Review

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Review

Engineering Allergenicity: A Review of Non-Thermal Processing Approaches to Reduce Immune Reactivity in Foods

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Abstract

Food allergy rates are escalating globally, driven by the interplay of inherited traits, environmental triggers, diet, and gut microbiome composition. Sensitization typically occurs through dermal, respiratory, or gastrointestinal routes, with epithelial barrier dysfunction and Th2-skewed immune responses playing key roles. Understanding the mechanisms and pathways of sensitization is critical to developing effective mitigation strategies. Efforts are underway to evaluate how both traditional heating and innovative non-thermal techniques can modify allergenic proteins in commonly allergenic foods. Thermal methods can denature allergenic proteins but may also compromise nutritional and sensory quality. Unlike heat-based processes, innovative non-thermal methods—including pressure-based, light-based, plasma, and sound technologies—can restructure proteins and diminish IgE reactivity without compromising food integrity. However, outcomes are highly context-dependent, influenced by food matrix, processing parameters, and assessment methods. Limitations in current in vitro and in vivo models, lack of standardized allergenicity testing protocols, and insufficient clinical validation remain key barriers. This review synthesizes current knowledge of allergy mechanisms with technological approaches for allergen reduction, highlighting promising interventions, existing constraints, and the need for integrated research to develop safe, hypoallergenic foods.

Keywords: food allergy non-thermal processing; allergenicity reduction; pressure-based (HHP) treatment; electric field-based (PEF) treatment; light-based (UV) treatment; ionized gas (cold plasma) treatment; sound wave-based (ultrasound)

1. Food Allergy and Sensitization: Mechanisms, Pathways, and Risk Factors

An atypical immune reaction to dietary proteins defines food allergy, which is reported in roughly 6% of pediatric and 3–4% of adult populations. Symptoms most often affect the skin, gut, or respiratory tract. These conditions are typically categorized into those involving IgE antibodies and those that are cell-mediated [1,2].

Typically, the immune system develops tolerance to food-derived antigens through a mechanism called oral tolerance; however, in cases of food allergy, this regulatory process breaks down. Although a wide range of foods can elicit allergic reactions, a limited number of foods account for most clinically significant cases. Common allergenic foods include dairy products, eggs, legumes like peanuts, various nuts, and seafood such as fish and shellfish [2–4].

A reliable diagnosis of food allergy necessitates a methodical approach grounded in clinical evidence. This usually begins with an in-depth medical history, supplemented by specific tests such as serum IgE assays, cutaneous testing, dietary interventions, and oral food challenges when warranted. Recent breakthroughs in molecular allergology have facilitated the identification of key

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allergenic components, expanding both diagnostic accuracy and therapeutic potential. Nevertheless, the current mainstay of management remains strict avoidance of known allergens, comprehensive patient education, and readiness to manage accidental exposures—primarily through the use of emergency epinephrine [2].

Food allergies can develop through various routes of sensitization. Proteins implicated in Class 1 food allergies are acquired through ingestion and often exhibit resistance to heat, low pH, and enzymatic breakdown during digestion [5–7]. These allergens are often introduced during infancy or early childhood, a period when the immune system is particularly susceptible to dysregulation. By contrast, class 2 food allergies typically stem from initial immune sensitization to airborne particles like pollen, which share structural features with certain proteins found in uncooked fruits and vegetables. This molecular resemblance can provoke cross-reactive immune responses, often manifesting as symptoms of pollen–food syndrome [5,6].

Emerging evidence indicates that exposure to specific class 1 food allergens, including peanuts and eggs, has the potential to trigger sensitization through transdermal routes, especially among those who have compromised skin barrier integrity [8,9]. A large proportion of class 1 food allergens are glycoproteins that are soluble and stable under various processing conditions have molecular masses within the range of 10–70 kilodaltons, and they typically resist both enzymatic degradation and heat treatment. Representative examples include milk caseins, egg-derived ovomucoid, peanut vicilins, and lipid transport proteins lacking substrate specificity present in fruits like apples (Mal d 3) and grains such as corn (Zea m 14) [5,6]. Bet v 1, the principal allergen in birch pollen, serves as a model for class 2 allergens. It can trigger respiratory sensitization and lead to oral allergic symptoms following ingestion of structurally similar proteins like Mal d 1 from apples or Dau c 1 from carrots. A large proportion of known food allergens are sourced from a relatively small group belonging to protein families, including those in the members of the Cupin and Prolamin superfamilies, along with various pathogenesis-related protein groups [5,6].

The clinical significance of particular sensitizations differs. For example, the peanut proteins Ara h 1, Ara h 2, and Ara h 3 are commonly involved in more severe IgE-mediated allergic reactions, whereas Ara h 8—a protein structurally related to Bet v 1—is typically connected to milder allergic manifestations, such as oral allergy syndrome [10]. Despite structural similarities between proteins, true clinical cross-reactivity is often less common than laboratory tests suggest [1,6].

Food allergies can result in a range of clinical disorders affecting one or more organ systems. Many gastrointestinal disorders attributed to food allergies exhibit overlapping symptoms, but they can often be distinguished through careful clinical evaluation and diagnostic testing [11–13]. Additionally, some pediatric gastrointestinal conditions—such as colic, constipation, and reflux—are occasionally suspected to have allergic etiologies (Figure 1).

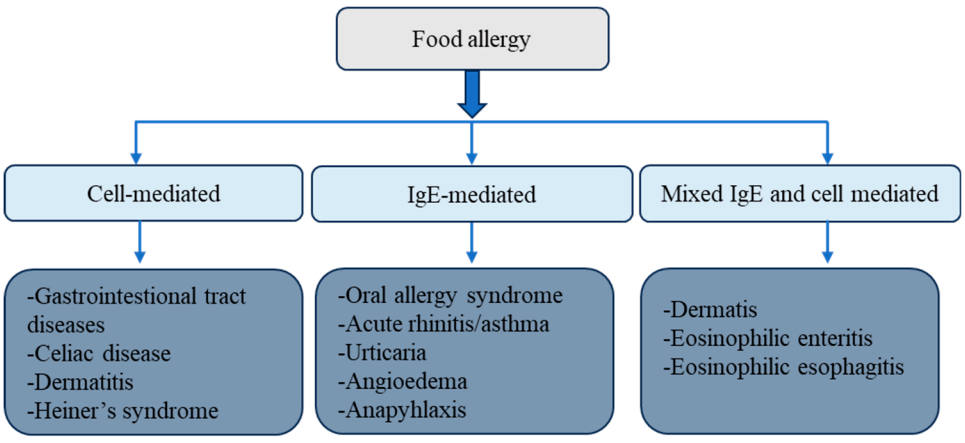

Figure 1. Grouping of food allergies (adapted from [169]).

Anaphylaxis represents a severe, potentially fatal reaction triggered by certain foods, often leading to severe complications and constituting a major reason for emergency interventions in

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outpatient care [14]. Life-threatening events are most frequently linked as a result of tree nut or peanut contact in youths and young adults who have both diagnosed food allergies and asthma, especially when epinephrine is not administered in a timely manner [12,15].

Adverse food reactions more broadly, termed food intolerances, arise from a variety of mechanisms. Unfavorable food reactions are typically classified into toxic and non-toxic categories. Among the non-toxic types, non–immune-based intolerances, including those resulting from enzyme deficiencies (like lactose intolerance) or from compounds such as biogenic amines, occur more frequently than true immune-driven food allergies. Nonetheless, immune-mediated food allergies, though less prevalent, affect millions globally and pose significant medical and economic burdens. In their most severe form, these allergies can cause life-threatening anaphylaxis [2,16,17]. According to the National Institute of Allergy and Infectious Diseases (NIAID), a food allergy is characterized as "a targeted immune response triggered by food, leading to repeatable adverse health outcomes" [18,19], encompassing both adaptive food allergens are sourced from a relatively small group of protein families, such as and innate immune pathways.

The term "allergy" was originally used to describe hypersensitivity reactions, including serum sickness observed in children treated with antiserum [20,21]. Later, hypersensitivity reactions were classified into four immunological types [22]. The predominant mechanism behind food allergies involves Type I hypersensitivity, where IgE antibodies target specific food proteins. These reactions involve complex immune cascades, including T cell activation and eosinophil recruitment [16]. Diagnosis often includes detection of specific IgE antibodies or IgE-related cellular responses [2].

Although there has been speculation regarding the involvement of food-specific IgG antibodies in Type II or Type III hypersensitivity reactions, current scientific evidence does not support a pathogenic role. Accordingly, professional societies discourage the use of IgG-based food allergy testing [23–25]. Type IV hypersensitivity reactions, mediated by T cells, contribute to conditions such as celiac disease, in which gluten proteins trigger autoimmune inflammation in the gut [26]. Food protein-induced enterocolitis syndrome (FPIES) is another condition driven by T cells, primarily impacting infants and occurring independently of IgE-mediated responses [27].

Recent studies also implicate the innate immune system in food-induced inflammation. Molecules such as wheat-derived amylase-trypsin inhibitors alongside specific milk oligosaccharides activate Toll-like receptor 4 (TLR4), initiating intestinal inflammation [4,28]. Such mechanisms may underlie disorders like non-celiac gluten sensitivity [4,29,30].

