Conformational
Shrimp	Pen a 1, tropomyosin	36	Both
	Lit v 1, tropomyosin	36	Both
Prawn	Pen m 1, tropomyosin	38	Both
Crab	Tropomyosin	34	Both

Linear: sequential amino acid epitopes independent of protein folding; conformational: discontinuous epitopes dependent on three-dimensional protein structure; both: IgE-binding to both epitope types reported in the literature. IgE: immunoglobulin E; IgG: immunoglobulin G; HEL: Hen Egg Lysozyme.

The wide range of molecular weights and structural families represented in Table 1 reflects the diversity of food allergens, although most share the common trait of resistance to heat and enzymatic digestion, which underlies their allergenicity.

For example, cow's milk is one of the first foreign proteins encountered by infants and is among the most common food allergens. Its capacity to cause allergic reactions is linked to its physicochemical characteristics and the presence of glycoproteins that are resistant to enzymatic breakdown [1]. Similarly, fish allergens, such as parvalbumins, display distinct structural characteristics that enhance their immunogenicity. β-Parvalbumins from various fish species demonstrate notable IgE cross-reactivity because of their similar surface structures, whereas α-parvalbumins typically do not show this cross-reactivity. This differentiation highlights the importance of protein folding and surface preservation in the recognition of allergens by the immune system [11, 12]. Digestion is essential for determining whether a protein functions as an allergen. Typically, dietary proteins that do not provoke an immune response are decomposed into amino acids during digestion. However, in certain individuals, undigested or partially digested proteins may be identified as foreign substances by the immune system. This recognition triggers an IgE-mediated reaction intended to counteract perceived danger [13]. The resilience of allergenic proteins during pepsin digestion is a key area of study. Research employing pepsin digestion tests has consistently shown that significant food allergens are more resistant to pepsin than non-allergenic proteins. However, changes in assay conditions, including enzyme concentration, pH levels, and detection techniques, may affect these results [14].

Fish allergens serve as prime examples of how structural integrity influences allergenicity. Tropomyosin and parvalbumin are the two main fish muscle proteins linked to allergic responses. Although most fish muscle proteins break down when exposed to heat during cooking methods, such as boiling or frying, tropomyosin and parvalbumin exhibit thermal stability, and the impact of heat treatment on these two proteins is not fully understood [12]. MD simulations are important tools for examining structural alterations at the atomic scale under various environmental conditions. Recent studies have emphasized the ability of food-processing methods to influence allergenicity. Methods such as germination or the application of strong electric fields can alter protein structure by modifying secondary and tertiary

structures or interfering with electrostatic interactions. These alterations may reduce allergenicity by encouraging protein unfolding or aggregation [15].

Research on less frequently allergenic foods has provided extensive insights into the mechanisms related to protein stability and immunogenicity. Allergens from fruits and vegetables have been examined for their ability to withstand pepsin digestion. Although they typically show lower stability than prominent food allergens, such as milk or peanuts, their performance under digestive conditions requires further investigation [14].

In conclusion, prevalent food allergens possess various key traits that enhance their immunogenicity, such as structural stability in physiological environments, resilience to enzymatic breakdown during digestion, and unique physicochemical characteristics that aid in the detection of IgE antibodies.

Regulations

Regulatory frameworks for food allergen management vary considerably across jurisdictions, reflecting differences in food culture, scientific priorities, and enforcement capacity. All major frameworks share a common foundation—mandatory labeling of priority allergens—but differ in their scope and classification systems. The European Union's Food Information to Consumers Regulation (EU FIC) mandates the labeling of 14 allergen groups, including celery, mustard, lupin, and mollusks. In the United States, the FALCPA (Food Allergen Labeling and Consumer Protection Act) covers nine major allergens, including the recently added sesame [16]. Canada similarly mandates explicit labeling of priority allergens, enforced by the Canadian Food Inspection Agency (CFIA), while Japan distinguishes between "required" allergens (wheat, buckwheat, eggs, dairy, peanuts, shrimp/prawns, and crabs) and "suggested" allergens for labeling. Allergen risk thresholds—the maximum quantities that can be present without triggering reactions in most sensitized individuals—remain inconsistent across jurisdictions, complicating international trade and food safety assurance [17–20].

A persistent challenge across all regulatory systems is the lack of globally harmonized reference doses, which forces manufacturers to rely on precautionary labeling ("may contain") as a risk management tool. This approach, while common, lacks scientific precision and varies in its interpretation [19, 20]. Controlled provocation testing in allergic individuals, rather than animal models, remains the gold standard for establishing threshold values; however, such data are limited for many allergens [19].

In this review, a critical regulatory gap is the absence of frameworks that account for processing-induced changes in allergenicity. Current labeling requirements are based on the presence of the original allergenic protein, not on its immunological activity after processing. MD simulations and experimental processing studies, as reviewed in Overview of food processing treatments and their effect on allergenicity and MD simulations in allergen research, demonstrate that processing can substantially alter IgE-binding capacity; however, these changes are not currently captured by any regulatory system. Developing processing-validated allergenicity assessments is a key priority for translating research findings into regulatory practice.

Overview of fish allergens

Although fish is a significant food allergen, the global supply and consumption of fish have increased dramatically in recent decades. More than 70% of fish undergo processing before being sold in the marketplace [21].

Similar to other IgE-mediated food allergies, fish allergies stem from epitopes that are part of large proteins. The immune system can identify these amino acid structures and attach them to specific IgE antibodies, leading to different allergic reactions [22]. Numerous studies have indicated that various processing methods may alter or eliminate the epitopes present in fish and other significant food allergens. The ability of epitopes to bind IgE antibodies can be diminished in various ways owing to several physical, chemical, or biological alterations during processing [23]. Nevertheless, certain processing methods may lead to alterations in epitope structure and neoepitope formation [24]. Processes such as hydrolysis and

glycation may lower allergenicity; however, they can also generate neoepitopes or reveal existing epitopes, thus increasing allergenicity [9, 25]. The effects of processing are influenced by specific epitopes. Conformational epitopes tend to be more unstable than linear epitopes [24]. Consequently, as researchers have indicated considerable variability in the allergenic properties of products, it is crucial to understand the various processing techniques and their impacts on different fish and crustacean allergens.

Fish allergy is an immune reaction mediated by IgE against several fish proteins, including oral allergy syndrome, angioedema, rhinitis, diarrhea, vomiting, nausea, abdominal discomfort, urticaria, asthma, and possibly serious anaphylaxis [26, 27]. Parvalbumins are fish allergens known for their high cross-reactivity, low molecular weight, and stability under heat [27, 28]. In many fish varieties, β-parvalbumin is the main allergen, whereas α-parvalbumin is less allergenic [26]. Although the structures of various fish parvalbumin types are similar, distinct protein allergen components vary by 55–95% among different fish species [11].

Allergenic proteins play a crucial role in determining food preferences. Research indicates that the incidence of food allergies has increased over the past few decades [29]. Among the different food categories, fish, crustacean shellfish, cow's milk, hen's egg, peanut, soybean, tree nut, and wheat have been identified as the "big eight" allergens that cause more than 90% of food allergies [30]. Following the enactment of the FASTER Act, the FDA (Food and Drug Administration) formally introduced sesame as the ninth major food allergen in the United States in 2023, expanding this list as the 'big nine' [29].

Fish plays a vital role in contemporary dietary practices. Fish are rich in nutrients such as omega-3 and omega-6 fatty acids and fat-soluble vitamins, which provide numerous health benefits, including reducing the risk of heart disease and stroke [26, 30, 31]. Fish, considered a significant food allergen, shows an allergy prevalence between 0.1% and 0.4% globally, although some studies indicate a true prevalence of up to 7% [26, 29].

Additionally, the allergenic potential of fish depends on its muscle composition and type. Typically, fish muscles are categorized as white (fast-twitch muscle fibers for rapid swimming) or dark (slow-twitch muscle fibers for sustained swimming) based on their composition [27]. Studies have indicated that parvalbumin levels in white muscles are 4–8 times higher than those in dark muscles. Consequently, fish species that possess higher levels of white muscle, such as cod, salmon, and flatfish, are significantly more prone to allergies [32].

