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Peanut Allergy: Characteristics and Approaches for Mitigation

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Abstract: Peanut allergy has garnered significant attention because of the high sensitization rate, increase in allergy, and severity of the reaction. Sufficiently reliable therapies and efficient mitigating techniques to combat peanut allergy are still lacking. Current management relies on avoiding peanuts and nuts and seeds with homologous proteins, although adverse events mostly occur with accidental ingestion. There is a need for hypoallergenic peanut products to protect sensitized individuals and perhaps serve as immunotherapeutic products. Alongside traditional practices of thermal and chemical treatment, novel processing approaches such as high-pressure processing, pulsed ultraviolet light, high-intensity ultrasound, irradiation, and pulsed electric field have been performed toward reducing the immunoreactivity of peanut. Covalent and noncovalent chemical modifications to proteins also have the tendency to alter peanut allergenicity. Enzymatic hydrolysis seems to be the most advantageous technique in diminishing the allergenic potential of peanut. Furthermore, the combined processing approach (hurdle technologies) such as enzymatic hydrolysis followed by, or in conjunction with, roasting, high pressure and heat, ultrasound with enzymatic treatment, or germination have shown a significant reduction of peanut immunoreactivity and may emerge as useful techniques in reducing the allergenicity of peanut and other foods. This study represents our current knowledge about the alterations in allergenic properties of peanut via different processing mechanisms as well as evaluating its future potential, geographical based data on increasing sensitization, clinical relevance, eliciting dose, and current management of peanut allergy. Furthermore, the molecular characteristics and clinical relevance of peanut allergens have been discussed.

Keywords: enzymatic hydrolysis, heat treatment, hurdle technology, immunoreactivity, peanut allergy, processing

Introduction

Peanut or groundnut is an edible seed of the legume family. Peanuts are an important food crop known for their high source of protein and oil, which contain a high content of mono- and polyunsaturated fats and fibers (Toomer, 2018; Wang, 2016). Consumption of peanuts on daily basis has been linked to the reduction of mortality risk by up to 20% from any cause (Bao et al., 2013). On the contrary, for certain individuals, ingestion of minute quantities of peanut or peanut protein residue can be risky as it can provoke fatal and deadly anaphylaxis (Bock, Muñoz-furlong, & Sampson, 2001; Hourihane et al., 2017; Jeong et al., 2017; Turner et al., 2016). Peanut allergy is one of the most discussed food allergies because of its high prevalence, reaction severity, as well as a lack of reliable therapies. The influence of peanut allergy in recent years negatively impacted allergic individuals and peanut processing industries. Rate of prevalence, severity, and hospital admissions due to peanut allergies has increased significantly in recent years (Cianferoni & Muraro, 2012; Mullins, Dear, & Tang, 2009; Prescott et al., 2013). Nearly 10% of the world's population is allergic to some form of food according to a new report (Sicherer & Sampson, 2018), thus it can be termed as a global health issue due to the lack of controlling strategies. There are many reports that summarized the increased sensitization and reaction severity of peanut allergy (Kotz, Simpson, & Sheikh, 2011; Sáiz, Montealegre, Marina, & García-Ruiz, 2013; Sicherer & Sampson, 2018; Tang & Mullins, 2017). Here, in Table 1, we summarize the studies focusing on the prevalence and increasing pattern of peanut allergies based on different geographical regions. Despite the sparse amount of data on peanut allergy prevalence among Asian and other developing countries, some studies in the last 10 years have reported that the Asian population has a mixed level of sensitization toward peanut that is low in comparison to the rates reported for Western countries (see Table 1). Several genetic and environmental conditions such as ethnicity, gender, genetics, and early childhood exposure to peanut are crucial factors for the increasing prevalence and severity toward peanut allergy (Foong & Brough, 2017; Leung, Wong, & Tang, 2018; Sicherer & Sampson, 2010, 2018). The absence of a cure or unstandardized desensitization methodologies for food allergies may have exacerbated the

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Table 1–Geographically scattered summary of populations sensitized and/or allergic to food and peanut, their increasing prevalence, and the most potent peanut allergen/s.

Territory/Country		Period of study	Current sensitized/allergic population	Increasing prevalence of peanut allergy recorded in % or fold	Most prevalent peanut allergen	References
America	USA	1997-2011	8% Food allergy sensitization **5.8 M, around 1.5 to 2% of total population found allergic to peanut	**Around 50% increase were reported for the age group below 18 years	Ara h 2, Ara h 1, Ara h 3, & Ara h 6	Gupta et al. (2011); Sicherer and Sampson (2010, 2018); Valcour et al. (2017)
	Canada	2002 to 2007	**7 to 8%% (prevalence of food allergy); 0.4 to 1.4% (prevalence of peanut allergy)	Almost stable	Ara h 1, Ara h 2, & Ara h 3	Al-Ahmed, Alsowaidi, & Vadas (2008); Soller et al. (2015)
Australian	Australia	1995 to 2007	* 8.9% prevalence of peanut sensitization, 3% peanut allergic	**5 fold increase in food allergy cases	–	Mullins et al. (2009); Osborne et al. (2011)
European	UK	2001 to 2005	**21 M (32%) have some form of food allergy sensitization and 7.8 M (12%) are sensitized to peanut	2 fold increase **2 M growth in between 2008 and 2009 (**9.5%)	–	Nicolaou et al. (2010)
	Denmark	–	1.70% (food allergic), out of that, 0.59% adults are allergic to peanut allergens	–	–	Osterballe, Mortz, Hansen, Andersen, and Bindslev-Jensen (2009)
	Norway	–	**6.8% (food allergy sensitization)	–	Ara h 2, 6, 3, & 1	Kvenshagen, Halvorsen, and Jacobsen (2009); Namork and Stensby (2015)
	Spain, Sweden	–	–	–	Ara h 2, Ara h 1, Ara h 3 (0 to 6 years), & Ara h 8 (>8 years)	(Vereda et al., 2011)
	France	–	0.3% to 0.75% peanut allergy prevalence	–	Ara h 9, and Ara h 8	Osterballe, Hansen, Mortz, Host, and Bindslev-Jensen (2005)
Asian	China	1999 to 2009	7.3% have IgE mediated food allergy sensitization (rare incident of peanut allergy)	**Twofold increase in 1999; 9.9% to 18% in 2009	Ara h 1, and Ara h 3	Cong et al. (2008); Hu, Chen, and Li (2010)
	Taiwan	–	**3.44% (age <3 years), 7.65% (age 4 to 18 years), 6.4% (age >9 years), 5.1% (age 0 to 6 years) are sensitized with food allergy and around 1% population, especially in children, show peanut allergen sensitization	–	Ara h 1, Ara h 2, Ara h 3, and Ara h 9	Lin, Wu, Cheng, Huang, and Yeh (2012); Wu et al. (2012)
	Japan	–	**5% to 10% infants and 4.5% school going children reported for food allergy prevalence. Peanut allergy sensitization: 2.8% of all food allergy	–	–	Ebisawa, Nishima, Ohnishi, and Kondo (2013); Urisu et al. (2014)
	Singapore	–	0.47% to 0.64% peanut allergy sensitization	–	–	Shek et al. (2010)

** Represents the significant amount due to higher prevalence and increasing rate. M is the abbreviation for million. Some of the data reported in numbers in the original study have been converted to percent (%).

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issues, thus, increasing prevalence of food allergy (Tang & Mullins, 2017).

Because of the absence of reliable therapies and other controlling strategies, the current management of safety relies only on avoiding peanut or foods with homologous proteins (nuts and seeds) from the diets of affected individuals. However, avoidance of peanuts, nuts, and seeds can be very difficult for the sensitized individuals due to several factors such as ubiquitous use of peanut and peanut ingredients in food processing industries, unintentional contamination, unawareness, and cross contaminations (Joyce et al., 2006). Most cases of adverse events are apparently due to the accidental ingestion that is increasing despite increased awareness (Al-Muhsen, Clarke, & Kagan, 2003; Cherkaoui et al., 2015; Joyce et al., 2006). In addition, undeclared allergens are a big cause of food recalls from the market according to the report by the US Food and Drug Administration (FDA) and others (Ashley et al., 2018; Vandekerckhove et al., 2017), which is another serious issue for food manufacturers.