The occurrence of IgE-mediated food allergies is estimated to affect approximately 3% to 8% of children and 1% to 3% of adults in developed countries [2,17,31]. These conditions impose more than merely physical health threats but also emotional and social burdens due to dietary limitations and lifestyle adjustments. Major food allergenic substances encompass dairy, wheat, eggs, tree nuts, peanuts, sesame seeds, fish, as well as various fruits and vegetables [24,31]. While sensitivities to milk, eggs, and wheat frequently diminish by adolescence, allergies to tree nuts, peanuts, and seafood are more likely to continue into adulthood [2].

Estimating the real occurrence of food allergies remains challenging due to variability in diagnostic methodologies and reliance on self-reported versus clinically confirmed cases [32]. However, research indicates a rising trend in both the frequency and intensity of food allergies, influenced by multifaceted interactions among genetic predispositions and environmental elements such as dietary habits and lifestyle choices [9,33–35].

Studies in epigenetics have identified unique DNA methylation signatures in CD4+ T cells of individuals, particularly children, suffering from IgE-driven food allergies, highlighting the influence of gene-environment interplay throughout the progression of these allergies [36]. The "hygiene hypothesis," first proposed by Strachan [37], posits that reduced microbial exposure—due to smaller family sizes and improved sanitation—impairs immune system development, increasing susceptibility to allergic disease. Supporting this, children raised in anthroposophic environments—with minimal antibiotic or vaccine exposure and adherence to organic diets—show lower rates of allergies [38,39].

 doi:10.20944/preprints202508.0397.v2

Reduced exposure to diverse diets and microbes during infancy may hinder the development of immune tolerance, potentially leading to a rise in immune-related disorders in industrialized populations [40]. Allergic sensitization refers to the immune system's initial response upon first exposure to an allergen [4,41]. Two primary routes of sensitization have been described. Primary food allergens—commonly referred to as Class 1 allergens associated with egg, milk, and peanut—induce sensitization through the gastrointestinal tract [42]. Secondary allergens like Bet v 1, derived from birch pollen, sensitize via the respiratory tract and can cross-react with homologous nutritional allergens like Mal d 1 present in apples, resulting in oral allergy syndrome [4,43–46].

Epicutaneous sensitization—where allergens enter through inflamed or damaged skin—has also been proposed as a sensitization route. In murine models, such exposure leads to intestinal mast cell expansion and systemic anaphylaxis [47–49].

Multiple factors influence the likelihood of sensitization:

- **Allergen structure and stability**: Resistance to gastrointestinal degradation enhances allergenicity [5,6,50].
- **Epithelial barrier integrity**: Genetic mutations (such as those in the filaggrin gene) along with environmental influences—like alcohol consumption, pathogen exposure, and use of NSAIDs—can impair mucosal barrier integrity, thereby promoting allergen entry [51–53].
- **Adjuvant effects of food matrices**: Nonallergenic components and microbial products in foods may act as immunological adjuvants, promoting sensitization [6].

Conversely, secretory immunoglobulins—particularly SIgA and SIgM—strengthen mucosal defenses and promote oral tolerance. The heightened intestinal permeability and susceptibility to allergen-induced anaphylaxis observed in mice lacking SIgA transport can be mitigated through the induction of regulatory T cells [54,55].

2. Mitigation Strategies for Food Allergens

2.1. Allergen Elimination and Control Strategies

Managing allergens in the food supply chain necessitates a comprehensive, preventive strategy aimed at their elimination or reduction. One approach involves genetic modification or selective breeding to eliminate or suppress genes encoding allergenic proteins, such as the targeted elimination of Ara h 2 and Ara h 6 proteins in peanuts through gene editing [56]. Enzymatic hydrolysis is another promising approach, in which proteolytic enzymes cleave allergenic epitopes to decrease immunoreactivity. This method has proven effective in hydrolyzed milk and wheat products [57,58]. However, proteolysis may not fully eliminate allergenic potential. For example, Sen et al. [59] observed that Ara h 2 allergen present in peanut retained IgE-binding regions of allergenic proteins post-hydrolysis, especially when disulfide bonds were intact. This highlights the importance of linear epitopes in allergen stability, and suggests that reducing disulfide bonds alone may not sufficiently degrade allergenic regions.

In addition to ingredient-level interventions, allergen control measures begin with careful raw material management. This includes supplier verification and traceability systems to ensure allergen-free inputs [60]. In manufacturing, dedicated lines, validated sanitation, and equipment separation are necessary to avoid cross-contact [61–63]. Hazard Analysis and Critical Control Points (HACCP), ingredient monitoring, and accurate food labeling are central to allergen risk management. Finally, workforce training and allergen awareness programs are vital for maintaining safety throughout the supply chain [64].

2.2. Influence of Processing Methods on the Mitigation of Food Allergens

Food processing is essential for altering allergenic characteristics, simultaneously improving safety, extending shelf life, and preserving nutrient quality. Physical, chemical, and biochemical processing methods can influence allergenicity based on food type and processing conditions [65–

 doi:10.20944/preprints202508.0397.v2

67]. The structural integrity and allergenic potential of proteins are significantly influenced by processing. Changes in physicochemical properties and protein stability can alter immunogenicity [57,68,69]. The food matrix can modulate these effects, at times enhancing allergenic responses [70,71]. These treatments could decrease, enhance, or leave allergenicity unchanged. Importantly, certain processing methods may generate neoallergens—novel allergenic molecules formed during food treatment—that have the potential to exacerbate allergic responses [72–74].

Both heat-based and non-heat-based processing methods can modify the structure of allergenic epitopes, potentially decreasing allergenicity by breaking down key immune-reactive sites or, conversely, generating new allergenic compounds. The phenomenon of neoallergen formation has been recognized since the 1970s, when it was reported that processed foods could elicit allergic responses even when raw forms did not [75]. Subsequent findings identified neoallergens in processed pecans [76] and wheat flour [77]. Processing typically reduces allergenicity in many foods. Fermentation boosts nutrient quality and food preservation, in addition to impacting allergenicity. Lactic acid bacteria, for example, degrade IgE-binding epitopes in milk proteins, such as β-lactoglobulin, thus reducing allergenicity [78]. However, fermentation's effects are product-specific. Soy sauce, a fermented product, retains allergenicity despite microbial activity [79], while fermented dairy products such as yogurt often show reduced immunogenicity due to acid denaturation and proteolysis [80,81].

2.3. Thermal Processing and Its Effects on Allergenicity

Thermal processing is a commonly employed technique throughout the food manufacturing industry that can substantially modify the molecular structure and allergenic characteristics of proteins. Heat-induced denaturation leads to unfolding of proteins, disruption of disulfide bonds, and potential destruction of IgE-binding epitopes. For example, heating egg proteins such as ovomucoid or ovalbumin at 100°C for extended periods reduces their IgE-binding capacity [81]. Similarly, heat-based processes like roasting or boiling peanuts can alter allergenic components, including Ara h 1 and Ara h 2, potentially decreasing or, in specific situations, increasing their allergenic effects (Table 1) [82,83].

Table 1. Influence of thermal processing on various food allergen types (adapted from [57]).

Allergen Type	Response to Thermal Processing
Proteins homologous to Bet v 1, such as Mal d 1 in apples and Pru av 1 in cherries	Highly sensitive to heat; prone to unfolding. Susceptible to chemical changes such as Maillard reaction products in high-sugar foods and interactions with polyphenols, leading to decreased allergenic potential.
Proteins belonging to the Prolamin superfamily, including non-specific lipid transfer proteins (nsLTPs), 2S albumins such as Mal d 3, as well as tropomyosin and parvalbumin	Moderately heat stable; proteins unfold to a limited extent but tend to regain their structure upon cooling. Maillard reactions may still enhance allergenic potential.
Proteins from the Cupin family, such as Ara h 1 found in peanuts, and lipocalins including β-lactoglobulin and α-lactalbumin present in milk	Partially resistant to denaturation; undergo partial unfolding and tend to aggregate. Can form structural networks (e.g., in emulsions or gels). Heat can also lead to Maillard reactions, which may increase allergenicity.
Flexible proteins (e.g., caseins in milk, gluten storage proteins in wheat, ovomucoid in egg)	Structurally dynamic and heat-resistant; maintain mobility and do not exhibit classic denaturation behavior under thermal conditions. Their allergenicity remains largely unchanged.

 doi:10.20944/preprints202508.0397.v2

Baking and extrusion may reduce the allergenicity of wheat and soy by Maillard reactions and protein aggregation, although new allergenic determinants can sometimes be formed [84]. In specific instances, heat processing can raise resistance of certain allergens to digestive enzymes, potentially enhancing their capacity to provoke allergic responses. Thus, heat treatments must be carefully optimized based on food type and target allergen.

Application of humid thermal methods has been found to lessen the immune-reactive characteristics of various food substances. For instance, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) revealed a marked loss of protein bands in canned tuna and salmon extracts compared to raw or conventionally cooked samples. Immunoblot analyses revealed very limited IgE-binding activity in canned fish extracts. Results from ELISA inhibition assays and oral food challenges involving subjects allergic to canned salmon demonstrated a reduction in allergenic reactivity, suggesting that some fish allergens may be more heat-sensitive than previously believed [60,85].