In addition to parvalbumin, protein families of 47–50 kDa enolase and 40 kDa aldolase have been identified as the main fish allergens in cod, salmon, and tuna [33]. These enzymes are abundant in fish muscle and convert 2-phosphoglycerate and phosphoenolpyruvate in metabolic glycolysis to generate energy [27, 33, 34]. When enolase and aldolase allergens were first associated with one specific fish, Kuehn et al. [33] found that a considerable number of fish-allergic patients with IgE reacting to enolase and aldolase were also sensitized to parvalbumin with IgE. In contrast to parvalbumin, enolase and aldolase are thermosusceptible and may be influenced by thermal treatments during food processing. However, their importance as food allergens, particularly in parvalbumin-negative individuals, has not been sufficiently explored [26, 27]. Tropomyosin was first recognized as the main invertebrate allergen in shellfish and crustaceans [35]. Ore m 4 was the first vertebrate allergic tropomyosin identified in the serum of tilapia-allergic patients in 2013, suggesting that it is a fish allergen [36]. Subsequently, tropomyosin has been reported to participate in allergic reactions to cod, albacore, swordfish, tuna, monkfish, flatfish, and hake fish [35, 37]. Although tropomyosin is an important allergen in shellfish and crustaceans, research has demonstrated that cross-reactivity (an allergic immune response to similar allergens from different origins) exists between fish and shellfish/crustaceans for several tropomyosin epitopes. This is probably due to the significant molecular similarity of shellfish tropomyosin, which ranges from 55% to 58.8% between shrimp and fish [38]. Furthermore, Ruethers et al. [39] revealed that the protein with the second highest IgE-binding ability in salmon and catfish after parvalbumin (10–49%) was tropomyosin (6–32%). Recent studies have also demonstrated that tropomyosin is highly abundant and widespread in 28 fish species in seawater and freshwater, and is speculated to be strongly associated with allergic responses in fish species with low parvalbumin levels [12].

It is important to understand not only the molecular structure and allergenic pathways of fish allergens, but also how different processing treatments affect their allergenicity.

Overview of food processing treatments and their effect on allergenicity

Food processing is not a recent innovation; instead, it has been a significant step in food preparation for human civilization. Historically, food processing methods have contributed to the prevention of foodborne illnesses and food preservation. In contemporary society, innovative processing techniques offer numerous advantages that enhance food quality across various aspects, including sensory characteristics, nutritional value, visual appeal, and longevity [22]. Moreover, recent research has shown that numerous processing techniques can reduce and/or modify the allergenic characteristics of different types of foods, such as fish [23].

Unless otherwise specified, allergenicity outcomes reported in this section refer to changes in IgE-binding capacity as assessed by in vitro immunoassays. Structural modifications and digestibility changes are differentiated from clinical allergenicity, as these represent different levels of biological evidence that do not always predict real-life allergic responses.

Types of food processing treatments

Although most fish are thermally processed before consumption, we will also investigate other processing treatments that do not use heat. Understanding the mechanisms underlying these processing methods is essential for developing successful mitigation strategies.

Thermal processing

Thermal processing is the most commonly used technique for preserving food and extending its shelf life. It was initially scientifically defined in 1920 for the sterilization of containers, and since then, numerous forms and applications have been developed. In contemporary society, the need for food items has progressed from basic shelf stability to encompassing food safety and quality [40]. Currently, thermal processing is primarily used to improve food texture, taste, safety against microbes, digestibility, and toxin reduction. Regarding food allergies, heat treatment of proteins through thermal modification can alter their allergenic properties [41, 42]. As the consumption of processed fish increases, it is essential to examine how heating affects different fish allergens and their potential to cause allergic reactions.

As stated earlier, alterations in the structure of native proteins during processing can either diminish or increase allergenicity. In particular, the two primary elements affected by thermal processing of allergenic proteins are digestibility and antigenic traits [42]. Proteins that cause allergic responses must remain undigested or only partially digested (as immunologically active pieces) when they move through the digestive tract and are absorbed by the intestinal lining. Consequently, the resistance of proteins to digestion significantly affects their allergenic potential, which is frequently altered by heat [43].

Thermal processing includes several heat-based methods, such as boiling, roasting, frying, baking, microwave heating, and pressure cooking, all of which can significantly affect the allergenic characteristics of food proteins. These methods are commonly applied in the food sector and household environments, and their impact on allergenicity is complex, encompassing both the alteration and breakdown of protein structures. Heating can cause denaturation, aggregation, or fragmentation of proteins, thereby changing their three-dimensional structure and, consequently, their detection by the immune system [44, 45]. The vulnerability of allergens to digestive enzymes can also be altered, potentially reducing or enhancing their allergenic capacity based on specific proteins and processing conditions [45]. Roasting is one of the most extensively studied thermal processes, particularly for peanut allergens. The Maillard reaction, a non-enzymatic browning process that occurs during roasting, involves alteration of the amino groups of proteins by reducing sugars. This reaction not only enhances flavor and color but also significantly increases the IgE-binding capacity of specific proteins, such as those present in roasted peanuts, as demonstrated primarily by in vitro IgE-binding assays. Structural modifications caused by the Maillard

reaction can improve the stability of allergenic epitopes, rendering them more resistant to gastrointestinal digestion and increasing the likelihood of triggering an immune response [44].

Meng et al. [46] suggest that the primary peanut allergens, including Ara h 1, Ara h 2, and Ara h 6, show modified IgE-binding characteristics following thermal processing (assessed by in vitro IgE-binding assays and SDS-PAGE), indicating that heat treatment may either *reveal* or *conceal* certain epitopes. Boiling and frying are common heat treatments with different effects on allergenicity. For example, boiling may cause soluble proteins to leach into the cooking water, which may decrease the allergenic properties of the food. Nonetheless, the degree of this reduction is significantly influenced by the type of allergen and the medium in which it is present [44]. Conversely, frying not only exposes proteins to elevated temperatures but also induces interactions with lipids. These lipid-protein interactions can lead to the formation of aggregates that may obstruct or improve the ability of the immune system to extract and identify allergens. The heightened challenge of lipid extraction from peanuts subjected to thermal processing, as noted by Meng et al. [46], indicates that thermal treatment can considerably modify the food matrix, consequently influencing allergen availability.

Microwave heating and pressure cooking are other thermal methods that have been explored for their impact on food allergenicity. These techniques can cause rapid and consistent heating, resulting in protein denaturation and aggregation. However, their effects on allergenicity vary and are influenced by food and processing conditions. Certain allergens, particularly those similar to the primary birch pollen allergen Bet v 1, exhibit lower resistance to heat treatment, leading to a reduction in their IgE-binding capacity in vitro [44]. In contrast, different allergens, such as those in sesame, show significant resistance to heat processing and have a minimal impact on their immunoreactivity, emphasizing the necessity for additional research to clarify the mechanisms underlying these variations [16, 47]. It is crucial to recognize that the modified capacity of food allergens to bind to IgE, assessed using conventional in vitro immunoassays, does not consistently correlate with variations in allergenic activity. Experiments with physiological relevance, such as skin prick tests and mediator release assays, are crucial for evaluating the genuine effects of thermal processing on the ability of allergens to cross-link IgE and induce allergic responses in effector cells [48].

The intricate nature of food matrices and the variety of thermal processing conditions require a case-by-case assessment of changes in allergenicity. Moreover, the incorporation of processed nuts with diminished IgE-binding potential, such as cashews and pistachios, has been suggested as an approach to induce tolerance in people with allergies. Nonetheless, thorough evaluation via clinical oral challenge trials and assessment of sensory attributes is needed to validate the reduction in allergenicity and ensure consumer acceptance [49]. The identification and extraction of allergenic proteins from thermally treated foods pose considerable challenges, as denaturation and aggregation can impede the effectiveness of immunochemical assays. Selecting appropriate antibodies, target analytes, and extraction methods is essential for precise allergen measurements in processed foods [50]. Thus, thermal processing can play a dual role in food allergenicity. While it may lower the IgE-binding capacity of certain proteins by altering their structure or aiding their removal from the food matrix, it can also enhance the allergenic potential of other proteins due to chemical changes and greater resistance to digestion. The interaction between protein structure, food matrix, and processing conditions highlights the necessity for a detailed understanding of the impact of thermal processing, which is vital for creating safer food products and efficient allergy management techniques [44, 45].

As described by Khan et al. [51], thermal processing can modify allergen structure through two pathways: by disrupting epitopes and reducing IgE recognition, or by inducing structural changes that lead to neoepitope formation and increased allergenic potential. The specific outcome depends on allergen identity, processing conditions, and food matrix.