Immunotherapy treatments have been considered as a potential future application to desensitization and to improve the quality of life for affected population. Different therapies and prevention mechanisms have been explored toward desensitization mechanisms to affected individuals including: oral exposure between 4 and 6 months of age can prevent the development of peanut allergy in high risk infants by 80% (Learning Early About Peanut Allergy, LEAP; Du Toit et al., 2015), early oral immunotherapy (E-OIT; Vickery et al., 2017), oral immunotherapy (OIT; Bird et al., 2018), probiotic and peanut oral immunotherapy (PPOIT; Hsiao et al., 2017), passive and direct oral exposure (Pitt, Becker, Chanyeung, Chan, & Watson, 2018), subcutaneous immunotherapy (SIT), sublingual immunotherapy (SLIT; Fleischer et al., 2013), epicutaneous immunotherapy (EPIT; Jones et al., 2017), anti-IgE immunotherapy (Sampson et al., 2011), cytokine immunotherapy (Kishida et al., 2007), TH-2 based immunotherapy (O'Konek et al., 2018; O'Konek, Landers, Janczak, Wong, & Baker, 2017), TLR9-based immunotherapy (Berin & Wang, 2012), and traditional Chinese medicine (TCM) herbal therapy (Patil et al., 2011). Many of these strategies have shown great potential for desensitization, especially immunotherapies via different routes, such as OIT, EPIT, and SLIT are recently considered as cutting edge therapy, although they are still not used in clinical practice and the persistence of desensitization efficacies are still in question (Yee & Rachid, 2016). As a consequence, no treatment has yet been authorized by the FDA and the European Medicines Agency for the treatment of food allergies. Thus, there is a need to explore alternative strategies at the same time, in order to prevent adverse clinical reactions and promote safety for allergic individuals.

Processed peanut and peanut products are consumed in a wide range of products with ubiquitous uses such as peanut oil, peanut butter, ice cream, cookies, flour, confectionaries, as an ingredient in many foods, and so on. Processing methods such as heat (roasting, boiling, and frying), chemical treatment, acidic and enzymatic hydrolysis, and other novel technologies such as ultrasonication, high pressure processing (HPP), irradiation, pulsed ultraviolet light (PUV), pulsed electric field (PEF), and combined processing (Hurdle technology) have been explored to reduce IgE reactivity and allergenicity of peanut. Processing technology has the capability of altering the protein structure, function, and physicochemical properties of peanuts (Dyer et al., 2018; Maleki, 2004; Maleki et al., 2003; Maleki & Hulburt, 2004; Nesbit et al., 2012; Nesbit, Chung, Hulburt, & Maleiki 2018). The alteration of allergenic proteins influences its immunoreactivity either positively or negatively by structural unfolding, aggregation, degradation, and crosslinking, which can influence the allergenicity. A key mechanism in reducing protein allergenicity is to alter its structural and linear immunoglobulin E (IgE) binding sites (epitopes) (Comstock, Maleki, & Teuber, 2016; Dyer et al., 2018; Huang, Yang, & Wang, 2014; Khan et al., 2018; Li, Yu, Ahmedna, & Goktepe, 2013; Maleki et al., 2003; Nesbit et al., 2012; O'Konek et al., 2018; Rahaman, Vasiljevic, & Ramchandran, 2016; Vanga, Singh, & Raghavan, 2017). Enzymatic hydrolysis and hurdle techniques have the greatest potential to alter allergenic proteins while covalent modifications such as crosslinking, aggregation, oxidation, reduction, alkylation, and acylation also impart significant alteration in allergenicity (Cabanillas et al., 2011; Chung & Champagne, 2009; Kasera, Singh, Lavasa, Prasad, & Arora, 2015; Mikiashvili & Yu, 2018; Yu, Ahmedna, Goktepe, Cheng, & Maleki, 2011). Some processing treatment methods may lead to an increase in IgE reactivity and produce unknown complexes (neoallergen) in peanut as an effect of the Maillard reaction (Gupta et al., 2018; Kumar, Verma, Das, & Dwivedi, 2012; Maleki, Chung, Champagne, & Raufman, 2000). Destruction of conformational epitopes may expose masked epitopes while linear epitopes may be altered by chemical and enzymatic hydrolysis (Kasera et al., 2015; Lee et al., 2016; Rahaman et al., 2016). Besides IgE epitopes, protein structure also plays a critical role and functions in the allergenic potential of proteins (Chruszcz et al., 2011, Nesbit et al., 2012, Dyer et al., 2018; Maleki, Kopper et al., 2000; Maleki et al., 2003, Maleki, 2004, Maleki & Hurlburt, 2004). Therefore, the alteration in allergen structure subsequently alters its physicochemical properties such as solubility and digestibility, which in turn plays a significant role in altering the immunoreactivity (Apostolovic et al., 2013; Mikiashvili & Yu, 2018; Plundrich et al., 2015; Szymkiewicz & Jędrychowski, 2009; Vanga et al., 2016). The degree of alteration of allergens depends on the types and conditions of processing. Recently, various approaches have been explored that diminish the IgE binding and/or allergenicity of peanut to a greater degree than previously reported, which may prove to be a step toward improving quality of life for the sensitized individuals. To opt for a suitable processing method with applicability to various forms of products, it is crucial to understand how these processes influence the allergenic potential of peanut allergens at the molecular level.

On the basis of recent research outcomes, this work reports an updated overview on the current understanding and future direction of peanut processing toward immunoreactivity reduction mechanisms including clinical reliability as well as the pro and cons of each method. This review also summarizes the immunoreactivity mechanism, clinical relevance, threshold dose, data on allergen-specific prevalence, as well as the molecular characteristics of peanut allergens.

Peanut Allergy: Mechanism, Clinical Relevance, and Current Management

The mechanism of peanut allergy sensitization and subsequent adverse reactions are attributed to IgE-mediated type I hypersensitivity where the exposure to the susceptible individuals to peanut protein causes the production of specific IgE antibodies by B cells against allergenic proteins (Alberts et al., 2003). The sensitization and exposure to peanut allergens may occur either by direct ingestion, inhalation, or by cutaneous exposure. Once the exposure occurs, the signaling and immune sensitization process follow several steps as shown in Figure 1A. Peanut allergens are recognized by the antigen-expressing cell (APC), which then presents the antigen

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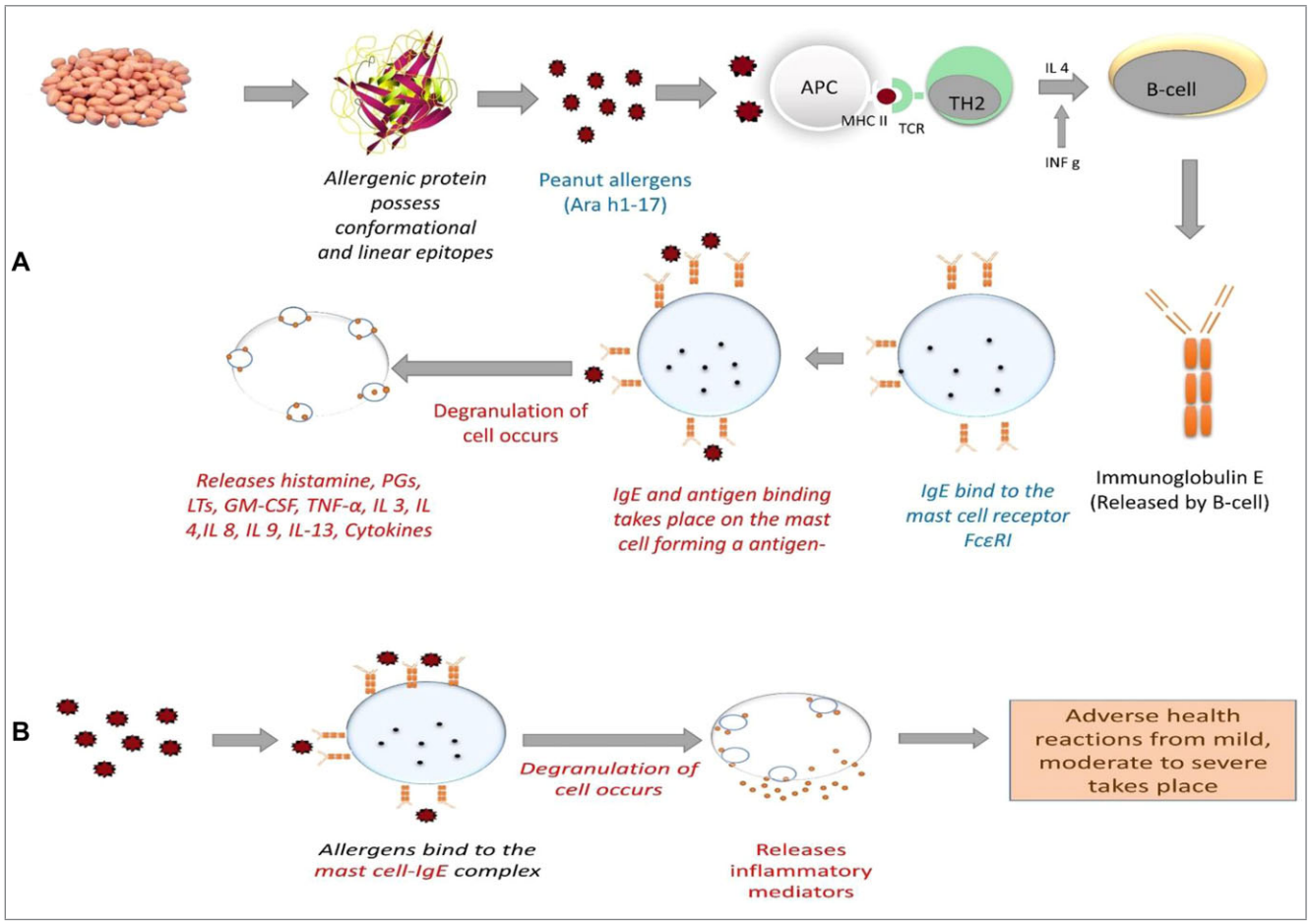