Similarly, kiwi fruit allergy provides a compelling case of heat-mediated allergenicity reduction. Differences in individual allergic responses often correlate with protein variability and cross-reactivity with airborne allergens such as pollen. However, steam-treating kiwi at 100°C for five minutes and then mechanically processing it markedly diminished allergic symptoms in certain pediatric cases [86–88].

For milk allergens, β-lactoglobulin's ability to bind IgE antibodies was evaluated both in its purified form and as part of whole milk using the FEIA-CAP inhibition method. While mild heating at 74°C caused only slight reductions, significant decreases in IgE binding were observed at 90°C. Although binding decreased, elevated levels of β-lactoglobulin were still capable of neutralizing comparable quantities of IgE antibodies regardless of the heat treatment applied [80].

Hypersensitivity responses arise when IgE antibodies recognize particular regions on allergenic proteins. The interaction causes clustering of IgE receptors on mast cells or basophils, prompting the secretion of histamine and various inflammatory agents [89]. Thermal sensitivity varies by epitope type: conformational epitopes are more easily denatured, while linear epitopes are more heat-resistant [57,90]. As a result, heating may reduce allergenicity in some foods like lentils, enhance it in others like peanuts, or have no effect, as observed in boiled peas [91,92].

Pollen-related allergens in fruits and vegetables often become less immunogenic after heating. However, allergens in foods like shrimp can remain heat-stable and retain immunogenicity post-cooking. The type of epitope also affects stability: conformational epitopes, reliant on three-dimensional protein folding, are more susceptible to denaturation during processing, whereas linear epitopes may resist heat but are subject to enzymatic or chemical alteration. Genetic engineering allows precise modification of such epitopes to reduce allergenicity.

Protein structures respond differently to thermal conditions: mild heat (70–80°C) disrupts secondary structures, moderate heat (80–90°C) affects disulfide bonding, and intense heat (90–100°C) induces aggregation [90,93]. Heat may also initiate Maillard reactions, where lysine residues form covalent bonds with sugars, generating advanced glycation end products that could increase allergenicity [90].

Heating can enhance digestibility and reduce allergenicity in legumes such as chickpeas, lentils, lupins, and black gram [94]. In contrast, it can diminish the digestive breakdown comprising peanut allergens, Ara h 1 and Ara h 2 [83,95]. Thermal processing may also produce neoantigens, which elicit stronger immune responses [69,83]. The Maillard reaction is a key mechanism behind this neoantigen formation, where heat-induced protein-sugar interactions yield glycation products with heightened immunogenic potential [96]. According to a study conducted by [97], blood sera collected from 57 individuals analyzed with suspected meat allergy exhibited enhanced IgE affinity was higher for raw meat extracts than for those heated at 140°C for 20 minutes, suggesting thermal degradation of most allergens. However, chicken was an exception, as six out of 24 sera exhibited a stronger reaction to heated chicken meat. Common allergenic proteins in raw and cooked chicken were identified at 17–

 doi:10.20944/preprints202508.0397.v2

66 kDa, while heat-labile proteins (45 and 150 kDa) disappeared and neoallergens emerged in the 14–90 kDa range.

Elevated temperatures and prolonged heating have been demonstrated to markedly diminish the IgE-binding ability of wheat proteins. When wheat flour and dough (flour mixed with 50% water) were subjected to heat treatments at 80°C, 100°C, and 120°C for 10, 20, and 60 minutes, both neutral and acidic protein extracts showed reduced IgE reactivity in skin prick tests (SPT) and radioallergosorbent tests (RAST) using sera from cereal-sensitive adults. No further reduction in allergenicity was observed beyond 10 minutes at 120°C, indicating a thermal stability threshold. Pooled sera RAST scores dropped shifting from class 4, which corresponds to untreated flour, down to class 2 following heat treatment. Notably, heated dough demonstrated lower allergenic potential than heated flour at 80°C and 100°C, though this difference was no longer evident at 120°C [98].

Heat treatment by microwave heating of kiwi fruit to 40, 60, 80, and 90°C resulted in a progressive reduction regarding allergenic potential evaluated through skin prick tests (SPT) among three individuals allergic to kiwi. At 90°C, SPT responses were negative in two patients and markedly reduced in the third, indicating significant thermal degradation of allergenic proteins [99].

Protein solubility in minced beef decreases with extended heat treatment. Following heating at 85°C for up to 2 hours, SDS–PAGE revealed faint bands at 17.8, 19, 14, 20, 45, and >60 kDa, which were still present in thoroughly cooked samples (80°C, 20 min) via immunoblotting. Among these, the 17.8 kDa protein showed the highest IgE-binding activity among individuals diagnosed with an allergy to cooked beef (positive DBPCFC). In minced beef heated at 80°C, bovine serum albumin and gamma-globulin became undetectable after for durations of 10 and 3 minutes, consequently. Conversely, purified serum albumin maintained its stability when heated to 95°C for 15 minutes, while purified gamma-globulin was completely denatured after 15 minutes at 65°C [100].

Dry heat processing can influence the capacity of foods to cause allergic reactions through complex reactions including the Maillard reaction and enzymatic browning. These processes can irreversibly alter or destroy conformational epitopes. For example, Gruber et al. [101] demonstrated that cherry allergen Pru av 1 loses its IgE-binding capacity due to the Maillard reaction and oxidation catalyzed by polyphenol oxidase. In a similar manner, roasting hazelnuts decreased their allergenic potential, especially in people sensitized to birch pollen–associated allergens like Cor a 1.04 and Cor a 2 [102]. However, five out of seventeen birch pollen–allergic individuals still reacted to roasted hazelnuts in food challenge tests conducted under double-blind placebo-controlled conditions, indicating that roasting does not eliminate allergenicity for all patients. These findings suggest that individual sensitivity to conformational versus linear epitopes is a critical consideration in evaluating the capacity to cause allergic reactions in thermally processed foods. Conversely, Maleki et sl. [103] found that thermal processing actually enhanced the IgE reactivity of key peanut allergens Ara h 1 and Ara h 2 indicating that heating may enhance peanut allergenicity in some cases.

Because processing alone may not eliminate all allergens, integrating multiple strategies is more effective [104]. Thermal techniques such as blanching, pasteurization, canning, and roasting offer accessible and cost-effective allergen mitigation and microbial control [105]. Freezing inhibits microbial growth below −9.5°C (15°F), contributing to food stability [60,79]. Drying enhances shelf life and lowers storage costs, though its allergen-modifying effects vary. Edible crickets with three major allergens identified: actin, hexamerin-like protein 2, and tropomyosin, particularly *Gryllus bimaculatus* (two-spotted cricket), are increasingly recognized for their high protein content; nevertheless, knowledge about how processing affects their allergenicity is limited. Evidence showed that tray drying caused a greater decrease in the allergenic potential of heat-sensitive proteins [106].

Fiocchi and colleagues [107] assessed immune reactions to beef processed by freeze-drying and homogenization in a group of ten children diagnosed with sensitivity to beef proteins. Each participant showed positive responses in skin prick testing for both uncooked and heated beef, as well as positive placebo-controlled, double-blind food challenge test outcomes after consuming 180 grams of beef subjected to heat treatment at 100°C for 5 minutes. Untreated bovine serum albumin elicited positive responses in all children in skin testing, with half also responding during the

 doi:10.20944/preprints202508.0397.v2

challenge. In contrast, only one child showed a positive skin test to freeze-dried beef, and none exhibited reactions in the challenge test. Beef prepared by homogenization did not provoke any positive test responses. In a related study, the same team [106] reported minimal immune reactivity to freeze-dried and homogenized beef and freeze-dried lamb meat, with just a small fraction (2 of 12) of children with confirmed beef protein sensitivity affected [88].

2.4. Non-Thermal Processing for Allergen Reduction

Non-heat-based processing approaches have attracted significant interest as effective approaches to lower food protein allergenic potential without compromising nutritional value, taste, and functional characteristics. Unlike traditional thermal treatments, non-thermal methods such as high-pressure processing (HPP), cold plasma, pulsed electric fields (PEF), ultraviolet (UV), and ultrasound (US), treatments provide more targeted approaches for modifying allergenic proteins. These techniques can disrupt protein structures, mask or cleave IgE-binding epitopes, and trigger biochemical changes that reduce immunoreactivity. With the global increase in food allergy prevalence, non-thermal interventions provide a compelling alternative for developing hypoallergenic food products while maintaining consumer acceptability and regulatory compliance (Table 2).

Table 2. Overview of nonthermal methods for allergen reduction (adapted from [90,95,104,105]).