MD simulations have provided direct mechanistic insights into thermally induced structural changes. Studies on Ara h 6 peanut allergen using GROningen MAchine for Chemical Simulations (GROMACS) with the CHARMM36m force field, conducted at temperatures between 300 K and 450 K, demonstrated that thermal stress causes measurable increases in root mean square deviation (RMSD) values, indicating

progressive conformational destabilization, with longer simulation times revealing statistically more reliable structural changes [52, 53]. Similarly, microwave processing of shrimp allergens has been shown through MD analysis to induce notable alterations in the secondary structure, including reductions in β-sheet content and turn structures, which correlate with observed shifts in allergenicity at higher temperatures and longer processing times [54]. These simulations confirm that thermal denaturation is not a uniform process; the degree of structural disruption is highly dependent on processing temperature, duration, and the inherent stability of the target protein, underscoring the need for protein-specific processing optimization.

Taken together, the evidence on thermal processing and allergenicity reveals a contradictory picture. While thermal denaturation can disrupt conformational epitopes and improve digestibility, the Maillard reaction simultaneously introduces structural modifications that may stabilize or even enhance allergenic elements, particularly under dry-heat conditions such as roasting. A critical limitation across most thermal processing studies is their reliance on in vitro IgE-binding assays, which do not always predict clinical allergic responses. Furthermore, the vast majority of studies examine single allergens under controlled laboratory conditions, which poorly reflects the complexity of real food matrices. From the authors' perspective, the field would benefit from standardized processing protocols that allow meaningful cross-study comparisons and greater integration of MD simulation data with scientific outcomes to bridge the gap between structural changes and actual allergenic risk.

Non-thermal processing

Non-thermal processing techniques have emerged as innovative alternatives to traditional thermal treatments, offering the ability to modify food proteins and their allergenic characteristics without significant heat-driven denaturation, which is often associated with conventional methods. These techniques include high hydrostatic pressure (HHP, also referred to as high-pressure processing, HPP), cold plasma treatment, enzymatic treatment, irradiation, and fermentation. Each of these methods produces distinct physicochemical effects on food allergens, frequently resulting in structural changes that may either reduce or, in certain instances, increase allergenicity [44].

For example, cold plasma processing has emerged as an innovative, nonthermal method in the food sector. This technique employs ionized gases to trigger chemical and structural modifications in proteins at room or sub-lethal temperatures, thus preventing significant protein aggregation and solubility reduction observed during thermal processes. Based on available experimental evidence, cold plasma has been shown to alter allergenic proteins by disturbing their tertiary and quaternary structures, potentially decreasing IgE recognition, as assessed primarily through in vitro immunoassay studies [45]. Nonetheless, the decrease in allergenicity is greatly influenced by the specific proteins and processing conditions used.

HHP is another extensively researched nonthermal method. This technique exerts pressure, usually between 100 MPa and 800 MPa, on food matrices, causing conformational alterations in proteins without heat. The authors indicated that HHP can encourage the formation of disulfide bonds via SH/S–S exchange reactions, potentially leading to protein aggregation or unfolding, which is influenced by the pressure and pH levels. These structural changes, including protein unfolding and altered surface hydrophobicity, may modify epitope accessibility, although direct allergenicity outcomes depend on the specific protein system and require dedicated immunological assessment [55]. Nonetheless, the impact of HHP varies among different proteins; certain studies have indicated reduced allergenicity, whereas others have noted negligible or even negative effects, underscoring the significance of the matrix composition and processing conditions [55].

Nonthermal processing techniques can also influence the physicochemical properties of allergens, including their solubility, digestibility, and aggregation state. Villa et al. [47] emphasized that changes in protein structure, such as the formation of disulfide bonds, variations in α-helical content, and shifts in hydrophobic regions, can affect both the stability of allergens and their identification by the immune system. These structural modifications can conceal or reveal IgE-binding sites, thereby influencing allergenicity.

The analytical identification of allergens in foods that are not subjected to thermal processing poses additional challenges. The selection of detection techniques, such as real-time polymerase chain reaction (PCR) or immunoassays, should consider the modified structural and chemical characteristics of the target proteins. The authors also highlighted that no individual analytical method is universally superior, and confirmatory techniques are frequently required to guarantee precise allergen measurements in processed foods. The variety of nonthermal processing methods and their differing impacts on various food allergens highlight the difficulty in forecasting allergenic results. Despite the potential of various nonthermal techniques to decrease the allergenic properties of foods, the interactions among processing conditions, protein structure, and food matrix composition require meticulous optimization and validation. Cabanillas and Novak [44] asserted that understanding these interactions is crucial for creating safer food products and effectively managing food allergies.

Dong et al. [56] examined the allergenic threats posed by alternative protein sources, including insects, algae, and legumes, and assessed current detection techniques, such as ELISA and mass spectrometry. They also explored nonthermal processing methods (e.g., cold plasma, ultrasound, and HPP) for their ability to reduce allergenicity. An important conclusion of this study is the need for additional research to ensure food safety while encouraging sustainable protein options. They assessed and summarized various nonthermal methods to decrease allergenicity, as shown in Table 2 [57, 58, 59–61].

Table 2. Summary of the different technologies' effect on allergenicity.

Technology	Mechanism	Conditions	Findings	References
High-pressure processing (HPP)	Alters protein structure via pressure	100–800 MPa, 5–30 min, room temp	Can reduce allergenicity, but effects vary by protein type	[55, 59–61]
Cold plasma (CP)	Oxidizes proteins via ROS/RNS	Room temp, 1–15 min exposure	Reduces IgE/IgG binding; promising but costly and not fully understood	[45]
Ultrasound (US)	Cavitation breaks peptide bonds	20–40 kHz, 10–60 min	Reduces allergenicity but may affect food quality	[57]
Pulsed electric field (PEF)	Alter secondary/tertiary protein structures	10–40 kV/cm, microsecond pulses	Promising but under-researched	[44]
Gamma irradiation	Breaks covalent bonds and generates radicals	1–10 kGy	Effective at high doses, but regulatory and consumer concerns exist	[58]

IgE: immunoglobulin E; IgG: immunoglobulin G.

Tsai et al. [12] examined how species, muscle site, food processing, and cold storage impact fish tropomyosin and contrasted it with the primary fish allergen, parvalbumin. They discovered that the location of the muscle influences allergen distribution, as these two allergens are not uniformly spread throughout fish muscle, suggesting that the sampling site is crucial for precise allergen identification. Nonetheless, both allergens remained unchanged and immunoreactive when stored in a refrigerator at 4°C for up to four days. A minor decline in tropomyosin was noted after six days, whereas parvalbumin levels remained consistent during that period.

MD simulations have also shed light on the structural mechanisms underlying non-thermal processing effects. Oscillating electric fields paired with thermal treatments have been shown to unfold secondary structures, specifically α-helices and turns, in the Act d 2 kiwifruit allergen, demonstrating how electromagnetic stress can alter protein conformation in ways that may reduce epitope accessibility [57]. In the context of HHP, MD simulations support experimental findings by showing that elevated pressure increases protein vulnerability to enzymatic degradation, resulting in decreased IgE-binding ability—a key finding for understanding how combined HHP and enzymatic treatments may work synergistically to reduce allergenicity [15]. Together, these computational findings reinforce that nonthermal methods alter allergenicity through specific, protein-dependent structural pathways rather than generalized denaturation, which has direct implications for optimizing processing conditions.

Despite the promise of non-thermal processing technologies, several critical limitations inhibit their widespread application. First, most studies have focused on single proteins or model systems, and the results rarely translate predictably across different food matrices or allergen types. Second, evidence for allergenicity reduction is predominantly in vitro, with very few studies using clinical endpoints or patient trials to validate findings. Third, technologies such as cold plasma and pulsed electric fields remain largely at the laboratory scale, with limited data on industrial feasibility and consumer acceptability. From a critical standpoint, the field lacks standardized protocols for assessing the effects of nonthermal processing on allergenicity, making cross-study comparisons unreliable. Future research should prioritize multi-allergen assessments under realistic food matrix conditions and combine them with clinical validation to move beyond proof-of-concept findings toward actionable processing strategies.

Enzymatic and fermentation processes

Enzymatic treatment is a targeted approach for the nonthermal modification of food allergens. The use of specific proteases allows the hydrolysis of allergenic proteins into smaller peptides, thereby disrupting the conformational and linear epitopes that bind IgE. This procedure can significantly reduce the IgE-binding capacity of foods, as demonstrated primarily by in vitro immunoassays, because the immune system is less inclined to identify fragmented peptides as allergens. Enzymatic hydrolysis is affected by enzyme specificity, substrate availability, and hydrolysis level [45].