Figure 1–Schematic representation of sensitization and subsequent adverse reaction to IgE mediated food allergens. (A) Schematic representing the sensitization mechanism within a susceptible individual when exposed to allergenic proteins. Upon ingestion, peanut allergens are recognized by antigen presenting cells (APCs), which then present the allergens to the Th-2 type lymphocytes via MHC II. The Th-2 cells then secrete IL-4 and IL-13, which promotes IgE production by plasmocytes (for example, B-cells). These IgE circulate in the blood serum bind on the surface of mast cells via high affinity FcεRI receptors with primary exposure or at the sensitization stage. (B) Finally, mast cell degranulation occurs that releases the inflammatory mediators such as histamines, PGs, LTs, and cytokines into circulation, upon a subsequent exposures to allergen.

on the surface of APC by the major histocompatibility complex (MHC) class II. This complex interacts with T-helper cells (Th-2) through cell–cell interactions, in response to cytokines, which express the surface protein CD4 (thus called $CD4^+$ cell). The CD4 transmits the signal to B-cells and other immune cells in the form of cytokines (Interleukin [IL]-4, IL-5, IL-6, and IL-13), which causes B-cell proliferation and antibody production (Deo, Mistry, Kakade, & Niphadkar, 2010; van Hateren, Bailey, & Elliott, 2017; Wieczorek et al., 2017; Zhang, Collier et al., 2016). The Th-2 phenotype expression is a key factor responsible for the allergic response to peanut allergens, while the Th-1 expression produces IFN-γ and IL-10, which results indicate tolerance to peanut in healthy individuals (Deo et al., 2010; Yu, Freeland & Nadeau, 2016; Zhang, Collier et al., 2016). Th-1 is predominantly expressed in healthy individuals in contrast to peanut allergic individuals (Van Overtvelt et al., 2008). The selective deletion of allergen-specific Th-2 cells without any significant change in the frequency of Th1 cells expression suggests the desensitized mechanism during immunotherapy (Wambre et al., 2014). During cutaneous exposure, the inflammatory cytokines including IL-25 and IL-33 are discharged by skin epithelium, and thymic stromal lymphopoietin (TSLP), which triggers the response toward Th-2 cell-related mechanisms (Paul & Zhu, 2010). The activation of IgE-producing B cells occurs in response to the released mediators by Th-2 and causes the secretion of IgE antibodies into the bloodstream against the allergenic proteins, thus, peanut or food allergies are termed IgE-mediated hypersensitivity. The allergenic protein and IgE interact in serum and surface-bound IgE via high-affinity IgE receptors FcεRI on the surface of mast cells (Amin, 2012; Youssef, Schuyler, Wilson, & Oliver, 2010). Mast cell degranulation releases mediators such as histamine, prostaglandin (PGs), leukotrienes (LTs), platelets activation factor, and cytokines (IL-4, IL-5, and IL-13), which cause allergenic inflammation and adverse health reactions (da Silva, Jamur, & Oliver, 2014; Paul & Zhu, 2010; Pettersson et al., 2017).

The newly produced mediators are often absent in the resting mast cells. Upon the primary exposure to and production of IgE against an allergenic food, the circulating IgE is loaded onto the surface of mast cells and basophils via FcεR1 receptors (high affinity IgE receptor). In the case of subsequent exposure (Figure 1B), the specific IgE-mediated mechanism is activated immediately in response to allergens and causes degranulation of the immune-active cells such as the mast cells and basophils. The allergenic protein attaches to IgE bound to the FcεRI of the immune-active cell (mast cell) and cross-links adjacent IgE molecules on the surface of the cell. The cross-linking of IgE molecules on the surface

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of mast cells enables cross-phosphorylation of FcεRI receptor and a signal transduction cascade that promotes and causes the immediate release of prestored mediators (histamine and other inflammatory mediators; Galli, Tsai, & Piliponsky, 2008; Stone, Prussin, & Metcalfe, 2010). As a result, an immediate hypersensitivity reaction occurs, in which allergic responses within the individuals can vary from mild to moderate and severe, including anaphylaxis. The clinical symptoms include cutaneous (acute urtricaria, angioedema, morbilliform rashes, and flushing), gastrointestinal (nausea, dysphagia, vomiting, colic, abdominal pain, and diarrhea), respiratory (sore throat, difficulty in breathing, wheezing, sneezing, rhinorrhea, and cough), and circulatory symptoms (cardiovascular collapse). The mild reactions include hives or itchy skin, tingling sensations, nausea, runny, and congested nose (multisystem reaction; Muraro et al., 2014; Sampson, 1999, 2004; Sicherer & Sampson, 2006).

The current safety management is based on the packaging and labeling legislation applied strictly in the form of declared allergenic food/peanut residues that warn the allergic individuals to avoid consumption of those foods. Allergic individuals rely on current food labeling and strict avoidance of an allergic food until the availability of reliable therapies or non-immunoreactive peanuts by means of processing. Thus, current allergen detection and identification methods are important tools for labeling and regulatory purposes. ELISA, which is a solution-based method, is the most widely used tool by manufacturers and authorities to detect and quantify the presence of peanut allergens, because of its high sensitivity, high throughput, high specificity, and ease of handling (Schubert-Ullrich et al., 2009). It is known that the majority of adverse reactions in allergic individual occur from accidental ingestions. Different commercial ELISA kits with varying sensitivity toward quantification are available in the market to assay peanut allergens (Jayasena et al., 2015); hence, depending on the food matrix being tested, the detection should be selected with utmost care. Moreover in recent years, genomic approaches (such as PCR; López-Calleja et al., 2013; Pierboni et al., 2018; Scaravelli, Brohée, Marchelli, & Van Hengel, 2009; Watanabe et al., 2006) and proteomic-based approaches (such as mass spectrometry [MS]; Boo, Parker, & Jackson, 2018; Chassaigne, Nørgaard, & Van Hengel, 2007; Wei, Gledhill, & Maleki, 2010) has gained prominence to detect peanut allergen components. DNA-based approaches may have an advantage over ELISA as the detection is based on expressed DNA, which has a higher stability in food processing methods, particularly since processing may influence the extraction efficiency of proteins, and may affect the quality of protein analysis methods due to alterations in allergens (Pierboni et al., 2018; Scaravelli et al., 2009). On the contrary, genomic approaches to detect DNA components beside the allergenic proteins may also be challenging if the DNA is altered by such processing, and the detection is based on the source DNA material rather than its allergenic protein that directly represent its IgE binding and allergenicity. Furthermore, while MS methods are gaining in popularity because of their high specificity and detection of multiple allergens simultaneously (Boo et al., 2018; Croote & Quake, 2016), having potential to become benchmark detection tool for allergen monitoring. In contrary they are not portable, require high skill level, and can be very costly.

The current labeling legislation does not apply to the quantitative determination of peanut, it only acts as a declaration that either peanut-containing ingredients are present in a food product or are present through cross-contamination within a food manufacturing plant. The dose required to cause adverse reactions is not very clear and in some cases appear to be dose independent. Further understanding of eliciting dose may be crucial for the implementation of immune therapy, processing, and allergen labeling to avoid critical adverse events. In some cases, even a dose down to 2 mg of peanut can induce severe reactions (Hourihane et al., 1997). In recent years, there has been a curiosity in studying the eliciting dose of reaction to peanut allergens. In a study, Taylor et al. (2010) found that the ED_{10} (eliciting dose anticipated to provoke a reaction in 10% of the population) value in long term distribution models among two population groups for peanut was 14.4 mg of the whole peanut. Another study reported a long-normal dose distribution on the European population showed the ED_{10} value was 2.8 mg of peanut protein, which is equivalent to 11.2 mg whole peanut that is almost in line with the previous study (Ballmer-Weber et al., 2015). A study by Klemans et al. (2015) also suggested that the ED_{05} (eliciting dose anticipated to provoke a reaction in 5% of the population) value for peanut protein was 2.6 mg. Another study reported that the ED_{05} dose of 1.5 mg is safe for peanut sensitized individuals as only 2% of the population was met with some objective symptoms (Hourihane et al., 2017). Another study recently represented a larger population size among Europeans and Australians aimed to determine the full range of reactivity toward peanut allergens and reported that the ED_{10} was 15 mg of the whole peanut for Australians and 20 mg for Europeans, whereas the ED_{50} value for Australians was 220 mg and for Europeans it was 340 mg (Arkwright et al., 2018). Thus, these recent studies suggest varying ranges of ED_{10} dose levels for a minimum reactive range in populations was around 2.0 to 2.5 mg for peanut protein or 8 to 10 mg for the whole peanut.