Technology	Mechanism of Action	Limitations
High-Pressure Processing	Disrupts non-covalent bonds within proteins; alters protein conformation through aggregation and gelation, affecting epitope exposure; enhances enzymatic hydrolysis.	Dual effects of pressure require accurate control; combining with other treatments may be necessary to optimize results.
Pulsed Electric Fields	Alters the secondary structure of allergenic proteins.	Often used alongside heat treatments to improve efficacy.
Pulsed UV Light	Delivers high-intensity UV pulses that cause photochemical modifications in proteins, including structural changes and epitope destruction	Limited penetration depth; potential degradation of sensitive nutrients; treatment uniformity can be challenging depending on food surface characteristics.
Cold Plasma	Reactive oxygen and nitrogen molecules (ROS and RNS) engage with antigens, leading to changes in protein structures.	High equipment costs; limited understanding of how cold plasma mitigates food allergens; potential cytotoxic effects of treated liquids warrant further investigation.
Ultrasound	Cavitation generated by ultrasonic waves breaks peptide bonds, leading to irreversible unfolding and structural disruption of allergenic proteins.	Can negatively impact product color, flavor, and nutritional quality.
Gamma Irradiation	Allergenic proteins absorb radiation, altering their 3D structures; free radical formation leads to water radiolysis and alteration of amino acid side groups.	Effective dose levels are not clearly established; not all protein-rich foods are currently approved for irradiation in the EU; concerns remain regarding food irradiation safety.

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2.4.1. High Hydrostatic Pressure and Allergenicity in Foods

High hydrostatic pressure (HHP) treatment is a preservation technique which exerts pressures between 100 and 600 MPa to food items, usually conducted at room or chilled temperatures without using heat. This treatment is particularly impactful on protein molecules, as it disrupts non-covalent interactions for example, hydrogen bonding, ionic bonds, hydrophobic forces, in addition to van der Waals interactions [108,109]. These interactions are fundamental to maintaining protein secondary, tertiary, and quaternary conformations. However, unlike thermal processing, HHP generally does not cleave covalent bonds such as peptide bonds or disulfide bridges unless extremely high pressures or extended treatment durations are applied [110]. As a result, while the primary amino acid sequence typically remains intact, HHP can lead to significant unfolding and denaturation of proteins, modifying their structural and functional characteristics [111].

One of the most critical implications of HHP-induced protein denaturation is its effect on allergenicity. Food allergens are proteins or glycoproteins that possess immunologically active regions—referred to as epitopes, which are identified by Immunoglobulin E (IgE) antibodies in sensitized individuals [112]. Epitopes can either be conformational, shaped by the protein's three-dimensional folding, or linear, constituted by a continuous sequence of amino acids. HHP has the potential to disrupt these epitopes, especially conformational ones, by unfolding the protein structure and eliminating the spatial arrangements necessary for epitope recognition. Consequently, the IgE-binding capacity of many food allergens is reduced after HHP treatment [84].

In addition to epitope disruption, HHP can also cause protein aggregation. During unfolding, reactive groups become exposed, leading to intermolecular interactions and protein cluster formation This aggregation can result in the concealment of linear epitopes or the creation of neoepitopes—new epitopic structures that may be more or less allergenic depending on the protein and processing conditions [113]. Furthermore, HHP-treated proteins often become more susceptible to proteolysis. The unfolding of the protein enhances accessibility for digestive enzymes such as pepsin and trypsin, promoting more effective breakdown during gastrointestinal digestion. This reduced stability and increased enzymatic degradation decrease the likelihood of allergenic epitopes surviving digestion and triggering immune responses [112].

The efficacy of HHP in reducing allergenicity is not uniform across all food proteins and is dependent on multiple variables such as pressure magnitude, duration of treatment, and temperature, pH, and the composition of the surrounding food matrix. For instance, pressures exceeding 400 MPa are generally required to significantly reduce allergenicity in robust proteins like β-lactoglobulin (milk originated), tropomyosin (shellfish originated), or ovomucoid (egg originated) [114]. Additionally, the existence of additional food constituents, including lipids and carbohydrates, or salts—can stabilize protein structures or shield epitopes, thereby diminishing the effectiveness of HHP [115,116]. These matrix effects must be considered when designing HHP-based allergen control strategies.

HHP may induce modifications in the structure of allergenic proteins, which can lower their immunoreactivity by disrupting conformational epitopes. However, in some cases, HHP can expose hidden epitopes or modify existing ones, resulting in unchanged or even increased allergenicity. The result varies according to the type of protein, the intensity of the pressure exerted, and the inclusion of additional treatments such as heat alongside HHP. For β-lactoglobulin (BLG), one of the principal milk allergens, exposure to 600 MPa resulted in lasting changes in its secondary and tertiary structures, accompanied by the liberation of free thiol groups and an increase in surface hydrophobicity [117]. These structural changes enhanced the protein's IgE-binding capacity, indicating increased allergenicity post-treatment.

Kato et al. [118] demonstrated that HHP, when combined with 8 M urea, significantly reduced rice allergen levels. The proposed mechanism involved structural damage to allergens caused by pressure, followed by enhanced extraction with urea to remove these proteins. Egg allergenicity was shown to decrease when pressure was applied in combination with heat. Furthermore, the joint application of pressure and heat markedly decreased the allergenic response to hen's egg, compared

 doi:10.20944/preprints202508.0397.v2

to either treatment alone [119]. HHP applied to soybean sprouts at treatment at 400 MPa produced considerable reductions in the levels of Gly m 1, a key soybean allergen [120]. The treatment also reduced antigenicity of soybean protein isolates, with modifications in protein structure likely responsible for the lowered immunoreactivity. Overall, HHP is a beneficial strategy for allergen mitigation in food systems. However, the effects are protein-specific and process-dependent. When combined with thermal treatments or other chemical agents, HHP may significantly reduce allergenicity, making it an important strategy in the development of hypoallergenic foods (Table 3).

Table 3. Impact concerning how high hydrostatic pressure influences the allergenicity of different food products.

Food	Treatment parameters	Major Observations	Sources
Soybean	300 MPa at 40°C for 15 min	HHP notably reduced allergenicity in soybean sprouts, with only an 18% decrease in essential amino acids and overall nutritional value. HHP may offer a viable method for producing low-allergen soybean sprouts.	[2]
Soybean	400 MPa	Treatment improved protein solubility and hydrophobicity, while decreasing β-sheet content.	[3]
Soy protein isolate (infant formula)	300 MPa for 15 min	Allergenicity reduced by 46.8% due to structural and interaction changes in SPI, suggesting enhanced safety in allergic individuals.	[4]
Peanut	150–800 MPa at 20–80°C for 10 min; 60–180 MPa	No major changes observed in allergen secondary structure under HHP. However, high-pressure microfluidization reduced Ara h 2 allergenicity by modifying its structure and increasing UV absorption and hydrophobicity.	[5,6]
Tofu	300 MPa at 40°C for 15 min	HHP did not change tofu protein composition but lowered intensity of some protein bands. Allergenicity remained unchanged.	[2]
Rice	100–400 MPa for 10–120 min; 300 MPa for 30 min + Protease N	Pressure facilitated allergen release into surrounding solution; with protease, allergens were nearly eliminated from rice grains.	[7]
Almond	600 MPa at 4–70°C for 5–30 min	No significant change in allergen concentration or IgE-binding capacity as per SDS-PAGE, WB, and ELISA.	[8]

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Whey protein isolate	200–600 MPa at 30–68°C for 10–30 min	The antigenic response of β-lactoglobulin increased with rising pressure, temperature, and duration of treatment.	[9]
Skim milk	200–600 MPa at 30–68°C for 10–30 min	β-lactoglobulin levels rose post-treatment; thermal addition mitigated allergenicity, but HHP-treated samples retained higher β-lg than controls.	[9]
Sweet whey	200–600 MPa at 30–68°C for 10–30 min	β-lg antigenicity rose at moderate temperatures and pressures; higher temperatures reduced but did not eliminate antigenicity.	[9]
Milk	>100 MPa + chymotrypsin/trypsin for 20 min;	Proteolysis of β-lg was accelerated under HHP, suggesting potential for hypoallergenic food production.	[10]
Apple	400–800 MPa at 80°C for 10 min	Mal d 3 allergen lost α-helical structure, becoming a random coil; immunoreactivity declined at ≥400 MPa.	[6]
Apple	700 MPa at 115°C for 10 min	Mal d 1 showed minimal changes at 20°C, but more pronounced at 80°C; Mal d 3 IgE reactivity dropped by ~30%.	[11]
Apple	600 MPa for 5 min	Mal d 1 immunoreactivity decreased by >50% with combined high pressure and heat.	[11]
Apple	600 MPa for 5 min; repeated consumption	Daily intake of HHP-treated apple gel for 3 weeks led to desensitization in highly allergic individuals; 90% negative skin tests post-treatment.	[12]
Carrot	500 MPa at 50°C for 10 min	Slight increase in β-sheet structure of Dau c 1; no change in immunoreactivity observed.	[13]
Carrot juice	400–550 MPa for 3–10 min; 500 MPa at 30–50°C for 10 min	HHP had no observable effect on the allergenic potential of carrot juice.	[13]
Celeriac	700 MPa at 118°C for 10 min	Allergenicity of Api g 1 was significantly reduced through combined pressure and thermal processing.	[11]
Celery	500 MP at 50°C for 10 min	Api g 1 structural changes were pressure-dependent but did not impact allergenicity.	[14]

2.4.2. Pulsed Electric Field (PEF) Technology and the Influence It Has on Food Allergenicity

Pulsed electric field (PEF) processing is an advanced, non-heat-based method in which brief, high-intensity electrical pulses—generally ranging from 1 to 50 kV/cm—are delivered to biological materials, including tissues and liquids. This approach results in electroporation, a temporary increase in cell membrane permeability caused by dielectric breakdown, facilitating improved mass transfer and promoting the release of internal constituents like proteins, lipids, and bioactive

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molecules [121–124]. PEF has gained broad acceptance across multiple domains within the food industry. Its applications range from microbial inactivation and juice extraction to drying pre-treatment, seed enhancement, detoxification of mycotoxins, and starch transformation [125–128]. Fundamentally, PEF's mechanism involves inducing electroporation in cell membranes through high-intensity, short-duration pulses. These pulses lead to reversible or irreversible structural modifications embedded in the membrane, thereby enhancing the transfer of substances and aiding in the recovery of intracellular compounds [123,124].

