Physical Deactivation Oral Immunotherapy Methods for Food Allergens

Subjects: Allergy

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Food allergies represent a serious health concern and, since the 1990s, they have risen gradually in high-income countries. Deactivation rather than degradation should be the way of attenuating the immune response. Methods involve both physical and chemo-enzymatic routes.

Keywords: food allergy ; thermal treatment ; high-pressure processing

1. Introduction

In a broad sense, the terms allergy and hypersensitivity are usually employed to describe inappropriate immune responses after exposure to substances that are harmless in most subjects. Although the immune system can respond to almost any foreign molecule, there is little doubt that food allergies represent a dominant manifestation of hypersensitivity reactions and a major health concern worldwide affecting both adults and children [1][2][3][4][5]. It is well established that this type of atopic or anaphylactic hypersensitivity occurs when the antigen (or specific allergens present in food) reacts with IgE antibodies bound to tissue mast cells and triggers the extrusion of granules from such cells. This mechanism causes the release of granular contents into the surrounding fluids, namely histamine, heparin, or enzymes causing potent local effects on muscles and blood vessels, among others. Moreover, the stimulated mast cells make other chemicals like prostaglandins and leukotrienes, which enhance the above effects further. Overall, the antigen-IgE-mast cell reaction is responsible for acute inflammation. While this process can be a local protective mechanism, the manifestations of an allergic reaction may be of variable intensity, ultimately ending up in life-threatening anaphylactic shock.

Deactivation rather than degradation should be the way of attenuating the immune response. Methods involve both physical and chemo-enzymatic routes. The potentiality, advantages and limitations will be illustrated through representative cases, especially those documented in recent times.

2. Physical Deactivation

2.1. Thermal Treatment

Heating is surely the oldest and most common protocol to modify the structure and function of native proteins [6]. It is pertinent to note that even baked matrices may increase tolerance to food allergens by modifying their structure, as evidenced in the case of muffins containing egg and peanut allergens [7]. Food cooking and processing will inevitably meet the ubiquitous Maillard reaction, the non-enzymatic browning involving the condensation of proteins and carbohydrates. Although multiple reaction products have been identified in Maillard reactions, which are influenced by numerous factors like temperature, pH, and substrates [8], in general, the glycation of individual proteins alters their functionality and in vivo behavior. As a result, digestibility, bioavailability, and immune response, including allergenicity, are affected as well. Thermal treatment, however, does not always lead to a hypoallergenic material. Thus, several studies on β-lactoglobulin, a key cow's milk protein, indicate that heating and glycation caused by Maillard reactions with mono- and disaccharides results in a greater inertness toward proteolysis, thereby increasing the allergenicity [9].

Maillard reactions may affect the way specific IgE binds to food allergens. This can be achieved through different mechanisms such as (a) conformational disruption of secondary and tertiary structures that impair the IgE binding potential of the protein; (b) formation of agglomerates that enhance degranulation and/or enzyme liberation from basophils, and (c) formation of new epitopes (i.e., part of the antigen recognized by the immune system) due to aggregation and side Maillard reactions.

Dry heating of cow's milk protein in the presence of lactose leads to losses in solubility and digestibility, casein being the most altered protein. At low temperatures (60 °C), the decreased solubility is mainly induced by H-bonding and hydrophobic interactions, without impairing protein hydrolysis nevertheless. At high temperatures (130 °C), covalent protein cross-linking arises from Maillard reactions to a great extent, and is, however, responsible for poor digestibility and low solubility [10].

The aggregation trend of thermally treated codfish parvalbumin evidences an extensive ligation of lysine residues with glucose. However, this protein surface modification does not affect the folding nor significantly impair calcium binding. Glucosylation likely results in a lower hydrophobicity of the denatured state that slows down the aggregation process. In fact, aggregation and shape are comparable to those of the unmodified protein, and the resulting material undergoes faster digestion, thereby pointing to an effective treatment against allergic responses [11]. In a related study, the allergenicity of tropomyosin, the prevalent shellfish allergen, is reduced (up to 60%) through Maillard reactions with oligosaccharides. Results are consistent with conformational changes of the protein epitopes, with glycation leading to α-helix disruption as the main mechanism accounting for allergenicity reduction [12].