Molecular Characteristics of Peanut Allergens

To date, 16 peanut allergen proteins have been reported and have been registered by the International Union of Immunological Society (IUIS), Allergen nomenclature subcommittee (allergen.org). Table 2 summarizes the different peanut allergens, their characteristics, and their IgE binding potency while Figure 2 represents the crystal structures of different peanut allergens elucidated to date. Allergens are classified into major and minor allergens. Those allergens that are found reactive to IgE in more than 50% of patient sera are suggested as major food allergens whereas below this range are considered as minor food allergens. Peanut allergens Ara h 1, Ara h 2, and, Ara h 3 are considered as major peanut allergens, and they also comprise the major proportion of peanut protein (Koppelman et al., 2001; Wu et al., 2016). Now days, Ara h 6 is also thought of as a major peanut allergen because of its similarity to Ara h 2 and recognition by serum IgE from allergic individuals (Koppelman, Hefle, Taylor, & De Jong, 2010; Kukkonen, Pelkonen, Mäkinen-Kiljunen, Voutilainen, & Mäkelä, 2015; Prodic et al., 2018). Besides the major/potent peanut allergens, research indicates that some other minor allergens can also potentially cause life-threatening symptoms, while other allergens may only cause oral symptoms (Arkwright, Summers, Riley, Alsediq, & Pumphrey, 2013; Mittag et al., 2004; Petersen et al., 2014). Specific features of these proteins such as structure, digestibility, solubility, resistance to heat and digestion, and other functional properties such as glycosylation and enzymatic activity impart immunological sensitization and are the basis for the varying IgE reactivity, thus, allergenicity (Besler, 2001; Bøgh & Madsen, 2016; Huby, Dearman, & Kimber, 2000; Platts-Mills & Woodfolk, 2011; Vanga et al., 2016; Vanga et al., 2017). The peanut allergenic individuals also possess an 86% risk of being sensitized toward other nut allergies due to cross-reactivity (Beyer et al., 2014; Maloney,

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Table 2–Summary of different peanut allergens registered to date and their characteristics.

Allergen group	Peanut allergen	Molecular weight and isoelectric point(pI)	Resistant to heat and digestion	Homology and cross reactivity	IgE binding potential	References
Cupin (Vicilin-type, 7S globulin)	Ara h 1	63 to 65 kDa pI 4.55	Highly resistant to heat (up to 88.3 °C)	Cross reactivity with other vicilin protein, Ara h 2, and Ara h 3	35 to 95%	Bublin et al. (2013); Burks et al. (1991); De Jong et al. (1998); Kleber-janke et al. (1999)
Cupin (Legumin-type, 11S globulin)	Ara h 3.01	60 kDa non reducing gel, 37 kDa in reducing gel pI 5.3	Highly resistant to digestion	47.2% homology with soybean glycinin; cross reactivity with other tree nut legumins, Ara h 1, Ara h 2, and Ara h 6	16 to 50%	Eigenmann et al. (1996); Flinterman et al. (2007); Jin et al. (2009); Vereda et al. (2011); Zhao et al. (2017)
	Ara h 3.02 (previously Ara h 4)	–	–	Ara h 3.02 share 95 to 98% homology with Ara h 3.01	–	
Conglutin (2S albumin)	Ara h 2	18 to 20 kDa pI 5.2	Highly resistant to heat and digestion	Ara h 2 shares 53% similarity with Ara h 6 and 35% with Ara h 7; Ara h 2 is cross reactive with some of the 2S albumin nuts and Ara h 1, 2 & 6	42 to 100%	Bublin et al. (2013); Flinterman et al. (2007); Hayen et al. (2018); Kleber-janke et al. (1999); Koppelman et al. (2010); Suhr et al. (2004); Valcour et al. (2017)
	Ara h 6	14.8 kDa pI 5.6	Highly resistant to heat and digestion	Ara h 6 is cross reactive with Ara h 1, 2 & 3	85% to 92%	
	Ara h 7	15 kDa pI 5.5	–	Ara h 7 shares homology with Ara h 2 and 6	43% to 80%	
Profilin	Ara h 5	14 kDa pI 14.6	–	Share homology with other profilin group proteins, sequence identity with grass profilin (Hev b 8), and high structural similarity with Bet v 2 protein	3% to 24% in birch pollen sensitized people	Wang et al. (2013); Wen et al. (2007)
Bet-v-1 (Pathogenesis related protein)	Ara h 8	17 kDa pI 5	Low heat and digestion stability	Structural similarity with other PR-10 allergens, high sequence similarity (84%) and sequence identity (68%) with soy Gly m 4 allergen	2.4% to 49% among birch pollen allergic people	Hurlburt et al. (2013); Mittag et al. (2004); Petersen et al. (2014); Valcour et al. (2017); Vereda et al. (2011)
Non-specific Lipid transfer proteins	Ara h 9	9.8 kDa pI 9.3	–	Cross allergenicity with peach and hazelnut nsLTPs (Pru p 3, Cor a 8)	Ara h 9 shares 8% to 60% strong association with peach allergy, 62% to 68% sequence homology with peach allergen Pru p 3 and a predominant allergen to the people of the Mediterranean area	Javaloyes et al. (2012); Lauer et al. (2009); Vereda et al. (2011)
	Ara h 16	8.5 kDa	–			
	Ara h 17	11 kDa	–			
Oleosin	Ara h 10	16 kDa pI 9.6	Weak resistivity to heat, ~59 °C is maximum tolerance	Cross reactivity with other soya and buck wheat group oleosins	–	Kobayashi et al. (2012); Petersen et al. (2015); Pons et al. (2002); Schwager et al. (2017)
	Ara h 11	14 kDa pI 10.4				
	Ara h 14	17.5 kDa				
	Ara h 15	17 kDa				
Defensin	Ara h 12	8 kDa reducing SDS	–	–	–	Petersen et al. (2015)
	Ara h 13	8 kDa reducing SDS	–	–	–	

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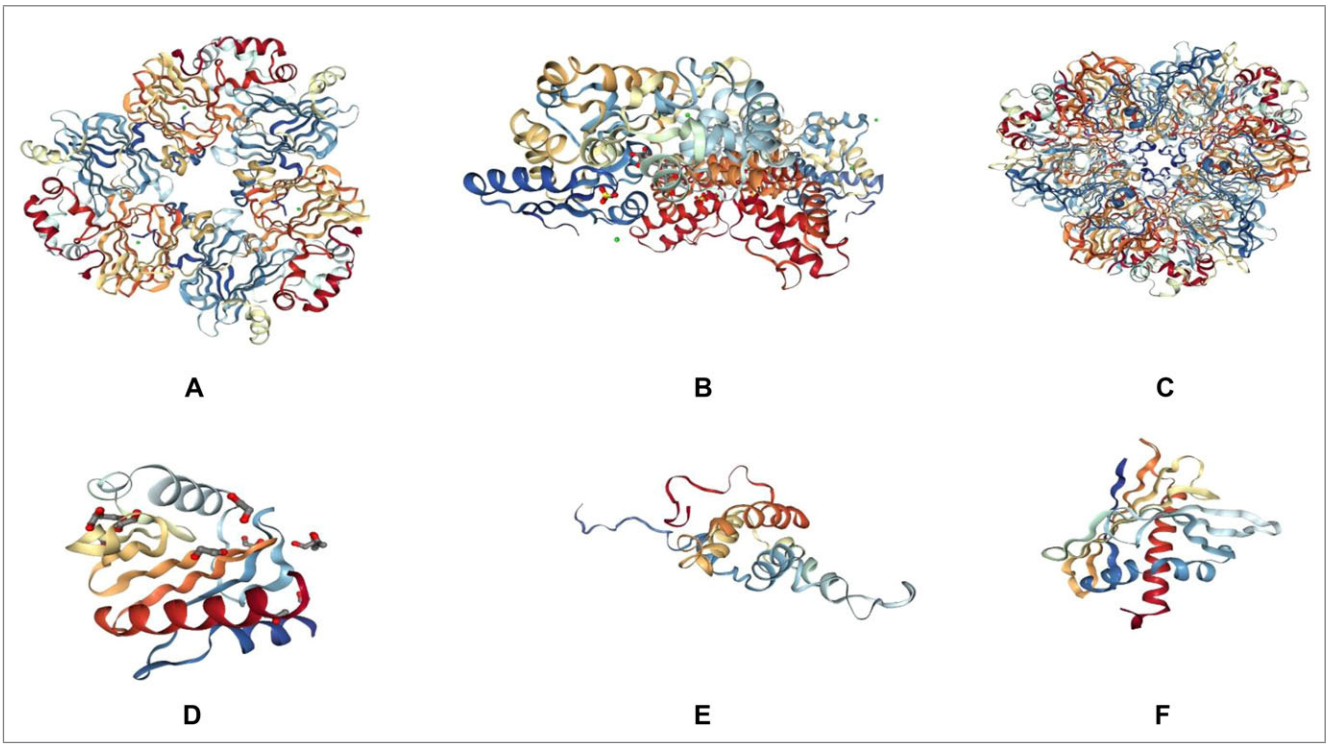