The performance of PEF systems is closely tied to the electrical behavior of cell membranes, which are largely composed of lipid bilayers acting as insulators. These membranes isolate the conductive environments inside and outside the cell, making each cell resemble a microscopic capacitor [122]. When immersed in a conductive medium and underwent an applied electric field, the cell alters the field's distribution, causing the membrane regions aligned with the field to experience intensified polarization at their poles [124].

A standard PEF system consists of a pulse generator capable of delivering voltages between 10 and 80 kV/cm, a treatment chamber equipped with insulated electrodes, a cooling unit, and a flow mechanism to ensure uniform treatment [122,125]. The system design is tailored through parameters like pulse duration, waveform, frequency, and electrode configuration. Pulse modes can be exponential decay, sinusoidal, monopolar, or bipolar. For instance, monopolar pulses have a constant polarity, while bipolar ones alternate it, minimizing polarization effects on electrodes. Different waveforms—such as square, decaying exponential, logarithmic, or oscillatory—offer varying benefits. Square waves, for example, ensure consistent membrane disruption, whereas decaying pulses can reduce energy use and thermal damage. Selecting the appropriate pulse configuration is essential to attain the targeted outcomes—such as microbial inactivation, compound extraction, or texture alteration—while maintaining the food's nutritional value and sensory attributes. Advanced control and safety systems are incorporated into industrial PEF equipment to ensure continuous, hygienic, and reliable processing [122,125,129,130]. Following exposure to the electric field, the cell—functioning as a dielectric sphere in a conductive environment—undergoes enhanced localized electric field intensity at its poles. These intensified regions are key sites for electroporation, often experiencing a field strength far exceeding the average applied to the bulk medium [124].

Although investigations into how PEF affects protein structures are still relatively scarce, multiple studies have documented significant structural and functional modifications that vary with the protein's nature [131], and evidence indicates that PEF may alter proteins' secondary and tertiary conformations. These structural changes are probably a result of the ionization of particular chemical groups and the disturbance of electrostatic forces [132,133]. Initially, protein molecules undergo polarization, which causes hydrophobic amino acid residues to be revealed to the surrounding solvent. This, in turn, promotes protein unfolding and aggregation when subjected to high-intensity electric fields [134]. In the food sector, PEF treatment has attracted significant attention due to its energy efficiency, capacity to retain nutritional value, and suitability for processing liquid-based food systems [135]. For instance, treatment of soybean protein isolates with PEF has demonstrated alterations in physicochemical properties, including denaturation and aggregation behaviors [136]. Similarly, studies on purified enzymes, including horseradish peroxidase and pectin esterase, showed reduced enzymatic activity after treatment, mainly as a consequence of alterations in the proteins' structural conformation [137]. However, it is important to consider that these changes may not result solely from the electric field itself; thermal effects due to associated Ohmic heating could also contribute to the observed outcomes [137]. Johnson et al. [138] examined how PEF treatment influences the structural integrity of specific food allergens, namely the peanut allergens Ara h 2 and Ara h 6, as well as the apple allergens Mal d 3 and Mal d 1b, all of which were produced through heterologous expression. Structural changes were assessed using circular dichroism spectroscopy and gel-filtration chromatography. The results indicated that PEF application caused no substantial changes in either the secondary structure or the degree of allergen aggregation. This outcome implies

 doi:10.20944/preprints202508.0397.v2

that PEF functions as a structurally gentle food processing technique, exerting minimal influence on the conformation of purified food allergens.

An investigation assessing PEF application at 25 kV/cm and 50 °C on Pru p 3, the primary allergenic protein in peach, showed that the treatment caused structural denaturation, as identified through ELISA with rabbit IgG. Nonetheless, this process did not alter the IgE-binding ability of Pru p 3, as demonstrated by a competitive fluorescent immunoassay using sera from individuals allergic to peach. Additionally, skin prick test results varied among individuals; more than 50% of participants exhibited increased skin reactivity following PEF treatment, indicating that patient-specific sensitization patterns may persist despite structural alterations in the allergen [139].

Experimental studies have demonstrated that PEF applications at high field strengths, particularly at 25–35 kV/cm for lengths spanning 60 to 180 μs, significantly impact the structural conformation of ovalbumin, including the α-helix content, thereby altering its immunogenic properties. The most substantial reduction in immunoreactivity was observed at 35 kV/cm with 180 μs treatment time [140], demonstrating the capability of this technology to lower the egg proteins' allergenic capacity.

In a molecular dynamics simulation study, Vanga et al. [141] explored the effects of dynamic and static-field conditions (2450 MHz frequency and 0.05 V/nm energy) at various temperature levels (300, 380, and 425 K) examining the structural characteristics of Ara h 6, one of the major allergenic proteins in principal peanut. The simulations revealed significant conformational modifications under all tested conditions, implying potential impacts on the allergen's functional properties. Subsequently, Vanga et al. [142] experimentally treated peanut flour with varying electric field applications of 10, 15, and 20 kV for 60–180 minutes. It was reported that time-dependent increases in α-helix conformational changes, probably resulting from the formation of novel random coil conformations and protein aggregates generated in the course of processing.

However, not all findings confirm a consistent reduction in allergenicity. For instance, Johnson et al. [138] reported no significant structural changes in allergenic peanut proteins of Ara h 2 and Ara h 6 (peanut 2S albumins), Mal d 1, and Mal d 3 (apple allergens) following electric fields application in the range of 0 to 35 kV/cm, with 2 Hz frequency and up to 130 kJ/kg energy input. Similarly, Paschke [143] found no notable reduction in celery allergen content when treated with a 10 kV PEF at 50 Hz.

A recent study investigated the modulation of ovalbumin (OVA) allergenicity using PEF treatment, highlighting structural changes linked to reduced immunoreactivity. PEF treatment at 6 kHz inhibited IgE and IgG1 binding by 30.41%, accompanied by visible microstructural surface cracks and loss of secondary structural elements in the protein. Spectroscopic analyses demonstrated a blue shift of the amide I spectral band, reductions in α-helical and β-sheet structural composition, and conformational changes in disulfide bonds. Increased fluorescence intensity suggested exposure of hydrophobic residues such as tryptophan and tyrosine. Molecular dynamics simulations further confirmed reduced structural stability and hydrogen bonding. These findings suggest that PEF disrupts allergenic epitopes by altering protein conformation, supporting its potential application in developing hypoallergenic egg products [144]. Compared with both thermal and alternative non-thermal methods, PEF seems to have a relatively modest effect on altering the structure and immunoreactivity of food allergens. These inconsistent outcomes highlight the need for further optimization of PEF parameters to achieve reliable and reproducible allergen mitigation.

2.4.3. Pulsed Ultraviolet (PUV) Light and Its Impact on Food Allergenicity

Pulsed light (PL) technology utilizes a series of brief, high-intensity bursts of broad-spectrum white light, predominantly containing 54% ultraviolet (UV) wavelengths followed by 26% light in the visible light spectrum along with 20% radiation in the infrared spectrum [145]. The PUV light spans wavelengths within the 200–1000 nm range, allowing it to deliver intense light energy within a very short duration [146]. As a result, PUV light can achieve energy intensities up to a thousand times greater than those of traditional continuous UV systems (Shriver and Yang 2011). The

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technology utilizes xenon flash lamps to generate short-duration (typically <1 ms), high-energy light bursts at a frequency ranging from 1 to 20 Hz. These pulses result in rapid microbial inactivation via photochemical, photothermal, and photophysical mechanisms, primarily targeting nucleic acids and protein structures. In addition to microbial control, PUV has been investigated for its potential to alter food allergens, degrade toxins, and induce structural and functional changes in biomolecules such as proteins and lipids, without significantly elevating product temperature or compromising sensory and nutritional qualities [147–150].

In food applications, PUV light has been investigated for its capacity to alter the structural configuration of allergenic proteins. This includes altering conformational epitopes and promoting protein aggregation, which may reduce allergenicity [146,150]. However, post-treatment re-association of peptide fragments might cause the development of novel epitopes—new structures that could increase the propensity of the food to cause allergies [151].

The functional mechanisms of PUV light inactivation can be grouped into light-driven photochemical, photothermal, and photophysical effects. These impacts play a role in chemical transformations and modifications in protein structure, influenced by rapid heating and the intermittent delivery of high-energy pulses [146,152].