Heating along with other physical treatments (i.e., high pressure, ultrasound or electric fields), can unfold native egg white proteins that improve their hydrolysis and digestibility. However, Maillard reactions have apparently opposite effects by reducing the digestibility and also lowering the IgE binding of ovalbumin, the latter potentially alleviating allergenicity. Supramolecular analyses suggest protein-protein interactions between unfolded states leading to aggregates of varied morphologies depending on the experimental conditions. Heat-induced spherical aggregates show lower accessibility of digestive enzymes than linear counterparts. However, higher supramolecular networks (i.e., gels) from linear aggregates are likewise reluctant to undergo enzymatic digestion [13].

Conformational changes are also behind the thermal deactivation of ovotransferrin, with reversible unfolding occurring at 55–60 °C. As temperature increases, the secondary structure is progressively disrupted and covalent disulfide bonds are cleaved above 80 °C. Overall, heating appears to have a positive effect by eliminating potentially allergenic epitopes of the protein [14]. Analyses of cow's milk and hen's egg white proteins reveal different patterns of allergenicity after heating. Most proteins become weaker, although ovomucoid remains stable. Time and temperature are key variables and the presence of wheat during heating decreases the IgE binding to proteins [15].

Despite numerous and often inconclusive statements, the cautionary lesson inferred from a recent review is that food (thermal) processing can either hide epitopes or change conformations of native proteins, though it is insufficient to safely protect from allergenic responses [16]. In that sense, another product used to improve the efficacy and safety profile in oral food challenges is dehydrated egg white. The allergenicity of commercially available dehydrated egg white is equivalent to raw egg white, but the former avoids microbiologically risk [17].

2.2. High-Pressure Treatment

High-pressure processing (HPP) emerges as a non-thermal procedure, sometimes an alternative to thermal treatment. Its applicability as a sterilizing method against microorganisms and foodborne pathogens is well established. Foods are exposed to high pressures, usually in the range of 300–700 MPa (from nearly 3000 to 7000 atm) for short periods (from seconds to a few min), with structural variations in biomolecules involving disruption of H-bonds and saline/dipolar interactions, hydrophobic interactions, and even weak covalent bonds, all resulting in conformational changes and protein denaturation [18][19][20]. The effects of HPP upon the Maillard reaction are complex indeed, and it would be incorrect to state that high pressure alone reduces the unwanted changes associated with browning. However, effects on protein unfolding have been documented [8][21]. The combined use of HP and high-temperature processing has been reported to retard Maillard reactions in whey protein-glucose model solutions [22].