Figure 2–The crystal structures of peanut allergens elucidated to date: The crystal structures of (A) Ara h 1 (PDB ID 3S71); (B) Fusion protein of Ara h 2 (PDB ID 3OB4); (C) Ara h 3 (PDB ID 3C3V); (D) Ara h 5 (PDB ID 4ESP); (E) Ara h 6 (PDB ID 1W2Q); and (F) Ara h 8 (PDB ID 4MAP); (PDB library retrieved from http://www.rcsb.org)

Rudengren, Ahlstedt, Bock, & Sampson, 2008). Cross-reactivity is an important consideration for peanut sensitized individuals as peanut proteins are homologous to proteins in other nuts, seeds, and vegetables (Cabanillas, Jappe, & Novak, 2018; Elizur, Bollyky, & Block, 2017), thus making dietary management more complex for allergic individuals. Interestingly, non-homologous peanut allergens also show different levels of cross-reactivity with each other due to similar exposed IgE epitopes on the surface (Bublin et al., 2013). The cross-reactivity of various nuts, peanut, and other allergens are covered widely in a recent report (Smeekens, Bagley, & Kulis, 2018). Allergenic proteins are classified into different families on their functional and structural basis that are discussed below in detail.

Vicilin-type 7S globulin

Ara h 1 is 7S globulin of vicilin-family proteins and it constitutes 12% to 16% of peanut protein. Ara h 1 is a major IgE reactive protein from peanut having a molecular weight of about 63 to 65 kDa and an isoelectric point (pI) of 4.55 (Burks et al., 1991; De Jong et al., 1998). The native Ara h 1 structure is a trimer composed of three identical monomers having similar structural features to other 7S globulins (Chruszcz et al., 2011; Maleki, Kopper et al., 2000, also see Figure 2; Cabanos, Urabe et al., 2011). The core amino acid sequence is very identical with other 7S globulins and has the thermal degradation temperature of 88.3 °C that is slightly above the average (87.5 °C) from other 7S globulins (Cabanos, Urabe et al., 2011; Fukuda, Maruyama, Salleh, Mikami, & Utsumi, 2008). The sequence shares 53% identity with soybean β-conglycinin (PDB: 1UIK) and adzuki (PDB: 2EA7; Cabanos, Urabe et al., 2011). Ara h 1 is highly stable to heat and digestion (Iqbal et al., 2016; Maleki, Schmitt, Galeano, & Hurlburt, 2014), but has been shown to form oligomers and to become chemically modified after thermal processing. Most of the Ara h 1 epitopes are exposed on the surface of the protein in its native structure (Cabanos, Urabe et al., 2011). To date, 27 linear epitopes have been recognized within the molecule of Ara h 1, of which the two most potent epitopes are found within the amino acid residues 139 to 147 and 175 to 183 (Matsuo, Yokooji, & Taogoshi, 2015). Recently, 36 T-cell epitopes of Ara h 1 have been identified (Ramesh et al., 2016), which is useful as T-cell epitopes may play a significant role in development of immunotherapy treatments (Tao et al., 2017). This information can help the food processing scientists to study the alteration in IgE binding epitopes to lower adverse events yet maintain T-cell epitopes for immunotherapy purposes.

Conglutin family 2S albumin-type protein

Peanut allergens Ara h 2, Ara h 6, and Ara h 7 have been registered under this family. Ara h 2 is a glycoprotein with a molecular weight of around 18 to 20 kDa and a pI of 5.2 and functions as a trypsin inhibitor (Burks, Williams, Connaughton, et al., 1992; De Jong et al., 1998; Maleki et al., 2003). Ara h 2 is the most allergenically potent peanut allergen and is recognized by approximately 90% of peanut allergic individuals (Valcour, Jones, Lidholm, Borres, & Hamilton, 2017). Ara h 2 constitutes 6% to 9% of peanut protein (Koppelman et al., 2001; Wu et al., 2016). It is a right-handed super-helix structure composed of five α-helices connected by several extended loops (Figure 2), resembling the trypsin inhibitor α-amylase (Mueller et al., 2011). The three-dimensional structure of Ara h 2 comprises four disulfide bridges, which contribute to its high stability against heat and digestion. Ara h 2 consists of two isoforms Ara h 2.01 (17 kDa) and Ara h 2.02 (19 kDa; Apostolovic et al., 2013). Ara h 2.02 is characterized for having an additional IgE binding epitope constituted by 12 extra

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amino acids and is a more potent IgE crosslinker than Ara h 2.01 (Chatel, Bernard, & Orson, 2003). Ara h 2 has been reported to have eight to 10 IgE binding epitopes (Otsu, Guo, & Dreskin, 2015; Shreffler, Lencer, Bardina, & Sampson, 2005; Stanley et al., 1997).

Ara h 6 shares secondary and tertiary structure similarity, sequence identity (59%), as well as IgE cross-reactivity with Ara h 2 (Flinterman et al., 2007; Koppelman et al., 2005; Lehmann et al., 2006). Ara h 6 has a molecular weight of 14.8 kDa and a pI of 5.6 (allergen.org; Eigenmann, Burks, Bannon, & Sampson, 1996; Offermann et al., 2015). Ara h 6 resembles Ara h 2 in structure sharing a four tightly coiled conformational core helical structure (Figure 2), which makes it heat and protease resistant (Sen et al., 2002). The structural features of Ara h 6 has high similarity with other isomers from the conglutin family that are all thought to be highly resistant to gastrointestinal digestion even after heat treatment (Apostolovic et al., 2013; Hazebrouck et al., 2012; Koppelman et al., 2010; Suhr, Wicklein, Lepp, & Becker, 2004).

Another allergen of this family is Ara h 7 that is found to be reactive in 13% of patients. Ara h 7 has a 35% sequence similarity with Ara h 2 and 53% similarity with Ara h 6 (Mishra, Jain, & Arora, 2016; Wen, Borejsza-Wysocki, DeCory, & Durst, 2007). Ara h 7 is thought to be less stable than Ara h 2 and Ara h 6 because of having only two conserved disulfide bonds (Schmidt et al., 2010). Ara h 7 has three isoforms (Ara h 7.01, 7.02, and 7.03), among them, Ara h 7.02 has an extra propeptide cleavage point that functions as an amylase/trypsin inhibitor (Hayen et al., 2018; Schmidt, Krause et al., 2010). Surprisingly, one recent study suggests a high frequency (80%) sensitization pattern with Ara h 7.02, nearly the same as the major allergens Ara h 2 and 6, due to a few unique epitopes present in the C-terminus of its allergenic loop. These unique epitopes may improve the accuracy of peanut allergy diagnosis (Hayen et al., 2018).

Cupin superfamily, 11S (legumin-type) protein

Peanut allergens Ara h 3 and Ara h 4 (renamed as Ara h 3.02) categorized under this family. Ara h 3 is also a major peanut allergen recognized by approximately 50% of peanut allergenic patients (Koppelman et al., 2003). Ara h 3 and soybean glycinin both are 11S proteins and resemble 47.2% in sequence identity (Jin, Guo, Chen, Howard, & Zhang, 2009). Ara h 3 crystalizes as a hexamer (Figure 2) with two trimer rings interacting in a face to face orientation. Its monomeric unit produces different molecular weight bands (48, 38, 36, and 24 kDa,) on SDS-PAGE (Wu et al., 2016). This hexameric globulin is composed of five different isomers, which attribute to its conformational epitopes. Four linear epitopes have been reported and mapped, of which, epitopes 3 and 4 were recognized more frequently by patient sera than epitopes 1 and 2 (Jin et al., 2009; Shreffler et al., 2005; Zhao et al., 2017; Rabjohn et al., 1999). In its native state, the third and the fourth epitopes of Ara h 3 are fully exposed while the other two epitopes alongside the side chains of the most critical residue are either entirely or partially buried (Jin et al., 2009). This possibly renders the IgE binding site for epitopes 1 and 2 in its intact form while epitope 4 and some part of epitope 3 is able to bind in its native state. The buried epitopes 1 and 2 may become exposed as novel IgE binding sites inside the gut when it is partially digested by enzymatic and acidic conditions. Ara h 3.02 shares a sequence identity up to 95% to 98% with Ara h 3.01 (Dodo, Viquez, Maleki, & Konan, 2004; Jin et al., 2009). Ara h 3.02 has a molecular weight of 58 to 61 kDa and a pI of 5.2 (Rabjohn et al., 1999).