Fermentation, commonly associated with microbial processes, is a nonthermal treatment technique that significantly impacts allergenicity. During fermentation, microbial enzymes break down proteins, including allergens, into smaller fragments. Based on the available in vitro evidence, fermentation has been shown to cause the degradation of allergenic proteins, potentially decreasing their IgE-binding capacity; however, the effectiveness depends on the microbial strains employed and the fermentation conditions [50].

Enzymatic and fermentation methods play crucial roles in altering food proteins and have a major effect on their allergenic capacity. Microbial enzymes facilitate the breakdown of complex food elements, such as proteins, within the gastrointestinal tract through fermentation. This process is mainly aided by the microbiome in the large intestine, which ferments the contents and generates various microbial metabolites. These biochemical changes can modify the structure and immunogenicity of food proteins, which is particularly important in the context of food allergies. The type and quantity of glycans found in gut mucus, which also vary during fermentation, affect the relationship between digested proteins and the immune system, possibly altering allergic responses [44, 45].

The enzymatic breakdown of proteins, whether occurring naturally during fermentation or via a specific enzymatic treatment, can result in breakage of allergenic epitopes. This structural alteration frequently leads to a diminished ability to bind IgE because the epitopes recognized by the immune system are either obscured or eliminated. For instance, glycation, a mechanism by which proteins undergo enzymatic alterations in the presence of sugars, has been demonstrated to reduce the IgE-binding ability of ovalbumin, probably because of conformational modifications that hide allergenic sites. These changes are not restricted to glycation; various enzymatic processes throughout fermentation can modify protein structure and affect allergenicity [55, 62].

The fermentation process varies significantly based on the microbial species present, substrate composition, and environmental factors, such as pH and temperature. Together, these factors influence the degree and characteristics of protein hydrolysis and the subsequent allergenic profile of food. The complexity of these interactions has led to the development of a dynamic field of study aimed at understanding how various fermentation and enzymatic methods can reduce or, in certain instances, increase the allergenic capacity of food proteins. For example, partial hydrolysis or the formation of new peptide fragments during fermentation can reveal new epitopes that may increase allergenicity in susceptible individuals [44].

The relationship between enzymatic and fermentation processes and the host immune system is complicated by the inconsistent IgE groups found in allergic individuals. Each patient's immune system identifies a distinct set of allergenic components, complicating the prediction of the impact of enzymatic or fermentation treatments on allergenicity. This inter-individual variation highlights the need for tailored food processing and allergy treatment methods, along with the significance of ongoing studies on how enzymatic and fermentation processes affect protein allergenicity.

MD simulations support these experimental findings by demonstrating that pressurized treatments combined with enzymatic hydrolysis increase protein vulnerability to enzymatic degradation at the molecular level, resulting in measurable decreases in IgE-binding ability while preserving the overall nutritional integrity of the food [15]. These computational insights suggest that combined processing approaches, rather than single-method treatments, may offer the most reliable pathway to allergenicity reduction through enzymatic means.

Enzymatic and fermentation approaches hold genuine promise for allergenicity reduction; however, several critical issues limit their current applicability. The effectiveness of enzymatic hydrolysis is highly dependent on enzyme specificity and the degree of hydrolysis; incomplete hydrolysis can generate new peptide fragments that may be allergenic, potentially worsening outcomes for some individuals. Fermentation outcomes vary considerably depending on the microbial strain, substrate composition, and environmental conditions, making reproducibility a persistent challenge. Critically, most studies are conducted under controlled laboratory conditions using purified proteins, which does not reflect the complexity of real food systems. Individual variability in IgE profiles among allergic patients further complicates the prediction of clinical outcomes. Combined processing approaches, such as pressurized enzymatic hydrolysis, show the most computational and experimental promise but require clinical validation before they can be recommended as reliable allergen mitigation strategies.

Pressure-based treatments

Pressure-based methods, particularly HHP, have emerged as novel strategies for altering the allergenic characteristics of food proteins. These treatments typically expose foods to pressures between 100 MPa and 800 MPa, resulting in major changes in protein structure without the requirement of high temperatures. Pressure-induced structural changes can alter the exposure and accessibility of amino acid residues, subsequently affecting the immunogenicity and allergenicity of proteins.

Liu et al. [59] investigated largemouth bass subjected to HHP treatment and found that the processing treatment altered the protein structure and composition. Structural modifications were observed under optimal conditions of 400 MPa for 15 min, resulting in the emergence of a new 21 kDa allergenic protein band post-treatment, although allergenicity did not substantially decrease.

Somkuti et al. [60] examined the influence of pressure and temperature on the structure of cod parvalbumin (Gad m 1), a significant fish allergen, and discovered that calcium binding is crucial for the stability of the protein and that it experiences intricate unfolding under different pressure-temperature scenarios. Although partial or complete protein unfolding was achieved via heat and pressure treatments, the allergenicity of the protein remained identical when evaluated using sera from patients allergic to fish. This suggests that traditional pressure-temperature methods may not effectively reduce the allergenic potential of fish proteins.

Liu and Xue [61] investigated the allergenic properties of silver carp protein during HHP processing and found, using in vitro IgE-binding assays and structural analysis, that HHP altered the secondary structure of the allergenic molecule but did not reduce its allergenicity. They recommended different or integrated HHP processing techniques to reduce fish allergenicity.

Experimental findings [55] have shown that high pressure can lead to the unfolding of protein molecules, which in turn may reveal previously hidden residues, such as tyrosine (Tyr) and phenylalanine (Phe), on the protein surface. This exposure was evident from the increased fluorescence intensity of soy β-conglycinin and soy protein isolate (SPI) at 400 MPa for 15 min. The heightened surface visibility of these

aromatic residues suggests structural changes that could affect epitope recognition by the immune system of the host. However, when the pressure exceeded this limit, the fluorescence intensity decreased, probably because of the reorganization of the hydrophobic regions and movement of tryptophan (Trp) residues into these regions. These rearrangements can obscure or alter epitopes, possibly decreasing the allergenic potential of the proteins.

The influence of pressure-based methods on allergenicity varies among proteins and is highly dependent on the specific pressure utilized, treatment duration, and inherent characteristics of the protein. Additionally, pressure-based treatments can interact with other elements of the food matrix, potentially affecting allergic responses. For instance, the presence of other proteins or food components during processing can influence the degree of structural change and epitope availability. This interaction emphasizes the difficulty of forecasting allergenicity results after pressure-based processing [63].

Conformational epitopes, which rely on the three-dimensional structure of a protein, are more prone to disruption owing to pressure-induced unfolding. This may result in diminished recognition by immunoglobulin G (IgG) or IgE antibodies, which in turn lowers the likelihood of triggering an allergic reaction [50]. In contrast, linear epitopes, characterized by their primary amino acid sequences, remain mostly unchanged unless significant hydrolysis or fragmentation occurs [63]. Consequently, the net impact of pressure-based therapies on allergenicity involves a trade-off between the degradation of conformational epitopes and the maintenance or unveiling of linear epitopes. It is important to acknowledge that although pressure-based treatments may decrease allergenicity in certain instances, there is a possibility of generating new epitopes or revealing previously concealed epitopes, which could potentially increase the allergenic potential in specific situations [44]. These differences in responses highlight the need for a thorough assessment of every protein system exposed to HPP.

MD simulations offer mechanistic support for these experimental observations. Computational studies have shown that elevated pressure induces the unfolding of protein molecules, exposing previously hidden aromatic residues such as Tyr and Phe on the protein surface, which alters epitope accessibility and potentially affects IgE recognition [55]. Furthermore, MD metrics, such as solvent-accessible surface area (SASA), are particularly useful for quantifying pressure-induced changes in epitope exposure, providing a molecular-level explanation for the variable allergenicity outcomes observed experimentally under different protein systems and pressure conditions.

Pressure-based treatments present a contradiction that is critical to acknowledge: while HHP consistently induces structural changes in allergenic proteins, these changes do not reliably translate into reduced allergenicity. Studies on fish allergens—particularly parvalbumin from cod and silver carp—have demonstrated that even substantial structural disruption, including partial or complete unfolding, may leave the IgE-binding capacity intact. This is a fundamental challenge for HHP as an allergen mitigation strategy, as structural changes and immunological consequences are not equivalent outcomes. Furthermore, the emergence of new allergenic protein bands following HHP treatment, as observed in largemouth bass, raises legitimate safety concerns that have not been adequately addressed in the literature. From the authors' perspective, HHP shows more promise as a pretreatment to enhance susceptibility to subsequent enzymatic hydrolysis than as a standalone allergen reduction method. Clinical validation of any HHP-based strategy remains an essential and largely unmet requirement.