HP processing of milk alleviates atopic dermatitis in a mouse model. Animals show lower levels of IgE in serum compared with untreated mice. Moreover, HPP decreases the cytokine production of T cells, especially Th1 and Th2 types, although no significant variations are observed for IL-2, IL-6, and IL-17A cytokines [23]. The effect of HP (at 550 MPa) on β-lactoglobulin evidences notable conformational changes, although the digestibility is not seriously compromised. The lower antigenic response seems to be caused by the hiding of conformational epitopes, which result from aggregation under hydrostatic pressure [24]. A comparative analysis between thermal pasteurization and HPP on bovine milk indicates that β-lactoglobulin and IgG undergo denaturation within the range of 400–600 MPa, while others (casein micelles) show minor conformational changes. The immunogenicity increases up to 600 MPa (at 30 °C for 15 min) due to protein aggregation, while at 600 MPa the secretion of Th-type cytokines diminishes, which can be related to hidden epitopes caused by sequential protein unfolding and aggregation. In contrast, thermal pasteurization (72 °C for 15 s) has little or no effect on immunogenicity [25]. The allergenicity of α-casein is reduced by means of HP (200–600 MPa), although a more pronounced effect can be induced by ultraviolet-C radiation. Changes in α-helicity, β-turn, and protein hydrophobicity appear to be the main structural motifs affected [26]. The allergenicity of HP-treated ovomucoid, based on the liberation of β-hexosaminidase from human prebasophils sensitized with sera from allergic patients, is reduced as pressure increases from 0.1 to 400 MPa. The higher figures are 88.4% and 80.7% inhibition at 400 and 500 MPa, respectively. Irreversible structural changes are associated with the unfolding of the tertiary structure of ovomucoid by exposing hydrophobic sites at the surface, whereas polar domains (tyrosine side chains in particular) increase [27]. HPP of ovoalbumin, compared with thermal treatment, induces structural deformations that are more significant in dilute aqueous solutions than in concentrated protein samples. Under hydrostatic pressure, water molecules will be able to disrupt the secondary structure by altering the hydrophobic interactions, which are however maintained in condensed phases [28]. It is worth pointing out that egg lysozyme exhibits enhanced catalytic activity under pressurization, albeit the pressures employed are much lower (ca. 150 atm) than those commonly used in food processing. This effect can however be advantageously harnessed in biocatalytic applications of enzymes [29].

HPP of almond milk (450–600 MPa) is more efficient in reducing immunoreactivity than thermal treatments unless they are conducted at high temperatures (85–99 °C). Loss of protein (amandin) solubility, mainly due to aggregation, rather than epitope destruction may account for the decreased immunogenicity [30]. The allergenicity of walnuts, however [31], decreases under the combined use of HP and heating (650 MPa, 100 °C, 15 min), thereby disclosing again the complicated issue of protein deactivation in allergenic samples through physical protocols.

Together with milk and egg-based allergens, the allergenicity of shrimp is a serious concern as well. While thermal treatment (roasting) reduces allergenicity by inducing protein unfolding, most epitopes are linear and bioassays in mice resulted in anaphylactic responses similar to those caused by raw shrimp protein. However, the combined action of roasting and pressure processing reduces significantly specific antibodies, mast cell degranulation, and other vascular effects with respect to mice fed with the raw protein [32]. Pressure sterilization is thought to cause protein aggregation, hiding digested epitopes inside the three-dimensional structure. The hypoallergenicity can also be ascribed to the low binding frequency of IgE to troponin C.

Allergy to fish is a quite common phenomenon, albeit allergenicity to processed fish and seafood is often rare. This can be exemplified by tuna species as patients do not show allergic responses to canned tuna, even if occasional cases have been reported [33]. Fish parvalbumin is actually a panallergen that is responsible for cross-reactivity among a variety of fish species. The major allergen in tuna fish is parvalbumin (Thu a 1), which is highly stable toward thermal treatment. HP treatments of yellowfin tuna, for instance, in the range from 200 to 600 MPa for short times (5 min) have been reported as a method to extend the shelf-life of this product during storage (between 4 and 15 °C) [34][35]. Pressure not only reduces the bacterial load but also retards the loss of volatile nitrogen and histamine.

2.3. Pulsed Electric Fields

Pulsed electric fields (PEF) represent likewise an alternative to conventional thermal processing, especially when operating at high fields (>20 kV/cm) for short times (μs–ms). Applications range from extraction and recovery of nutritional substances to drying, freezing, and pasteurization processes, or detoxification, among others [36][37]. Compared with other physical methods, the assessment of PEF on allergenicity is still in its infancy and no clear-cut conclusions have been attained. Even if experimental setups involve essentially the coupling of a PEF generator to batch or continuous reactors, the outcomes are significantly influenced by parameters such as field intensity, voltage, or frequency. It seems that PEF at low electric strength (<10 kV/cm) does not induce any structural change of allergens, unlike the effects caused by HP or thermal processing [38]. However, the ability of ovalbumin to bind IgE and IgG1, together with the release rate of β-hexosaminidase is substantially inhibited at 10 kV/cm, while no effect takes place in the absence of electric pulses. These effects point to conformational changes and masking of sensitized epitopes that globally reduce allergenicity [39]. In addition, PEFs have been shown to inhibit the formation of Maillard products with respect to thermal treatment [40].