When applied to peanut samples—including raw, roasted, and peanut butter extracts—PUV light at distances of 10.8, 14.6, and 18.2 cm for 1–6 minutes caused a marked lowering of the abundance of Ara h 1, Ara h 2, and Ara h 3 allergenic proteins, as evidenced by SDS-PAGE analysis. Increased energy doses (111.6 to 223.2 J/cm^2) and longer exposure times further enhanced this reduction, while greater distance from the light source diminished the effect. ELISA results showed that IgE binding decreased approximately threefold in raw peanut extracts and sevenfold in peanut butter slurry compared to untreated controls. These effects are attributed to changes regarding protein solubility and the generation of insoluble aggregates as a result of PUV processing [153].

PUV light treatment of soy extracts for 2, 4, and 6 minutes led to a time-dependent decline in major allergens such as glycinin and β-conglycinin. Indirect ELISA using sera from soy-allergic individuals showing decreases in IgE binding of 20%, 44%, and 50%, respectively. The detected decrease is likely associated alongside the creation of aggregated structures of allergenic proteins when subjected to PUV [146]. PUV application also reduced the immune response potential of the soy allergens Gly m5 and Gly m6 when applied at a distance of 8–10 cm for up to 6 minutes. Gel bands corresponding to Gly m5 disappeared after just 2 minutes of treatment, while Gly m6 bands remained visible even after 6 minutes, indicating differential susceptibility to degradation or precipitation [154].

Similarly, ultraviolet-C (UV-C) treatment has shown promising effects in reducing the capacity of milk proteins to cause allergic reactions. Significant reductions in IgE binding were also observed in milk allergens including α-lactalbumin, α-casein, and β-lactoglobulin after 15 “minutes of UV-C exposure. The decrease in immunoreactivity is likely due to alterations in discontinuous epitope structures. The enhanced effect in whey solutions has been linked to the greater pulse intensity and energy associated with PUV treatment [155]. The enhanced allergen reduction observed in whey proteins subjected to PUV treatment could be linked to the higher energy and pulse rate involved in the process [156].

For egg white proteins, ultraviolet exposure caused both aggregation and backbone cleavage; however, no substantial changes in immunoreactivity were observed compared to untreated controls. This suggests that the structural changes induced by UV light may not significantly affect allergenic epitopes in egg proteins [157].

Exposure of raw and shrimp extracts prepared by boiling, 5 mg/mL, to PUV light at a pulse duration of 360 μs, at a frequency of three pulses per second, and a distance of 10 cm led to a notable and irreversible reduction in allergenic reactivity. This reduction has been attributed resulting in the creation of high molecular weight protein complexes, likely due to cross-linking between tropomyosin and other heat-sensitive proteins [145,146]. This effect was correlated with the

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generation of high–molecular weight compounds through cross-linking between tropomyosin and other heat-sensitive proteins during the treatment [145].

PUV light has shown the capacity to lower allergenic potential in soy, peanut, milk, and shrimp products, though it appears ineffective for modifying egg allergens. Although these findings are encouraging, specifically aimed at producing hypoallergenic foods, additional research—such as clinical trials and in vivo experiments—is required to validate its wider use in food processing [142]. However, not all studies report a reduction in allergenicity with UV processing. For instance, Manzocco et al. [157] found that although ultraviolet exposure led to aggregation and backbone cleavage in egg white proteins, these structural changes did not significantly affect their immunoreactivity compared to untreated samples, suggesting that the epitope structures remained largely unchanged.

Overall, the evidence suggests that PUV light may be capable of lowering allergenic potential in foods like soy, milk, shrimp, and peanuts, while it appears to have limited impact on egg allergens (Table 4). Although the technology shows potential for developing hypoallergenic food items, additional research —particularly clinical and in vivo studies—is essential before broader adoption across the food processing industry [104].

Table 4. Outcome of UVC processing on allergenity of various foods (adapted from [169]).

Food	Allergen of interest	Treatment	Impact on immune reactivity	Sources
Milk	α-casein, α-lactalbumin	UVC treatment for 15 min	25% α-casein reduction	[166]
	β-lactoglobulin	UVC treatment for 15 min	27.7% whey fractions reduction	[166]
Egg	Ovalbumin	UV processing with (0.61 kJ/m² energy	No effect	[168]
	Ovomucoid	UV processing with 63.7 kJ/m² energy	No effect	[168]
Shrimp	Tropomyosin	PUV sterilization for 4 min	Reduced	[170]
Peanut	Ara h 1, Ara h 2, Ara h 3	PUV treatment on butter slurry for 1–3 min; peanuts in raw and roasted form for 2–6 min	67% reduction IgE binding of peanut butter slurry; 12.5 folds reduction, 100% reduction total extracts	[164]
Soy	Gly m5	PUV treatment (1–6 min)	100% reduction Gly m5 reduced	[165]
	Gly m6	PUV treatment (1–6 min)	Gly m6 retained	[165]
	Soy extracts (e.g., glycinin, β-conglycinin)	PUV treatment 2 min	20% reduction	[164]
		PUV treatment 4 min	40% reduction	[164]
		PUV treatment 6 min	50% reduction	[164]

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2.4.4. Gamma Irradiation and Its Impact on Food Allergenicity

Radiation refers to the transfer or release of energy across space or a medium as waves or particles. It is broadly classified into two types: Nonionizing radiation possesses lower energy compared to ionizing radiation. Nonionizing radiation does not have sufficient energy to dislodge electrons from atoms or molecules and is typically considered non-harmful, causing little to no chemical changes. This category includes ultraviolet light, visible-spectrum radiation, infrared energy, microwaves, and radio waves—each possessing lower energy levels and typically not employed in food processing. In contrast, ionizing radiation carries sufficient energy to dislodge electrons, resulting in the formation of ions. This form includes energetic electromagnetic waves and subatomic particles capable of altering molecular structures. Among ionizing methods, gamma irradiation is considered one of the most straightforward techniques [164,165]. The reduction in immunoreactivity was linked to protein denaturation, assessed through parameters such as turbidity, surface hydrophobicity, and chromogenic reactivity. These findings suggest that clumping of allergenic proteins could have a crucial role in minimizing allergenicity post-irradiation [166]. Exposure of wheat germ agglutinin (WGA) to irradiation was found to initially cause polypeptide chain fragmentation, followed by the creation of large, insoluble, non-crystalline aggregates, ultimately leading to a decrease in allergenicity [167].

Radiation has been established as a practical approach for preserving food while maintaining its nutritional value and sensory qualities. It induces structural modifications in food proteins—including fragmentation, aggregation, cross-linking, and alterations to amino acids—that can influence their immunogenic properties [168]. These changes are largely mediated by reactive oxygen species formed during the radiolysis of water when proteins are irradiated in aqueous environments.

Exposure to ionizing radiation at doses of 10 and 50 kGy (administered at 10 °C with a dosage rate of 10 kGy per hour) altered the conformational epitopes in peanuts, leading to a significant reduction in IL-4 cytokine production by splenocytes from sensitized mice [169]. Similarly, Luo et al. [168] demonstrated that irradiation of purified peanut allergen proteins and whole peanut extract (at 1, 3, 5, and 10 kGy, 10 °C) resulted in a notable decline in IgG binding, as detected by ELISA, with higher radiation doses enhancing the effect. Interestingly, up to 5 kGy, the IgG response for whole peanut extract exceeded that for Ara h 6 alone, potentially due to the presence of other components in the extract that shielded Ara h 6 epitopes.

A comparable pattern was reported by Zhenxing et al. [170] in shrimp, where protein extracts irradiated at doses between 3 and 15 kGy (10 °C, 1 kGy/h) showed reduced IgE binding. However, intact shrimp muscle initially exhibited increased IgE reactivity up to 5 kGy, which then declined at higher doses. A dose-dependent response to irradiation was observed in a study on cow milk allergy by Lee et al. [156], where IgE binding to isolated β-lactoglobulin (β-lg) increased up to a dose of 5 kGy. Beyond this level, protein agglomeration occurred, likely masking specific epitopes and causing a decline in allergenic potential. In contrast, Kaddouri et al. [171] reported enhanced recognition of anti-β-lg IgG antibodies following irradiation with 3–10 kGy at 13 Gy/min rate of both liquid-form and freeze-dried bovine milk and whey. These differing results may be attributed to the sample form—whether β-lg was isolated or present in a milk-based matrix—and the class of antibodies applied for detection. These findings suggest that the allergenic response of purified proteins and whole food extracts to irradiation can vary, which holds relevance for food industry applications. Furthermore, differences in dose rate among studies indicate that the impact of varying dose rates at a constant radiation dose on protein allergenicity remains unclear.

In other research, irradiation of whole almonds, cashews, and walnuts did not cause modifications in the structure of allergenic proteins or affect their capacity to trigger allergic reactions [172]. Conversely, investigations have demonstrated that irradiation might elevate immunological responsiveness of gliadin and wheat flour [173]. These opposing results may stem from the physical state of the samples—solid versus solution—which can influence how effectively irradiation reduces allergenicity. Overall, applying irradiation to food allergens in liquid form appears to be the most promising approach for minimizing their immunoreactivity.

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Ovalbumin, an egg allergen, was reduced after gamma irradiation with cobalt-60 at 100 kGy, whereas treatment at 10 kGy produced no notable effect [174]. This reduction was believed to result from alterations in the protein's molecular weight. Gamma irradiation is known to promote protein crosslinking—such as disulfide bond formation—and enhance hydrophobic interactions, both of which can lead to protein aggregation [175]. However, a 100 kGy dose exceeds levels typically considered safe for food processing, as doses up to around 10 kGy are generally recognized as safe for consumption [176]. In a separate study, Seo et al. [177] also reported reduced ovalbumin immunoreactivity in white cake samples irradiated at 10–20 kGy.