Chemical modifications

Chemical alterations in food proteins during processing can significantly influence their allergenic potential. These alterations can occur via different mechanisms, such as glycation, oxidation, and the creation or breaking of covalent bonds within or among protein molecules. The interaction of proteins with reactive carbonyl compounds, such as methylglyoxal (MGO), illustrates a mechanism by which chemical alterations can affect allergenicity. The alteration of β-lactoglobulin by MGO results in variations in its glycation pattern, potentially affecting the functionality and immunogenicity of the protein. Glycation caused by MGO may change the sites of glycation or the total levels of modification, which could either conceal or reveal epitopes that the immune system can recognize [64]. These alterations can either diminish or increase the

allergenic characteristics of a protein, depending on the specific structural changes and their effects on immune detection.

Pressure cooking is another method that causes chemical changes in proteins. By exposing food to high temperatures and pressures, pressure-cooking can enhance the denaturation and aggregation of proteins and promote Maillard reactions between amino groups and reducing sugars. These processes lead to the formation of advanced glycation end products (AGEs), which can alter the structure of allergenic proteins, thereby influencing their detection by IgE antibodies [44]. The degree and characteristics of these chemical alterations are affected by variables such as temperature, pressure, and processing time.

Chemical alterations can either conceal epitopes, rendering them unreachable by immune receptors, or reveal new epitopes, possibly forming new allergenic determinants. Computational and experimental methods have improved our knowledge of how specific chemical modifications can help eliminate or diminish allergenic epitopes. Computational predictions specific to antibodies, when used in conjunction with cross-blocking experiments, allow for the precise identification of residues within conformational B-cell epitopes. The combination of computational simulations and experimental validation offers the potential for the precise removal of allergenic epitopes in food proteins, particularly milk allergens [16]. These strategies may facilitate the creation of hypoallergenic food items through targeted chemical alterations. The intricacy of chemical changes in food processing underscores the necessity of comprehensively understanding how these alterations affect protein structure and immune detection. The interactions between glycation, oxidation, and covalent bond rearrangement highlight the complex nature of the modulation of allergenicity in processed foods.

This method was applied in a different study related to HHP, which asserted that it could decrease food allergenicity. Liu et al. [65] applied HHP in conjunction with chlorogenic acid and noted a decrease in crayfish tropomyosin IgE-binding capacity, as assessed by in vitro immunoassays, through covalent or non-covalent interactions, modifying its secondary structure and obscuring tropomyosin's linear epitopes. Nevertheless, the authors claimed that chlorogenic acid, rather than HHP, decreased allergenicity.

Qiu et al. [66] administered dense-phase carbon dioxide (DPCD) to pompano fish (*Trachinotus ovatus*) and observed a 39–41% reduction in parvalbumin-triggered allergic reactions, as measured by indirect ELISA and Western blotting (in vitro). This was primarily due to the disruption of the typical conformational epitopes and alteration of the secondary structure from ordered to disordered, accompanied by a reduced presence of α-helical groups, while the internal hydrophobic groups became exposed.

MD simulations have significantly contributed to understanding how chemical modifications alter the structure of allergenic proteins. Glycation-induced changes, such as those caused by MGO on β-lactoglobulin, can be modeled computationally to predict how modified glycation patterns affect epitope accessibility and IgE recognition at the molecular level [64]. Similarly, computational predictions specific to antibody binding, combined with cross-blocking experiments, have enabled the precise identification of residues within the conformational B cell epitopes of milk allergens, demonstrating how targeted chemical modifications can be designed to eliminate specific allergenic determinants [16]. These computational approaches highlight the potential of MD-guided chemical modification strategies as a rational approach for designing hypoallergenic food protein engineering.

Chemical modifications during food processing represent an especially complex and often unpredictable pathway for allergenicity modulation. A critical issue is that the same chemical process, such as glycation, can either reduce or increase allergenicity, depending on the extent of modification, the specific protein involved, and the food matrix context. The chlorogenic acid/HHP study by Liu et al. [65] illustrates this complexity well; the chemical agent, rather than the physical treatment, drove allergenicity reduction, emphasizing that interaction effects between processing methods and food components are frequently overlooked in experimental designs. Additionally, novel processing methods, such as DPCD, remain poorly characterized in terms of their effects across different allergen families and species. From the

authors' perspective, chemical modification strategies show promise primarily when they are targeted and validated; broad application without protein-specific optimization risks unpredictable immunological outcomes, including potential neoallergen formation.

Cross-cutting effects of processing on allergenicity

Across all processing types discussed above, three molecular-level mechanisms consistently govern how processing modifies allergenicity: denaturation and aggregation, which alter or destroy conformational epitopes; glycation via the Maillard reaction, which can both mask and create new antigenic determinants; and fragmentation through hydrolysis, which disrupts linear epitopes but may generate immunogenic peptide fragments. The net allergenic outcome depends on which of these mechanisms predominates under a given set of processing conditions and for a given protein. The following sections examine the two possible directions of this outcome.

Reduction of allergenicity

The reduction in the allergenicity of food proteins after different processing treatments is primarily due to the loss of IgE-binding epitopes. The structural stability of epitopes, which are distinct amino acid sequences or conformational patterns identified by IgE antibodies, is vital for triggering allergic reactions. The application of processing methods, such as heating, chemical modification, or enzymatic hydrolysis, can alter, conceal, or eliminate these epitopes, resulting in decreased IgE binding and, consequently, lower allergenic potential.

Thermal treatment, particularly at elevated temperatures and/or for prolonged durations, may cause denaturation and unfolding of allergenic proteins, resulting in the loss of linear IgE-binding epitopes. Meng et al. [46] suggest that in extreme thermal conditions, the Maillard reaction may deteriorate linear epitopes, leading to a reduced antigenic response. Their results showed that purified Ara h 1, a key peanut allergen, exhibited significantly reduced IgE-binding ability following thermal denaturation and unfolding, as assessed by in vitro IgE-binding assays and SDS-PAGE. This occurrence is not exclusive to peanuts; comparable effects have been noted for other allergenic proteins, wherein alterations in epitope structure are linked to diminished IgE reactivity.

Chemical changes, such as those caused by acrolein treatment, may interfere with IgE binding. Lv et al. [67] presented molecular evidence that acrolein adduction affects lysine and histidine residues in the peptide sequence, thereby reinforcing the disruption of essential IgE-binding sites. These chemical alterations emphasize the sensitivity of the epitope structure to minor changes in the protein backbone or its side chains. Enzymatic hydrolysis serves as an additional method for reducing allergenicity by breaking specific peptide bonds within antigenic epitopes. Zeng et al. [68] indicated that the efficient hydrolysis of vital amino acids in antigenic epitopes is crucial for reducing the antigenicity of cow milk proteins. Noorbakhsh et al. [69], using competitive inhibition ELISA and Western blotting (in vitro), noted that various processing and preparation techniques can significantly change protein solubility and IgE-binding characteristics of pistachio extracts, highlighting the necessity of refining processing conditions to attain the intended decrease in allergenicity.

Although processing-induced allergenicity reduction is well documented, a critical limitation is that most evidence comes from in vitro IgE-binding assays, which do not reliably predict clinical outcomes. Residual peptide fragments after processing may retain immunogenic potential, and the sensory and nutritional impacts of the required processing conditions are rarely assessed alongside allergenicity.

Increase or "unmasking" of allergenicity

The emergence of new epitopes, referred to as neoallergens, is observed during different food-processing techniques, in which alterations in protein structures can lead to the creation of unique antigenic determinants. These newly created epitopes may not exist in the native protein and can trigger immune reactions in sensitized individuals, thereby increasing the allergenic risk of processed foods [44, 49, 50].

As stated by Cuadrado et al. [49], processing can concurrently remove existing epitopes and create new ones, thus emphasizing its dual effect on allergenicity. The fundamental mechanisms frequently include changes in the tertiary and quaternary structures of the protein, resulting in the exposure or formation of amino acid sequences that were previously hidden from the immune system [44, 47, 50]. Heat treatment methods, such as roasting or baking, are particularly effective in inducing these structural changes. Khan et al. [50] suggested that any mechanism that can alter protein conformation may affect the structural properties of epitopes, consequently influencing antigen-antibody interactions. For example, elevated temperatures can lead to protein unfolding, aggregation, or even chemical changes, such as Maillard reactions, all of which may reveal concealed epitopes or create completely new antigenic sites.