Profilin group

Ara h 5 is a member of the profilin group of allergens, and has a very low molecular weight of 14 kDa and has a pI of 4.6 (Kleber-janke et al., 1999; Wen et al., 2007). IgE against Ara h 5 has not been observed frequently in patient sera. A study conducted with 40 patients reported that 13% individuals were sensitized to Ara h 5 (Kleber-janke et al., 1999), this low sensitization frequency from Ara h 5 might be due to its low content in peanut. Furthermore, a study using a quantitative proteomics approach to quantify Ara h 5, reported undetectable levels of the allergen in peanut (Johnson et al., 2016). Thus, the authors suggest a further evaluation with different extraction protocols and heavy labeled peptides are needed before evaluating its clinical feature. Many other species of the profilin group also contain allergenic proteins such as the pollen allergens like Bet v 2, thus, people with pollen allergies have a greater tendency to cross-react with peanut protein allergens (Wang et al., 2013). The crystal structure of Ara h 5 (Figure 2) has seven strands of antiparallel β–sheets and two α-helices at one side as well as another helix on the other side (Wang et al., 2013), which is highly similar to Bet v 2 protein. The sequence of Ara h 5 most likely resembles other profilin proteins such as in *Hevea brasiliensis* or latex (Hev b 8; Wang et al., 2013).

Oleosin group

Currently, four oleosin proteins Ara h 10, Ara h 11, Ara h 14, and Ara h 15 from peanut are found to be allergenic, with a molecular weight of 16 kDa, 14 kDa, 17.5 kDa, and 17 kDa, respectively (allergen.org). Peanut oleosins consist of a highly conserved central hydrophobic domain (approximately 70 residues) and hydrophilic N- and C-termini, which differ in the primary sequence of amino acids. Oleosins are major oil bodies that stabilize oleosomes, which impart structural stabilization and also impart an enzymatic role during the germination process (Maurer et al., 2013; Parthibane, Rajakumari, Venkateshwari, Iyappan, & Rajasekharan, 2012). The crystal structures of oleosin allergens are unknown. Some of their linear IgE epitope sequences, including SDQTRTGY and IADKARDVKDRAKDYAGAGRAQE, have been identified (Kobayashi, Katsuyama, Wagatsuma, Okada, & Tanabe, 2012; Schwager et al., 2017). The sequence SDQTRTGY was reported for having high cross-reactivity with buckwheat, which may induce anaphylactic shock (Kobayashi et al., 2012). The peanut oleosin group has also been found to cause severe allergenic symptoms (Schwager et al., 2017; Zuidmeer-Jongejan et al., 2014), thus, this group of allergens might also require special consideration for detection purposes and their behavior with physical and chemical processing needs to be studied. The thermal denaturation temperature for the 14 and 16 kDa oleosin proteins lies at around 50 °C, while the 18 kDa oleosin proteins denature at 59 °C (Cabanos, Katayama, Tanaka, Utsumi, & Maruyama, 2011), which suggests that the thermal stability of oleosin allergens is not so high, however it has been reported that the allergenicity of these allergens increases upon roasting with an increase in its IgE binding ability (Petersen et al., 2015; Schwager et al., 2017).

Pathogenesis related group (PR-10)

PR-10 proteins are composed of the Bet v 1 superfamily of proteins. It includes the peanut allergen Ara h 8 that has a molecular weight of 17 kDa and two isomers (Ara h 8.01 and Ara h 8.02) that are officially registered by the IUIS. Ara h 8 is a low abundant

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protein in peanuts and regarded as a minor peanut allergen (Pons et al., 2002; Riecken, Lindner, Petersen, Jappe, & Becker, 2008). However, in the birch pollen allergic individuals, Ara h 8 is a major and the most significant allergens in Oral Allergy Syndrome (OAS), which causes oral cavity symptoms and other more severe reactions (Mittag et al., 2004). A recent study revealed a higher IgE binding response for Ara h 8 among a U.S. population where 2.4% of children, 49.4% of adolescents, and 42.9% of adults produced IgE against Ara h 8 (Valcour et al., 2017), suggesting this requires further serious study. Ara h 8 shares homology with other PR-10 related allergens; for example, Api g 1, Dau c 1, Fra a 1, Gly m 4, and Pru av 1 (Hurlburt et al., 2013). Five linear antigenic epitopes and three conformational epitopes were predicted for Ara h 8 on a prediction modeling basis (Mishra et al., 2016). The Ara h 8 structure is composed of three α-helices that flank a seven-stranded anti-parallel β-sheet creating a large cavity with a metal binding site (sodium; Figure 2). The most significant structural difference between Ara h 8 and other members of the Bet v 1 family was observed in the conformation of the $\beta 3$, $\beta 4$ strand, and the connecting loop between them (Hurlburt et al., 2013). Ara h 8 sequence was found to be most similar to Gly m 4, the soybean allergen, with 84% sequence similarity (Hurlburt et al., 2013). Although having low stability to heat (roasting) and very low stability to gastric digestion, Ara h 8 still possess IgE reactivity after heat treatment (Mittag et al., 2004). It is suggested that the low digestibility of Ara h 8 is a main cause for the dominant oral symptoms displayed by sensitized individuals (Mittag et al., 2004; Petersen et al., 2014). The epitopes and their behavior with processing treatments is a subject to study in detail.

Nonspecific lipid transfer protein allergens

Nonspecific lipid transfer protein (nsLTP) is named because of its ability to arbitrate the transfer of phospholipids in membranes. This group of proteins is highly stabilized by four disulfide bonds (Douliez, Michon, Elmorjani, & Marion, 2000; Kader, Julienne, & Vergnolle, 1984). The nsLTP from peanut registered by IUIS are Ara h 9, Ara h 16, and Ara h 17 with molecular weights of 9.8, 8.5, and 11 kDa (SDS-PAGE reducing), respectively. Ara 16 and Ara 17 are recently categorized for their allergenic potency (allergen.org, allergome), hence, there is no profound supportive material regarding the characteristics of these allergens. Ara h 9 has two isoforms, Ara h 9.0101 and Ara h 9.0201, having 90% sequence homology with each other. Both isoforms of Ara h 9 have 62% to 68% sequence similarity and high cross-reactivity with Pru p 3 (the peach nsLTP allergen). Ara h 9 is found to be a significant peanut allergen, especially in people from the Mediterranean area. Interestingly, most of the Ara h 9 sensitized individuals do not show IgE binding toward the major peanut allergens Ara h 1, Ara h 2, and Ara h 3 (Bublin & Breiteneder, 2014; Lauer et al., 2009). Ara h 9 was found to be responsible for many severe clinical symptoms such as peanut-associated bronchospasm, chronic atopic diseases, and more (Arkwright et al., 2013). Ara h 9 is also a significant peanut allergen and may be associated with severe adverse reactions in some countries. Its structural features and effects upon various processing treatments is still unknown, thus, further study is required.

Defensin group of allergens

Defensins are cysteine-rich, small, and highly stable groups of proteins that play defensive roles against plant pathogens as a part of the inherent immune system (Lay & Anderson, 2005). Although studies on peanut defensins are lacking, IUIS has registered two peanut proteins as allergenic in this category, Ara h 12 and Ara h 13. Ara h 12 and Ara h 13 have molecular weights of 8, 12 kDa and 8, 11 kDa, respectively. An intensive protein spot of 8 kDa (Ara h 12) was reported on a 2D gel apart from some other peanut allergens. Three bands of about 10, 11, and 12 kDa (Ara h 13) were observed in a native gel, which appeared as one band of about 8 kDa in a reducing SDS-PAGE. Testing for IgE binding to these bands with a Western blot revealed that some of the patient's sera bound to these proteins (Ara h 12, Ara h 13), although IgE binding tended to the reduced band was lower (Petersen et al., 2015). According to the research (Petersen et al., 2015), the amino acid sequence of these three bands (12, 11, and 10 kDa) showed that the sequences between the first two bands were similar with a difference of only three amino acids. The sequence of the last band differs by 27 amino acids or a 43% sequence identity with the other two bands.

As we observed from the above section, the prevalence, severity, molecular characteristics such as physicochemical properties and structural feature, behavior toward heat, and digestion of different peanut allergens vary greatly. Most of the peanut allergens especially potent peanut allergens are highly heat and digestion tolerant, due to having various covalent bonded secondary and tertiary structure and disulfide bridges. Some of the allergens are characterized while the characteristics of many of them are less known and should be studied.