In studies involving crustacean allergens, such as shrimp tropomyosin, gamma irradiation at doses of 7 kGy and higher resulted in the disappearance of the characteristic 36 kDa tropomyosin band on SDS–PAGE, indicating structural degradation. Additionally, IgE binding was significantly diminished at the highest doses tested [178]. Multiple studies have demonstrated the effectiveness of gamma irradiation in modifying allergenic proteins. To illustrate, Seo et al. [174] reported that ovalbumin, a principal allergen. Gamma irradiation may promote protein cross-link formation through the creation of disulfide linkages and intensified nonpolar molecular interactions, which promote aggregation and possible loss of immunoreactive epitopes (Table 5) [166].

Table 5. Mechanisms by which PUV affects protein structure and allergenicity.

Mechanism	Details	Impact on Allergenicity	Sources
Photo-oxidation	PUV emits intense UV-C light (200–280 nm) that causes oxidative modifications, particularly in amino acids like tryptophan, tyrosine, and methionine.	Oxidative damage to amino acid residues alters the structure of allergenic epitopes.	[158]
Disruption of Disulfide Bonds	High-energy UV pulses can break disulfide bridges that maintain protein conformation.	Destabilization of tertiary structure reduces IgE-binding ability.	[160]
Epitope Modification	PUV alters conformational and linear epitopes through structural denaturation.	IgE-binding is reduced due to loss of native allergenic regions.	[68,101]
Protein Aggregation / Fragmentation	UV treatment may lead to cross-linking or fragmentation, depending on exposure time and intensity.	Aggregation can mask epitopes; fragmentation may eliminate allergenic potential.	[101,160,161]
Surface Effects	PUV has limited penetration depth (~microns); primarily impacts food surface proteins.	Effective for surface allergens; limited impact on allergens embedded within the food matrix.	[162,163]
Dose- and Matrix-Dependent Effects	Allergen reduction is influenced by pulse energy, duration, distance, and the optical properties (color, opacity) of the food.	Matrix composition may shield proteins or influence energy absorption, thus modulating allergenicity outcomes.	[164]

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2.4.5. High-Intensity Ultrasound and Its Impact on Food Allergenicity

Ultrasound technology has garnered considerable recognition in the area of food processing due to its non-thermal and energy-efficient nature. It involves the application of acoustic waves typically at frequencies beyond human auditory perception between 20 kHz and 100 kHz in food systems [180]. During the use of ultrasound at high intensity, it induces an occurrence described as acoustic cavitation—the process in which microbubbles form, expand, and collapse violently in a liquid environment [181]. The core mechanism involves cavitation, wherein alternating compression and rarefaction cycles form microbubbles that collapse violently, generating localized temperatures approaching 5000 K and pressures attaining 1000 atm [182]. These extreme conditions facilitate protein denaturation, potentially altering allergenic structures and epitopes. This collapse generates localized hot spots with extremely extreme heat conditions (up to 5000 K) and pressures (up to 1000 atm), although these effects are confined to the microscopic scale and last for only a few microseconds [183]. Alongside thermal effects, cavitation leads to intense shear forces, microjetting, turbulence, and formation of reactive species like hydroxyl radicals from water sonolysis), all of which can contribute to physical and chemical transformations in food matrices [184].

High-intensity ultrasound (HIU) has been explored for various food processing operations, including peeling of fruits and vegetables [185], reduction of oil content and shelf-life extension of fried products [186], and shortening of parboiling time in rice processing [187]. More recently, its potential to alter protein structure and reduce food allergenicity has attracted scientific interest. The primary mechanism by which HIU affects allergens lies in its capacity to alter the spatial structure of allergenic proteins. During sonication, proteins can undergo unfolding, aggregation, or fragmentation due to mechanical stress and localized heating, leading to changes in epitope exposure and antigenicity. Additionally, free radicals generated during cavitation may oxidize specific amino acid residues, contributing further to conformational changes [58,166].

Processing of shrimp samples to high-intensity ultrasound were conducted at a frequency of 30 kHz for durations ranging from 130 to 180 minutes and this treatment significantly reduced IgE-binding capacity in both isolated tropomyosin (the major shrimp allergen) and within unrefined shrimp protein extracts. In particular, IgE binding to the isolated tropomyosin was reduced by 81.3–88.5%, whereas binding in the shrimp extract decreased by approximately 68.9%, based on ELISA assays. Immunoblotting also revealed that prolonged ultrasound exposure brought about the formation of new, lower-molecular-weight protein bands, suggesting fragmentation of the allergenic protein. This structural disruption likely contributed to the observed decrease in allergenic reactivity. Interestingly, the treatment duration was relatively long, yet no significant temperature rise or quality deterioration of the shrimp product was reported [187]. This highlights one of the advantages of HIU as a non-thermal method for allergen mitigation. Zhenxing et al. [170] further confirmed that shrimp allergenicity decreased more significantly at elevated temperatures (50 °C) during ultrasound treatment. In another study, Zhang et al. [188] subjected shrimp tropomyosin (TM) to 15 minutes of ultrasound (100–800 W), reporting a substantial degradation of TM and associated reduction in allergenicity, as evidenced through ELISA and immunoblot analyses with sera obtained from allergic individuals. However, some shellfish allergens appear more resistant to ultrasound. Chen et al. [189] found minimal degradation and unchanged IgE-binding activity of arginine kinase (AK) in crayfish even after ultrasound treatment at 200 W and 30 °C for up to 180 minutes. Dairy proteins also showed limited response; Tammineedi et al. [155] found no meaningful alterations in SDS-PAGE profiles of α-casein, β-lactoglobulin, and α-lactalbumin after treatment with 500 W ultrasound at 20 kHz for up to 30 minutes. Similar findings were reported in other milk allergen studies [155,190].

In soy protein, Tammineedi et al. [155] demonstrated a 24% decrease in immunoreactivity following treatment using high-power ultrasound at 37 kHz for a duration of 10 minutes. The observed reduction is linked to changes in structural arrangements at the secondary and tertiary levels. Similarly, significant allergen reduction has been observed in roasted peanuts; Li et al. [187] reported decreases of 84.8% in Ara h 1 and 4.88% in Ara h 2 after exposure to 50 Hz ultrasound for two hours. Alterations concerning the architecture of the α-helical IgE-binding regions of these

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proteins are believed to account for this change [191,192]. Zhenxing et al. [170] further confirmed that shrimp allergenicity decreased more significantly at elevated temperatures (50 °C) during ultrasound treatment. In another study, Zhang, He et al. (2018b) subjected shrimp tropomyosin (TM) to 15 minutes of ultrasound (100–800 W), reporting a substantial degradation of TM and associated reduction in allergenicity, as evidenced by ELISA and immunoblotting employing sera obtained from individuals with allergies.

The efficiency of ultrasound appears to increase when combined with thermal treatment, suggesting a synergistic effect. Ultrasound processing offers several benefits, including reduced energy consumption, minimal chemical usage, enhanced mass transfer, and better retention of sensory qualities [193]. Given these advantages and its proven potential in reducing immunoreactivity in soy, peanuts, and shellfish, ultrasound is a compelling alternative to conventional processing methods. Nevertheless, optimization of key parameters—including frequency, length of treatment, and temperature—is essential to maximize its efficacy in allergen reduction across various food types. Overall, high-intensity ultrasound offers a promising approach to reduce the potential to trigger allergies in food proteins through an integration of physical and chemical effects (Table 6). While more data is needed to understand optimal parameters and food matrix-specific responses, current evidence supports its utility as part of an integrated strategy for allergen control in food systems [165].

Table 6. Effects of gamma radiation treatment on food allergens.

Food system	Radiation Dose (kGy)	Impact on allergen	Mechanism/Observation	Sources
Egg (Ovalbumin)	10–100	Reduced allergenicity	Change in molecular weight; protein aggregation and cross-linking (disulfide bonds)	[174,177]
White Cake (with egg)	10–20	Reduced ovalbumin reactivity	Reduction in IgE binding	[174]
Shrimp (Tropomyosin)	7–10	Undetectable tropomyosin band; reduced IgE binding	Protein denaturation, aggregation; turbidity and surface hydrophobicity changes	[145]
Shrimp (Tropomyosin)	1–15 + heat (100°C)	5–30-fold reduction in IgE binding	Synergistic effect with heat treatment	[170]
Egg (Ovomucoid)	10 + heat	Almost undetectable levels of ovomucoid	Irradiation more effective than heat alone due to ovomucoid heat stability	[156]
Tree Nuts (Almonds, Cashews, Walnuts)	1–25 ± heat	Minimal change in allergenicity	Allergens stable under irradiation and heat	[179]

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2.4.6. Cold Plasma and Its Impact on Food Allergenicity

Cold plasma is primarily used for the purpose of microbial reduction and food decontamination owing to its lower energy expenditure and reduced required temperature ranges compared to traditional methods [199]. Plasma, a form of matter composed of ionized particles, is frequently described as the fourth state of matter encompasses a blend of reactive entities, for example ultraviolet photons, charged ions, electrons, and reactive radicals, molecules, and excited atoms. These components are formed through the energy absorption and ion production of gases and are capable of interacting with proteins, resulting in conformational changes. Since most food allergens are proteins, it is hypothesized that cold plasma treatment could similarly alter their structures through these reactive agents [165,199]. Various research efforts have revealed the potential of plasma treatment in reducing food allergenicity. For example, Venkataratnam et al. [200] applied cold atmospheric plasma (80 kV) for different durations (0 to 60 minutes) to dry, fat-extracted peanut flour and intact peanuts. Competitive ELISA using bovine serum albumin showed up to a 43% reduction in antigenicity, accompanied by secondary structure changes detected through circular dichroism. In another investigation, direct-application dielectric discharge-generated cold plasma treatment for 5 minutes at 30 kV and 60 Hz led to a 76% decrease in shrimp allergen tropomyosin's immunoreactivity [166].