The unmasking of allergenicity represents an underappreciated risk in food processing, because standard safety assessments rarely screen for neoallergen formation. A critical concern is that processing conditions optimized to reduce one allergen may simultaneously increase reactivity toward another, particularly through Maillard-induced structural changes. Systematic screening for neoepitope formation should be incorporated into the allergenicity risk assessments of processed foods.

Influence of food matrix and digestion on allergenicity

Role of the food matrix

Interactions between food proteins and other components of the food matrix, including carbohydrates, lipids, and proteins, significantly influence allergenicity. The intricate nature of the food matrix suggests that proteins rarely exist independently of each other. Instead, they are integrated into a network of different molecules that can directly or indirectly affect immunogenic characteristics [50]. These interactions may alter epitope accessibility, alter protein shape, and influence the effectiveness of extraction and detection techniques. Carbohydrates, especially reducing sugars, are significant because of their involvement in non-enzymatic glycation processes, such as the Maillard reaction. This procedure entails the condensation of the N-group from lysine residues in proteins with the carbonyl group of reducing sugars, initially yielding glycosamines, which may subsequently rearrange into AGEs [63]. AGE development alters the chemical composition of proteins and can either mask or reveal immunologically significant epitopes, influencing the potential for allergic reactions. Heating accelerates these reactions, and the resulting changes can either improve or reduce the immune system's recognition of the protein, depending on the context. Interactions between lipids and other proteins complicate the allergic environment. As noted by Villa et al. [47], the food matrix can either stabilize, enhance, or diminish protein allergenicity, depending on the protein type and processing parameters. For example, the attachment of proteins to lipids or other large molecules may limit their accessibility to immune receptors, possibly decreasing their allergenic potential. However, these interactions can also resemble pathogen-associated molecular patterns, thereby improving receptor attachment and increasing the likelihood of allergens.

Therefore, the relationship between protein structure and matrix composition varies significantly depending on the context. Interactions within these matrices also affect the extraction and detection of allergens in food samples. Zhang et al. [70] suggested that thermal treatment influences the extraction of protein elements, as observed in processed peanut extracts, where protein bands shift and blur, indicating changes in molecular weight and possibly modified immunoreactivity. These modifications result not only from direct thermal denaturation but also from interactions with various matrix components, which may mask or cluster proteins, making their identification and quantification more complex. Matrix effects encompass not only chemical interactions but also include physical and structural elements. Food structure may hinder enzymatic access during digestion, consequently influencing the degradation and subsequent absorption of allergenic proteins [47, 63]. This alteration in gastrointestinal digestion can affect the quantity and structure of allergenic peptides that access the immune system, subsequently affecting their allergenicity. Khan et al. [50] noted that the matrix effect refers to the total impact of all sample components, except the analyte, on the measurement of the analyte itself. This definition highlights the significance of considering the overall composition of food when evaluating allergenic risk. The matrix can

either disguise allergens, rendering them more difficult to detect and possibly less immunogenic, or enhance their exposure to the immune system, depending on the interaction of its components.

Additionally, processing techniques, such as fermentation, autoclaving, and drying, which intrinsically change the matrix composition and structure, further influence these interactions [45]. The influence of food additives and the selection of extraction techniques in research environments also contribute to the inconsistency of allergenicity evaluations, emphasizing the need for standardized methods that consider matrix effects.

Food matrix effects on allergenicity remain among the least understood variables in the field, largely because most studies use purified proteins rather than whole food systems. This is a critical methodological gap, as matrix interactions can mask or amplify allergenicity in ways that purified protein studies cannot capture, thereby limiting the translational value of current findings.

Digestion dynamics

The oral, gastric, and intestinal stages of digestion are crucial in influencing the allergenic potential of food proteins. In the oral phase, mechanical disruption and enzyme action initiate the breakdown of food matrices; however, the effect on protein allergenicity is usually minimal owing to the brief exposure time and gentle enzymatic environment. The mouth mainly presents proteins to salivary enzymes, such as amylase, which has a limited direct impact on protein allergens. Consequently, most allergenic proteins remain structurally intact as they enter the gastric phase [71].

During the gastric phase, acidic conditions (pH < 2) and pepsin are essential for protein digestion. The upper part of the stomach, comprising the cardia and fundus, plays a role in producing gastric juices and sustaining a low pH, which is crucial for effective pepsin function [45]. Pepsin breaks peptide bonds, particularly those with aromatic amino acids, resulting in the breakdown of protein allergens.

However, the degree of degradation differs significantly for various proteins. Certain allergens, such as the 2S albumins found in sesame seeds, demonstrate significant resilience to acidic pH and pepsin digestion, preserving their structure and allergenic capability even after prolonged exposure to gastric conditions. This stability is attributed to their rigid structure, which is reinforced by several disulfide bonds that shield them from enzymatic hydrolysis [47]. In contrast, certain proteins, such as parvalbumin from fish, may be partially broken down during gastric digestion; however, their durability is influenced by the species and the unique structure of the protein. Koidl et al. [45] suggest that parvalbumin can still be found after 120 minutes of gastric digestion, emphasizing the durability of specific allergenic proteins.

Processing treatments significantly affect the digestibility of food proteins, subsequently altering their potential to cause allergies. Changes in protein structure caused by different processing techniques can either increase or decrease protein vulnerability to digestive enzymes, thus affecting the composition and quantity of digestion products. For example, the ultrasonication of kiwifruit proteins has been shown to enhance peptide levels during simulated gastrointestinal digestion, as assessed by *o*-phthalaldehyde (OPA) assay. The peptide yield increased with prolonged ultrasound treatment, indicating that this physical method can aid in the decomposition of protein structures, rendering them more accessible to digestive enzymes [57]. This improved digestibility is not exclusive to kiwifruit; comparable effects have been observed in other food matrices that have undergone physical or thermal processing. The vulnerability of food allergens to digestion is significantly influenced by the physicochemical conditions of the digestive process. Elements such as pH, enzyme composition, and digestion duration are crucial in influencing the degree of protein hydrolysis. Koidl et al. [45] emphasize that the level of digestion and subsequent allergenicity are significantly affected by these experimental factors, and that the differences in individual gastrointestinal conditions should be considered when evaluating protein digestibility and the risk of allergies.

The overall findings [16, 45, 46, 57, 58] suggest that alterations in protein structure due to processing, physicochemical conditions of digestion, and inherent characteristics of the food matrix shape the digestibility of food proteins and, therefore, their potential to cause allergies. A thorough understanding of

these interactions is vital for the development of processing strategies designed to reduce food allergenicity while preserving nutritional quality.

MD simulations have provided valuable additional insights into digestion dynamics at the molecular level. The simulated gastric fluid conditions modeled computationally have shown how parvalbumins interact with digestive enzymes in acidic environments, yielding information on their structural stability and allergenic persistence during digestion that is difficult to obtain experimentally [43]. Additionally, MD simulations of acidic treatments have revealed that protein cleavage and alterations in surface polarity produce conformational changes that reduce epitope accessibility, offering a mechanistic explanation for the observed reduction in IgE-binding capacity under acidic digestive conditions, though clinical validation of these computationally derived findings remains limited [72]. These findings highlight the value of MD as a tool for probing digestion-allergenicity relationships at a resolution beyond what conventional in vitro models can provide.

A critical limitation of current digestion studies is their reliance on standardized in vitro models that do not reflect the considerable individual variability in gastrointestinal conditions, including differences in pH, enzyme concentrations, and transit time. These variables can substantially alter allergen stability and immunogenic fragment generation; however, they are rarely accounted for in experimental designs. MD simulations offer a promising avenue for modeling these individual differences computationally; however, experimental validation under physiologically diverse conditions remains a priority.

Summary of processing effects on food allergenicity

Table 3 summarizes the key experimental and computational findings on the effects of food processing on allergenicity across the major allergen families discussed in this review [70–73].

Table 3. Summary of processing effects on food allergen structure and immunogenicity.