Effects of Processing on Peanut Allergens and Immunogenicity

The immunoreactivity of allergenic proteins may be based on the nature of their recognition and binding with specific IgE or IgG antibodies (Verhoeckx et al., 2015). The protein region, which is recognized as a binding site by specific IgE, can be a short chain of few amino acids or the unique three-dimensional structural motif, which is known as sequential and conformational epitope, respectively. Thus, food processing that causes structural and chemical alteration in the IgE binding can alter the allergenic potency of foods. The influence on the immunoreactivity in relation to processing varies with the nature of the allergen, food matrices, and processing conditions (Long et al., 2016; Tian, Rao, Zhang, Tao, & Xue, 2018; Vanga et al., 2016). The processing technology may alter the protein properties and functionality as a result of denaturation, unfolding, reconstituting of the disulfide bonds, formation of new intra/intermolecular bonds, hydrolysis, aggregation, cross-linking, oxidation/reduction, glycation, glycosylation, and interactions with other components. Figure 3 demonstrates the effect of physical and chemical processing on the peanut allergens. Recently, in the search for a step toward mitigating peanut allergenicity alongside traditional heat treatments, the effect of nonthermal treatments, chemical modifications, and enzymatic methods have been explored (Comstock, Maleki, & Teuber, 2016; Long et al., 2016; Mikiashvili & Yu, 2018; Rao et al., 2016; Yang, Mwakatage, Goodrich-Schneider, Krishnamurthy, & Rababah, 2012; Yu, Liu, Shi, Liu, & Wang, 2016). Peanut has a tendency to trigger severe immunoreactivity even in minute quantities; hence, most processing methods aim to increase the safety of allergic individuals until better methods are developed. The other concern seems to be that the post-processing evaluations of the allergic potency of peanut is mostly based on *in vitro* IgE binding assays, however, some *ex vivo* methods such as basophil activation tests (BAT) are being optimized to enhance these measurements to be more clinically relevant. Furthermore, the purified peanut allergen alone imparts lower immune-stimulation

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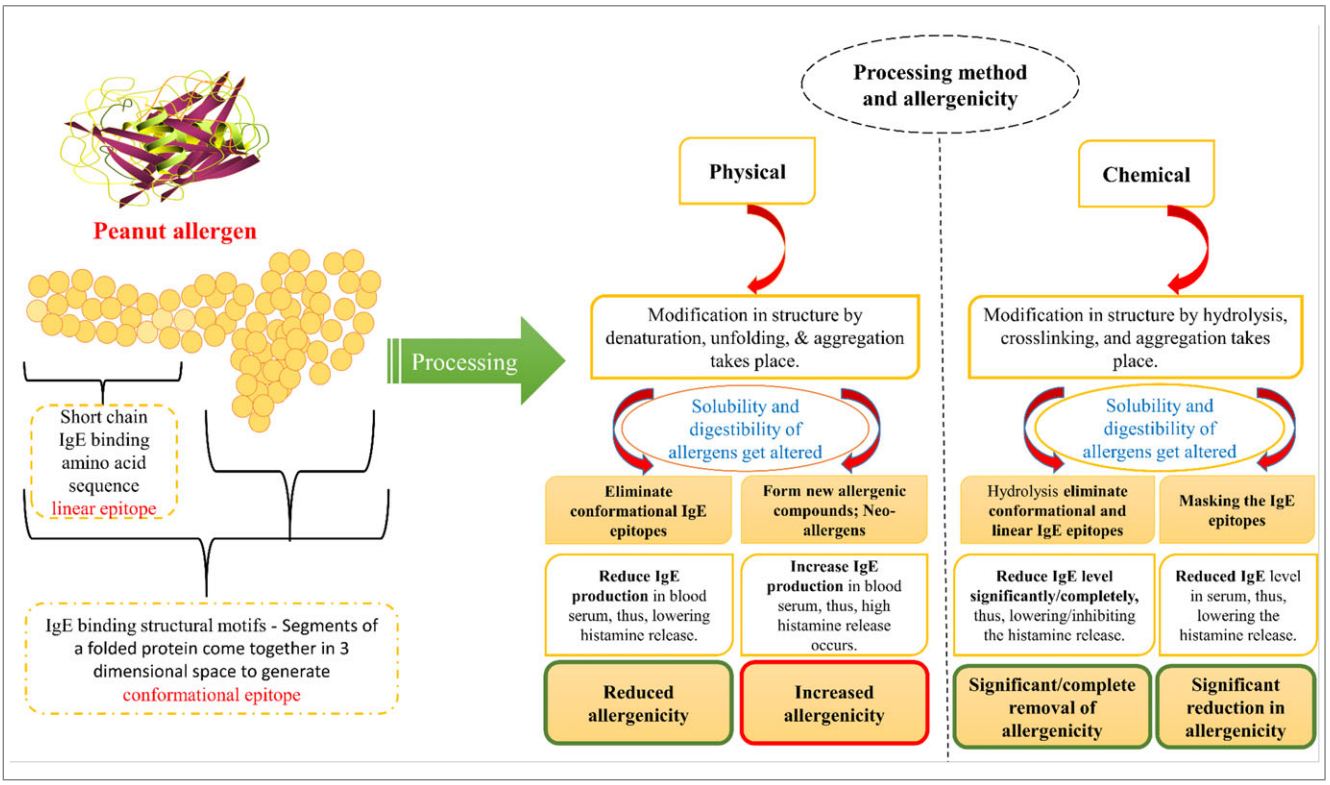

Figure 3–The potential paths/mechanisms of attenuating peanut allergenicity by means of different processing methods. The recognition and binding of allergens to IgE depends on linear and conformational epitopes that is altered to various degrees by means of physical and chemical processing and, thus, altering the allergenic reactions differently.

activity than the whole matrix (total protein plus the other ingredients) according to a study in balb mice using oral ingestion and popliteal lymph node assays (van Wijk et al., 2005). This is most likely due to the fact that there are multiple allergens in peanuts or most allergic foods and the method and matrix in which they are processed can alter allergenic potency of the whole food. This study indicates that different food matrices can also affect the immunoreactivity of individual peanut allergens, which suggests that the whole matrix influence should also be taken into consideration while assaying immunoreactivity.

Thermal processing

Thermal processing is adopted from traditional cooking methods, and it is still among one of the most broadly utilized techniques in food processing with the basic aim of reducing microbial hazards to increasing the shelf-life, to achieve the desired modification, and to enhance the quality of food products. The consumption pattern of peanuts has a long history with traditional ways of thermal processing such as boiling, roasting, and frying being the most commonly used thermal processing methods. It has been a perception that the rare incidences of peanut allergies among the Chinese population may be due to their consumption mode, that is, boiled peanuts, in comparison to the western countries, which mostly consume peanuts in a roasted form (Beyer et al., 2001), but currently the evidence does not match up to this belief. The effects of various thermal processing on peanut allergens are summarized in Table 3. Thermal processing includes boiling and frying, roasting and baking, as well as Ohmic heating (Vanga et al., 2017). Thermal processing induces modifications to food proteins such as unfolding, denaturation, hydrolysis of peptide bonds, disulfide induced aggregation, and generation of new intra/intermolecular bonds and adverse reactions with other food components (Davis & Williams, 1998; Maleki & Hurlburt, 2004; O'Konek et al., 2018; Rao et al., 2016; Schmitt, Nesbit, Hurlburt, Cheng, & Maleki, 2010). The modifications by thermal processing include altered tertiary and secondary structures of the native proteins (Maleiki, 2004; Maleki et al., 2003; Nesbit et al., 2012; Zhang, Zhu, Zhang, Cai, & Chen, 2016), consequently altering the allergenic potency to varying degrees. One of the mechanisms for reduction in IgE reactivity/allergenicity by heat processing is due to the disruption of conformational epitopes that lowers IgE binding, while the increase in IgE reactivity may be due to the exposure or generation of new epitopes (neo-allergen) (Gupta et al., 2018; Maleki, Chung et al., 2000).

In general, boiling and frying in oil showed the potential to alter the content and immunoreactivity of major peanut allergens up-to various degrees, depending on the processing conditions. Several studies have revealed the remarkable reduction in the content of Ara h 1, Ara h 2, and Ara h 3 in peanut samples as well as the significant decrease in immunoreactivity of soluble proteins as an effect of boiling and frying (Beyer et al., 2001; Mondoulet et al., 2005). As peanut proteins and allergens are not eliminated and do not evaporate upon thermal treatment, this observation is attributed to reduced solubility of the proteins due to thermal processing, which causes protein cross-linking, aggregation, degradation, unfolding, and reduced solubility (Schmitt, Nesbit et al., 2010). As most immunological assays measure the content of soluble material, it appears as the immunoreactivity of the allergen is reduced. A recent study reports that the boiling treatment of peanut reduced the contents of Ara h 2, 6, and 7 with a remarkable

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Table 3–Effect of thermal processing and chemical modification on the immunoreactivity of peanut allergens.