In one study, the impact of both direct and remote effects of cold atmospheric pressure plasma (CAPP) treatment on soy protein isolate immunoreactivity was examined [165]. Researchers measured factors such as mass of the sample, surface temperature, pH level, and hydrogen peroxide concentration. SDS-PAGE analysis revealed a noticeable decline of protein band density associated with to the primary allergenic components of soy, β-conglycinin (Gly m5) and glycinin (Gly m6). The most substantial decrease in immunoreactivity—ranging from 91% to 100%—was observed in the fraction of soluble proteins following direct CAPP treatment. Remote CAPP exposure also reduced immunoreactivity, though to a slightly lesser extent, with reductions of up to 89%. These reductions in immunoreactivity are linked to the disturbance or concealment of conformational epitopes by free radicals produced during plasma exposure, which hinders IgE antibody recognition [201]. Meinlschmidt et al. [154] reported that CAPP treatment of soy protein extracts for up to 10 minutes at 9, 10, and 11 kVpp (3.0 kHz) nearly eliminated Gly m5 allergen reactivity. This was likely due to alterations in conformational and linear epitopes or destruction of antibody binding sites, as shown through a sandwich ELISA employing human sera and monoclonal antibodies.

Alternatively, application of cold atmospheric plasma to milk α-casein at 13.56 MHz radiofrequency and 30.7 L/min argon gas flow for 5, 10, and 15 minutes. They found no significant reduction in immunoreactivity, possibly due to limited plasma exposure or insufficient treatment intensity [155]. These varied findings across food types suggest that cold plasma's effectiveness in allergen reduction is not uniform and is influenced by factors including sample composition along with processing parameters. To date, reductions in allergen levels have been observed in soy, peanuts, shrimp, and wheat after cold plasma treatment, while no notable changes have been reported in milk proteins. Furthermore, concerns remain regarding the quality degradation associated with this method. Several studies have linked cold plasma processing to undesirable effects, including accelerated lipid oxidation, nutrient loss (such as vitamins), and compromised sensory qualities [202–204]. Therefore, while cold plasma holds potential, its limitations highlight the need for continued research and the development of alternative food processing technologies.

2.4.7. Genetic Modification and Its Role in Reducing Allergenicity

Genetic modification (GM) involves intentionally changing an organism's DNA through biotechnological techniques, enabling the introduction, deletion, or modification of specific genes to achieve desired traits, allowing for the insertion, deletion, or silencing of specific genes to achieve desired traits. In the context of food production, GM is employed to increase crop productivity, boost resistance against pests and diseases, and elevate nutritional or functional qualities. Over recent years, its potential to reduce food allergenicity has become a growing area of research, although

 doi:10.20944/preprints202508.0397.v2

concerns regarding safety, stability, and consumer perception remain significant. One strategy to reduce allergenicity in foods through GM involves silencing the expression of specific allergenic proteins by employing post-transcriptional gene silencing or co-suppression mechanisms. This approach aims to prevent the production of allergenic proteins during gene expression. However, uncertainties persist with respect to the long-term stability of these modifications. Incomplete inhibition or loss of gene silencing could reintroduce allergens, posing potential health risks to sensitized individuals. Additionally, removing key proteins might impact the structural and functional integrity of food products, as many allergenic proteins serve essential functions in metabolism and development of the source organism [79].

Public skepticism also surrounds the possibility that modifying protein structures may inadvertently result in the emergence of new allergenic determinants or epitopes that the immune system may fail to recognize as harmless. Therefore, a more targeted strategy—such as altering specific IgE-binding epitopes while maintaining the general configuration and function of the protein—could provide a safer and more efficient approach [205].

Experimental studies provide insights into these strategies. For instance, Rupa et al. [206] modified the IgE-binding sites of ovomucoid, a major egg allergen, by introducing glycosylation at known epitope locations. This post-translational modification significantly reduced IgE-mediated immune responses in a mouse model, demonstrating the feasibility of epitope-focused interventions. However, since the modified proteins were expressed in yeast cells rather than in the natural egg matrix, it remains unclear how such changes would impact the final food product. In a study targeting peanut allergens, Chu et al. [207] applied RNA interference to suppress the expression of Ara h 2 and Ara h 6, two major peanut allergens. Although this intervention significantly decreased the binding of IgE antibodies to these allergens, the whole peanut extract still retained immunoreactivity, likely due to the presence of other allergenic proteins. Interestingly, the silencing of these genes did not lead to any noticeable morphological changes in the peanut plants. Similarly, Dodo et al. [56] reported reduced IgE binding following genetic silencing of Ara h 2. In another example, Herman et al. [208] established that silencing Gly m Bd 30, a key soybean allergen, nearly eliminated IgE binding. Detailed protein analyses and microscopic evaluations confirmed that the modified soybeans were structurally and compositionally similar to their non-GM counterparts and did not produce any new allergens. Although these findings highlight the potential of GM approaches to reduce allergenicity (Table 7), broader questions concerning consumer acceptance, regulatory approval, and long-term safety must still be addressed.

Table 7. A review of recent studies examining how ultrasound technology affects allergenicity during food processing (adapted from [194]).

Food	Target Allergen	Treatment	Immunoreactivity	Sources
Soy	Proteins	37 kHz, 10 min	Reduced by 24%	[195]
Milk	α-casein	20 kHz and 500 W (10–30 min)	No noticeable impact	[155]
	β-lactoglobulin	20 kHz and 500 W (10–30 min)	No noticeable impact	[195]
	α-lactalbumin	20 kHz and 500 W (10–30 min)	No noticeable impact	[190]
Peanut	Ara h1	50 Hz applied for 5 hours	Reduced by 84.8%	[196]
	Ara h2	50 Hz applied for 5 hours	Reduced by 4.88%	[197]

 doi:10.20944/preprints202508.0397.v2

Shrimp (boiled)	Proteins	30 kHz with 800 W at 0–50 °C range for 0–30 min	Reduction up to 40%–50%	[197]
Shrimp (raw)	Proteins	800 W with 30 kHz at 0–50 °C for 0–30 min	Reduction up to 8%	[197]
Crayfish	Tropomyosin	100–800 W for 15 min	Reduced	[198]
Shrimp and allergens	Multiple (including tropomyosin)	800 W and 30 Hz for 30–180 min	Reduction up to 75%	[170]
Crayfish	Arginine kinase	200 W at 30 °C for 10–180 min	No noticeable impact	[189]

3. Conclusions

The rising prevalence of food allergies necessitates the creation of efficient mitigation approaches that guarantee both consumer safety and food quality. A mounting body of evidence highlights the complex interplay between the molecular characteristics of food allergens, processing conditions, and the surrounding food matrix in determining allergenic potential. Multiple processing approaches, including thermal and non-thermal—induce distinct physicochemical modifications in food proteins, thereby influencing their digestibility, bioavailability, and IgE-binding capacity.

Thermal treatments such as moist heat generally reduce allergenicity by disrupting protein conformation and enhancing proteolytic susceptibility, while dry heat methods (e.g., roasting and baking) may increase allergenicity through the formation of neo-epitopes via Maillard reactions. Non-thermal technologies, including HPP, PEF, cold plasma, PUV, US, and gamma irradiation, offer promising alternatives for allergen mitigation by inducing conformational changes without compromising nutritional and sensory attributes. Additionally, microbial fermentation and enzymatic hydrolysis can effectively target linear epitopes, further reducing allergenic potential.

Despite these advances, translating reductions in IgE reactivity observed in vitro to clinically meaningful outcomes remain a significant challenge. The variability in digestive stability, structural resilience of allergenic proteins, and individual immune responses necessitates the use of complementary in vivo assessments including skin prick testing and oral food challenge procedures, and mediator release assays to validate hypoallergenic claims. Furthermore, a comprehensive understanding of both conformational and linear epitope modifications is essential for the design of processing strategies that minimize elicitation thresholds without compromising safety.

Future research should focus on standardizing allergenicity assessment protocols, integrating multi-hurdle approaches for simultaneous allergen and microbial risk reduction, and expanding studies to encompass emerging alternative proteins. Ultimately, a multidisciplinary approach that bridges food processing technology, immunology, and clinical validation is crucial for developing safe, effective, and industry-relevant solutions to mitigate food allergenicity and improve public health outcomes.

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