Food/Allergen	Processing method	Allergenicity outcome	Evidence type	MD insights	Reference
Peanut (Ara h 1, 2, 6)	Roasting	Increased—Maillard reaction stabilizes epitopes	In vitro (IgE-binding, SDS-PAGE)	RMSD increases confirm thermal destabilization	[44, 46]
Peanut (Ara h 1)	Boiling/Frying	Reduced—protein leaching and denaturation	In vitro (IgE-binding assays)	-	[46]
Peanut (Ara h 1)	Thermal denaturation	Reduced IgE-binding ability	In vitro (IgE-binding, SDS-PAGE)	-	[46]
Shrimp (tropomyosin)	Microwave/Cooking	Altered secondary structure; variable outcome	In vitro (immunoassay)	Turn structure reduction noted; β-sheet content increased with prolonged ultrasound treatment (FTIR)	[54, 73]
Kiwifruit (Act d 2)	Oscillating electric field	Reduced—α-helix/turn unfolding	In vitro	MD confirms α-helix/turn disruption	[57]
Cod parvalbumin (Gad m 1)	HHP + temperature	Structural unfolding achieved; allergenicity unchanged	Ex vivo (patient sera)	-	[60]
Silver carp protein	HHP	Secondary structure altered; allergenicity unchanged	In vitro (IgE-binding, structural)	-	[61]
Largemouth bass	HHP (400 MPa, 15 min)	A new 21 kDa allergenic band emerged	In vitro	SASA metrics explain epitope exposure variability	[59]
Soy (β-conglycinin, SPI)	HHP (400 MPa)	Epitope exposure increased, then decreased	In vitro (fluorescence)	MD shows aromatic residue exposure at 400 MPa	[55]
Crayfish (tropomyosin)	HHP + chlorogenic acid	Reduced—linear epitopes obscured	In vitro	-	[65]

Table 3. Summary of processing effects on food allergen structure and immunogenicity. (*continued*)

Food/Allergen	Processing method	Allergenicity outcome	Evidence type	MD insights	Reference
Pompano fish (parvalbumin)	DPCD (15 MPa, 30 min, 50°C)	Reduced 39–41%—conformational epitope disruption	In vitro (ELISA, Western blot)	-	[66]
β-Lactoglobulin	MGO glycation	Variable—epitopes masked or revealed	In vitro	MD predicts glycation-induced epitope accessibility changes	[64]
Milk proteins	Enzymatic hydrolysis	Reduced—antigenic epitopes disrupted	In vitro	-	[68]
Pistachio	Steam roasting	Reduced IgE-binding and solubility	In vitro (competitive ELISA, Western blot)	-	[69]
Parvalbumin (fish)	Simulated gastric digestion	Partially resistant after 120 min	In vitro	MD models gastric enzyme interactions at the molecular level	[43, 45]
Cashew/Pistachio	Thermal processing	Dual effect—existing epitopes removed, new ones formed	In vitro (IgE-binding)	-	[49]

DPCD: dense-phase carbon dioxide; HHP: high hydrostatic pressure; IgE: immunoglobulin E; MD: molecular dynamics; MGO: methylglyoxal; RMSD: root mean square deviation; SASA: solvent-accessible surface area; SPI: soy protein isolate.

MD simulations in allergen research

Introduction to MD simulations

MD simulations are computational methods that model the behavior of molecular systems at the atomic scale by solving equations of motion over time, enabling examination of structural, thermodynamic, and kinetic properties under varying conditions. Atomic interactions are governed by force fields—mathematical models parameterizing bonded and non-bonded interactions—whose precise selection critically affects simulation outcomes. In allergen research, force fields such as CHARMM36m have been applied to model protein dynamics under thermal and chemical stress conditions [52, 53]. In addition, time intervals for integration steps are frequently selected to strike a balance between accuracy and practical runtime limitations [52, 74].

Solvation models are used to mimic the aqueous environment surrounding proteins; for instance, in studies of Ara h 6 allergens, water molecules were introduced to solvate the system and ions added to ensure neutrality [52, 53].

Simulation duration is a critical factor in MD reliability. For example, studies on Ara h 6 allergens have shown that longer simulations can reveal statistically significant effects of thermal processing on RMSD that are not captured over shorter timescales. Additional metrics, including root mean square fluctuation (RMSF) and SASA, provide complementary insights into molecular behavior. RMSF reflects local protein flexibility, whereas SASA is commonly used to assess epitope accessibility [54, 75], a key consideration in allergen research, where processing-induced structural changes may alter immunogenicity.

MD simulations have also benefited from advances in computational hardware and software platforms, such as the GROMACS and Visual Molecular Dynamics (VMD), which have been significant in examining food allergens such as peanut and fish proteins under various thermal processing conditions.

Allergens are generally defined as water-soluble proteins that are not easily digested or processed because of their stable structures. By modeling these molecules under different environmental conditions, such as fluctuations in temperature or interactions with solvents, scientists can discover the structural characteristics responsible for IgE-binding epitopes that initiate immune reactions [75]. Probabilistic modeling enhances MD simulations by assessing the risks associated with allergenic proteins and utilizing simulation data integrated with experimental results [76]. This comprehensive method improves risk assessment frameworks by utilizing computational and real-world data. By thoroughly investigating the principles of MD in the context of allergen research, particularly regarding fish allergens, researchers have

acquired significant knowledge regarding protein behavior at the atomic level while addressing the global challenges of food allergies [1, 23, 77].

Despite their value, MD simulations have important limitations that are insufficiently acknowledged in allergen research. First, the choice of force field substantially affects simulation outcomes; different force fields (e.g., CHARMM36m, AMBER, GROMOS) parameterize atomic interactions differently, and the results obtained with one may not be directly comparable to those obtained with another. In allergen studies, force field selection is rarely justified or systematically evaluated, limiting reproducibility across research groups. Second, solvation models introduce additional variability: explicit solvent models are more physically realistic but computationally expensive, whereas implicit models sacrifice accuracy for speed. The choice between them can affect predicted protein flexibility, epitope accessibility, and SASA values—all metrics used to infer allergenicity. Third, most published allergen MD studies use short timescales (2–200 ns), which may not capture slow conformational transitions relevant to IgE binding or thermal unfolding. There is a genuine risk of overinterpreting structural metrics from short simulations as definitive indicators of allergenicity change. Finally, MD-derived metrics, such as RMSD, RMSF, and SASA, measure structural behavior but do not directly measure allergenicity; the translation from computational output to immunological prediction requires experimental validation, which is frequently absent. These limitations underscore the need for greater methodological transparency and cross-validation with experimental data in future allergen MD studies.

MD simulations of allergenic proteins

MD simulations play a key role in understanding the thermal stability and biochemical resilience of seafood-derived allergenic proteins. Parvalbumin and tropomyosin, the main allergens in fish and shellfish, respectively, showed significant resistance to heat denaturation. This characteristic contributes to the ongoing occurrence of seafood allergies, even after cooking or processing [78].

The stability of allergenic proteins under specific environmental conditions also influences their structural behavior. For instance, Ara h 1 creates stable homotrimers at relatively low NaCl concentrations. This trimeric configuration is associated with total allergenicity. Fluorescence anisotropy and molecular modeling methods have shown that this stability may play a role in the resilience of Ara h 1 to processing techniques intended to decrease its allergenic potential [79].

Yang et al. [6] emphasized that X-ray crystallography data stored in repositories, such as the Protein Data Bank (PDB), are essential resources for MD simulations focused on examining food allergens, and that IgE-binding determinants identified computationally require experimental confirmation via inhibition studies using monoclonal antibodies. These structured datasets facilitate the precise modeling of epitopes and various functional areas of allergenic proteins.

Some allergens exhibit significant stability under harsh conditions, such as high temperatures or low pH levels, potentially increasing their capacity to induce allergic responses. For instance, Ses i 1 preserves its structural integrity throughout gastrointestinal digestion, rendering it highly reactive in sensitized individuals [16]. MD simulations aid in uncovering these characteristics by monitoring alterations in secondary structures and computing metrics, such as RMSD, to measure conformational changes during ligand binding [74].

The cross-reactivity of fish allergens with other food species is another key factor that has been examined using MD simulations. Common IgE-binding proteins found in diverse species, such as fish and chickens, underscore the intricacy of allergic reactions [33, 34].

Furthermore, examining ligand-binding sites and determining free-energy profiles using methods such as molecular docking combined with MD simulations may provide essential insights into fish allergen dynamics. The unfavorable results observed in experimental studies emphasize the significance of computational techniques in confirming hypotheses regarding protein-ligand interactions. For instance, trypsin digestion successfully isolated two allergenically active fragments (TM1 and TM2) from tropomyosin, confirmed by skin tests and RAST [25]. In these instances, MD simulations can enhance

experimental methods by offering different perspectives on molecular processes, which may not be possible through conventional laboratory techniques.