2.4. Ultrasound and Microwave Irradiations

The use of power ultrasound, with usual acoustic frequencies between 20 kHz and 500 kHz, is clearly an enabling technique that has gained considerable interest and application in food processing [41][42]. Most physical and chemical effects arise from cavitational collapse, i.e., the rapid formation, growth and implosion of microbubbles in liquids, thus generating enough kinetic energy, accompanied by shear forces and formation of reactive species that trigger subsequent reactions. Low-frequency sonication and short-time heating can be combined to ensure the stability of protein-based dairy formulations, for which prolonged heating causes thickening or gelling [43]. Ultrasound can promote the denaturation of proteins, albeit long irradiation times also lead to molecular degradation. Enzyme inactivation is dependent on acoustic parameters, especially frequency and power density, together with enzyme type, temperature, concentration, and pH.

Deactivation of enzymes that degrade food quality can be accomplished by ultrasound alone or in conjunction with other processes such as mild heating and low pressure to shorten the radiation time [44].

As expected, cavitation can alter the supramolecular organization of biomolecules in the vicinity of microbubbles and induce aggregation through noncovalent interactions. This phenomenon appears to be responsible for changes in the properties of bean proteins [45]. Short-time sonication has been reported to modify the secondary structure of fruit proteins, namely loss of α-helices and increase in β-sheets, which cause a reduction in the IgE binding while enhancing protein digestibility [46].

Sonication (25-kHz probe, 900 W, 20 °C) of milk casein in the presence of a nonionic emulsifier (tween 80) at different concentrations results in colloidal casein with high transparency. Transmission electron microscopy (TEM) reveals how ultrasound disrupts the proteinaceous material and leads to particles with diameters less than 100 nm. ELISA assays show that the IgE-binding ability of such colloidal casein decreases, whereas the LAD_2 mast cell line degranulation demonstrates the hypoallergenic character of ultrasound-treated casein as well. Such properties lasted for more than 30 days [47].

Unlike ultrasound, microwave (MW) irradiation constitutes an efficient thermal activation based on dielectric heating, involving the alignment of polar molecules in a rapidly oscillating electric field. This low-energy radiation can likewise modify the textural organization and molecular conformation of proteins. Reduction in the allergenicity of tropomyosin (up to 75%) present in shrimp has been described after MW heating at 125 °C for 15 min [48]. Although ultrasound (lacking quantum character) and MW (electromagnetic radiation) are very different in physical nature; synergistic effects have been observed by coupling both fields in simultaneous or sequential modes. Enhancements caused by the juxtaposition of such radiations derive from a fast thermal activation, while ultrasonic agitation improves mass transfer in heterogeneous mixtures [49]. Sequential MW and sonication have been applied to peach lipid transfer protein, which causes severe allergic reactions in sensitive patients. The protein in question is quite resistant to thermal processing and proteolysis. Unfortunately, the combined radiation is insufficient to reduce significantly IgE binding, as autoclave treatment does not decrease protein allergenicity either [50]. The effects of either MW or US on the secondary structure of egg white protein are chiefly influenced by temperature and irradiation times. Avidin activity decreases by MW heating from 60 to 80 °C (1 min vs 5 min-irradiation) and the unordered structures remain constant. Ultrasonic processing increases the proportion of β-sheets after 1 min-irradiation compared to the silent control, although the secondary structure content does not change appreciably after prolonged sonication [51].

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