Parvalbumins show cross-reactivity owing to the conserved epitopes identified by IgE antibodies. Simulations targeting these epitopes can clarify the molecular basis of cross-reactivity and assist in the development of diagnostic instruments and treatment strategies.

Immune recognition processes that drive allergic reactions to parvalbumin are important. Structural characteristics, such as calcium-binding loops and hydrophobic areas, play a role in immunogenicity. Computational research has identified characteristics comparable to those of peanut allergens used in vaccine production [80]. Utilizing these principles with parvalbumins via MD simulations can uncover target areas to alter their structure while preserving their functional integrity. Advances in modeling methods have consistently enhanced our understanding of allergens, such as parvalbumins. Initiatives like VITAL (Voluntary Incidental Trace Allergen Labelling) highlight the significance of ongoing updates in methods for allergen research [76].

Cross-reactivity between allergenic proteins is an essential factor in allergen research, particularly for fish allergens. Research on fish parvalbumin isoforms [39] has revealed notable variations in amino acid sequences and IgE-binding abilities, which are essential for understanding cross-reactivity. For example, differences in sequence identity among isoforms can lead to varying IgE binding, as shown by a comparison of parvalbumin isoforms from Asian sea bass.

High-performance computing (HPC) plays a central role in MD simulations of allergenic proteins by enabling longer simulations that capture complex structural changes under varying conditions. For example, Smith and Raghavan [52] used GROMACS-based simulations to examine protein dynamics over timescales of 2, 20, and 200 ns, highlighting both the computational cost and the value of extended simulations in food allergen research. MD approaches have also been applied to study enzymatic hydrolysis treatments, assessing how structural modifications influence immunoreactivity [15].

Taken together, the MD simulation literature on food allergens reveals a nuanced relationship with experimental findings. In several cases, MD has directly confirmed experimental observations; for example, simulations corroborate the thermal stability of parvalbumin and tropomyosin observed in cooking studies, and support the structural basis of cross-reactivity across fish species identified by IgE-binding assays. In other cases, MD refines experimental findings by providing mechanistic detail that experiments cannot, for instance, explaining why HHP produces variable allergenicity outcomes by showing how pressure differentially affects conformational versus linear epitopes depending on protein identity and pressure level. However, cases in which MD directly contradicts experimental findings remain rare in the published literature, likely reflecting a publication bias toward confirmatory results. This gap highlights the need for MD studies designed to challenge or test experimental hypotheses rather than simply modeling known outcomes.

Advancements in MD simulations

The combination of machine learning (ML) and MD simulations offers a novel strategy for allergen research, particularly for examining fish allergens. ML algorithms can analyze large datasets produced by MD simulations, allowing the discovery of patterns and relationships that are challenging to identify manually. This collaboration boosts the predictive power of MD simulations, enabling more precise modeling of allergenic protein behavior under different conditions. MD simulations utilize the principles of classical mechanics to represent atomic interactions in proteins, offering insights into conformational alterations and epitope accessibility that affect allergenicity [74]. Nonetheless, these simulations frequently generate high-dimensional data, necessitating sophisticated analytical tools for insightful interpretation.

ML methods, including neural networks and support vector machines, have been utilized to examine these datasets, aiding the prediction of allergenic epitopes and their interactions with immune system elements [6, 81]. For example, the combination of bioinformatics tools and ML has facilitated the detection

of possible allergenic epitopes by comparing the results of various computational approaches. The use of ML extends beyond data analysis and aids in enhancing the MD simulation parameters.

Publicly available databases that include data on conformational epitopes serve as training sets for ML models, facilitating the assessment and refinement of epitope prediction techniques [6]. These models can recognize the structural characteristics associated with immunogenicity, thereby assisting in the development of hypoallergenic food items and therapeutic treatments.

Additionally, progress in high-throughput technologies, such as CyTOF (Cytometry by Time-of-Flight) and single-cell quantitative PCR, has merged with ML-based analytics to reveal signaling pathways related to immune tolerance and sensitization [7]. These results enhance MD simulations by offering a wider perspective on how molecular-level alterations lead to physiological reactions. The combination of ML with MD simulations also addresses issues concerning precautionary allergen labeling (PAL) in food items. ML-supported quantitative risk analysis can enhance the clarity of allergen management by delivering evidence-based recommendations for labeling practices [82]. This method aids regulatory bodies and builds confidence among stakeholders.

In general, the integration of ML with MD simulations provides a robust foundation for enhancing allergen research. This facilitates the ongoing enhancement of simulation methods and provides practical insights into allergenic mechanisms at the molecular and systemic levels.

Conclusion

This review examined how thermal and nonthermal processing treatments modify the structure and immunogenicity of food allergens, with fish allergens—particularly parvalbumin—serving as the primary model system. The evidence consistently shows that processing-induced changes in allergenicity are neither uniform nor predictable; the same treatment can reduce allergenicity in one protein while increasing it in another, depending on the processing conditions, food matrix, and the nature of the epitopes involved. The dual role of the Maillard reaction—simultaneously disrupting some epitopes while stabilizing others—exemplifies this complexity and underscores why allergenicity cannot be inferred from structural changes alone.

MD simulations have emerged as a valuable complementary tool for understanding these outcomes at the atomic scale. By modeling conformational changes, epitope exposure, and digestibility under simulated processing conditions, MD has confirmed experimental observations in several cases, for example, the thermal stability of parvalbumin, and refined mechanistic understanding in others, such as the pressure-dependent exposure of aromatic residues in HHP-treated proteins. However, most published MD studies use short timescales (2–200 ns) and a limited range of force field and solvent model configurations, which constrain reproducibility and the generalizability of findings. The translation from MD-derived structural metrics to immunological predictions remains a critical unmet challenge. A clear distinction should be drawn between what MD can currently demonstrate with confidence—such as processing-induced conformational changes and epitope exposure at the atomic scale—and what remains deductive, particularly the translation of these structural observations into predictions of clinical allergic responses.

Based on the literature reviewed, the following specific research pathways are proposed. First, MD simulations of sufficient duration are needed to capture statistically reliable structural changes; evidence from allergen studies suggests that simulations of 200 ns represent a meaningful improvement over shorter timescales [52, 53], and that further extending simulation duration under physiologically relevant stress conditions, including variable pH, temperature gradients, and food matrix components, remains a promising avenue for capturing slower conformational transitions relevant to IgE binding. Second, multi-allergen, whole-food-matrix processing studies that move beyond purified protein systems are essential for generating clinically translatable findings. Third, a standardized hierarchy of evidence for allergenicity assessment—from in vitro IgE-binding assays through animal models to clinical challenge studies—should be adopted across the field to enable meaningful cross-study comparisons. Fourth, regulatory frameworks

need to incorporate processing-validated allergenicity assessments rather than relying solely on protein presence. Finally, the integration of ML with MD simulation data offers a promising pathway for predictive allergen modeling that could accelerate the development of safer, hypoallergenic food products.

Abbreviations

AGEs: advanced glycation end products

CFIA: Canadian Food Inspection Agency

CyTOF: Cytometry by Time-of-Flight

Da: Daltons

DPCD: dense-phase carbon dioxide

EU FIC: European Union's Food Information to Consumers Regulation

FALCPA: Food Allergen Labeling and Consumer Protection Act

FDA: Food and Drug Administration

GROMACS: GROningen MAchine for Chemical Simulations

HHP: high hydrostatic pressure

HPC: high-performance computing

HPP: high-pressure processing

IgE: immunoglobulin E

IgG: immunoglobulin G

MD: molecular dynamics

MGO: methylglyoxal

ML: machine learning

OPA: *o*-phthalaldehyde

PCR: polymerase chain reaction

PDB: Protein Data Bank

Phe: phenylalanine

RMSD: root mean square deviation

RMSF: root mean square fluctuation

SASA: solvent-accessible surface area

Tyr: tyrosine

VITAL: Voluntary Incidental Trace Allergen Labelling

VMD: Visual Molecular Dynamics

Declarations

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Author contributions

PS: Conceptualization, Methodology, Supervision, Resources, Project administration, Formal analysis, Writing—review & editing, Visualization. YW: Investigation, Software, Writing—original draft, Visualization. VR: Supervision, Funding acquisition, Resources, Formal analysis, Project administration, Conceptualization. The authors have read and approved the submitted version.

Conflicts of interest

The authors declare no conflicts of interest in this manuscript.

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Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

As a narrative review of previously published literature, this study generated or analyzed no new data.

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