Processing method	Effect on immunoreactivity	Peanut allergen studied	Mechanism	Advantage or disadvantage	Reference
Boiling and Frying	Reduced IgE binding for boiling. Most studies measured IgE binding to soluble material only in fried samples.	Ara h 1, 2, 3 & 6	Alteration in secondary structure (α-helix and β-sheet); denaturation and loss of allergens in small fragments observed in cooking water	Require high temperature treatment; may alter the nutrients and bioactive compounds	Beyer et al. (2001); Comstock et al. (2016); Mondoulet et al. (2005); Tao et al. (2017); Vanga et al. (2016); Schmitt, Nesbit et al. (2010)
Roasting	Increased IgE binding	Ara h 1, 2, 3, 6 & 8	Formation of unknown stable fragments, agglomeration, increased random coiling in secondary structure, decreased digestibility, and Maillard reaction occurs (formation of new allergens may takes place)	Increased the IgE binding potency	Beyer et al. (2001); Comstock et al. (2016); Maleki et al. (2003); Mondoulet et al. (2005); Petersen et al. (2014); Vanga et al. (2016); Vissers et al. (2011)
Enzymatic hydrolysis	Significant reduction in IgE binding potential around 90% to 100%. several only measured for soluble material only		Allergens cleaved into fragments (proteolysis), epitopes are inaccessible by IgE, and/or increased digestibility. One of the most potent applications to alter peptide strands and structural epitopes.	May completely dysfunction the IgE reactivity, simple processing step with no/low energy input, no harmful chemical	Astwood et al. (1996); Burks et al. (1992); Cabanillas et al. (2011); Hazebrouck et al. (2012); Kasera et al. (2015); Mikiashvili and Yu (2018); Yu et al. (2011)
Phytic acid treatment	Reduced IgE binding to up to sixfold to soluble material	Ara h 1 & 2	Forms complex with allergens that may hinder epitope recognition by IgE	May produce indigestible complexes; advantage of additional bioactive supplement	Chung and Champagne (2007)
Phenolic compound	Reduced the immunoreactivity of roasted peanut to 10- to 16-fold to soluble material	Ara h 1 & 2		May produce indigestible complexes; advantage of additional bioactive supplement	Chung and Champagne (2009)
Tannin acid	Significantly reduced immunoreactivity to soluble material	Ara h 1 & 2	possible masking of allergenic epitopes by complex formation	Produces undigestible complex	Chung and Reed (2012)
Polyphenol	Reduced IgE binding to soluble material	Ara h 1, 2, 3 & 6	Alteration in the secondary structure of protein and possible masking the epitopes	Less energy required; additional bioactive fortification	Plundrich et al. (2014)

lowering in the IgE binding against these allergens using patient sera (Turner et al., 2014). In addition, boiled peanuts (processed) reduced the probability of an adverse reaction during immunotherapy and successfully desensitized individuals toward Ara h 2, 6, and 7, thus, serving as a better candidate as a hypoallergenic peanut for immunotherapies (Turner et al., 2014). Besides altering major peanut allergens (Ara h 1, 2, 3, & 6), another study showed boiling and frying also significantly reduced the content of other peanut allergens such as Ara h 8 and Ara h 9 (Comstock et al., 2016). Dot blot analyses demonstrated the immunoreactivity of boiled and fried peanut samples up to varying degrees, which suggests that boiling and frying may not have the potential to diminish the immunoreactivity and generate hypoallergenic peanuts (Comstock et al., 2016). Furthermore, a recent study suggests that extensive boiling of peanut for 8 hr can reduce the IgE binding activity up to eightfold while extended boiling for 12 hr significantly reduced the immunoreactivity by up to 19-fold (Tao et al., 2017). The transfer of these allergens to boiled water could explain the loss of these allergens from the peanut that leads to a significant reduction in immunogenicity (Beyer et al., 2001; Mondoulet et al., 2005; Zhang, Zhu et al., 2016). Another study reported that fried peanut samples had the least IgE binding activity for Ara h 2 in comparison to boiling and glycation treatments with a reduction in IgE binding activities by 70%, 50%, and 37%, respectively (Zhang, Zhu et al., 2016). Turner and co-workers (2014) demonstrated that the reduction of immunoreactivity of thermally processed peanuts via *in vivo* studies correlates well with *in vitro* allergenicity reduction studies. A recent study conducted in the murine model showed that the declining trend of immunoreactivity *in vivo* and the clinical symptoms affected by Ara h 2 in mice was reduced significantly by thermally treated peanuts, and the strongest reduction was observed in the case of fried peanut (Zhang, Zhu et al., 2016). Additionally, the content of histamine and Th-2 type cytokines such as IL-4, 5, and 13 were upregulated while the level of IgG2a and the Th-1 cytokine IFN-γ were downregulated, suggesting that thermally treated Ara h 2 may induce anaphylaxis to varying degrees. Findings varied as some studies conducted by Cabanillas and co-workers (2015) and Tao et al. (2016) performed skin prick tests (SPT) with different thermally processed peanuts in peanut allergic patients showed a significant reduction ($P < 0.001$) in SPT wheal sizes with boiled peanut, while another study showed higher skin test reactivity to thermally processed peanuts (Maleki et al., 2010).

To explain the mechanism behind the changes in the immunoreactivity/allergenicity of thermally treated peanut, several researchers have studied the alteration to structure/epitopes and other physicochemical properties such as solubility and digestibility associated with immunoreactivity. Maleki's group (Schmitt, Nesbit et al., 2010) demonstrated that the aggregation and reduced solubility of some allergens following thermal processing made them unavailable in solution and resulted in reduced IgE binding or SPT results to the soluble material. Atomic force microscopy (AFM) imaging revealed the transformation of spherical proteins into the large rod-like branched aggregate chain structures in thermally treated peanut that caused a reduction of small molecular weight proteins (Cai, Zhang, Zhu, & Chen, 2016; O'Konek et al., 2018). Circular dichroism (CD) spectroscopy and Fourier transform infrared spectroscopy (FT-IR) studies revealed the collapse of the tertiary structure of peanut allergen proteins caused by unfolding, denaturation, and aggregation when subjected to thermal treatment (Koppelman, Bruijnzeel-Koomen, Hessing, & de Jongh, 1999; Rao et al., 2016; Tian et al., 2018; Zhang, Zhu

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et al., 2016). It was reported that the secondary structure of peanut allergens Ara h 1, 2, and 6 is significantly modified by the application of high-temperature treatment with a clear reduction in the α-helices, a slight increase in extended β-sheet structures, and an increase in the content of irregular coils (Koppelman et al., 1999; O'Konek et al., 2018; Rao et al., 2016; Tian et al., 2018; Vanga et al., 2016; Zhang, Zhu et al., 2016). However, CD analysis demonstrated that Ara h 1 and Ara h 2 proteins purified from roasted peanuts maintained their secondary structure and were not unfolded, although higher order structures were seen in the insoluble material, which indicate aggregation (Maleki et al., 2003; Nesbit et al., 2012). Whether these aggregates are composed of unfolded or folded proteins is not known at this time. It may be possible that the folded oligomeric proteins are still soluble and extractable, and the unfolded aggregates become insoluble. The effect of heat treatment on the primary structure of allergens is rarely studied. Sayers and co-workers have made an effort to report the effect of thermal treatment on the peptides of the peanut allergens Ara h 1, 2, 3, 6, and 7 (Sayers et al., 2016). They reported the complex behavior of targeted peptides after thermal treatment with some modifications on different targeted peptides that might lead to the hydrolysis, unfolding, aggregation, and some alteration in disulfide bridges and secondary structure. The unpredictable behavior indicates a lack of sensitive peptide targeting analysis of processed allergens by MS, on the other hand, variation in sample extraction may also affect the measurement of allergenic peptides (Sayers et al., 2016). Alteration extraction of total protein and allergens in post-processed peanuts has also been reported previously (Schmitt, Nesbit et al., 2010). This study indicates that water and oil-based heating alone can significantly reduce the immunogenicity of soluble peanut extracts, while some *in vivo* studies suggest that it may also decrease the clinical manifestation, but cannot completely eliminate the peanut allergenicity. Most importantly, the recent research suggests that extensively boiled peanut may serve as a safe product for immunotherapy as it decreases the immunoreactivity and maintains its T-cell proliferation ability (Tao et al., 2016). The initial trial on immunotherapy through sequentially boiled peanuts indicates that extensively boiled peanuts reduce the chance of adverse events during the course of the immunotherapy, while still effective in desensitizing the patients (Tao et al., 2017; Turner et al., 2014).

Roasting is most often a dry heating method that is a very commonly applied process for peanuts, most dominantly in the Western countries either before its consumption directly or prior to its utilization in a product for the roasted peanut flavor. The effect of roasting on peanut allergenicity has been studied widely. Despite the ameliorating effect of boiling on the treatment of peanut allergens, it has been noted that the roasting of peanuts can enhance its IgE binding potential up to 90-fold (Beyer et al., 2001; Maleki, Chung et al., 2000; Mondoulet et al., 2005; Rao et al., 2016). Increased IgE binding in roasted peanut has been explained in several studies, and unlike boiling and frying, roasting does not impart for significant structural changes (Rao et al., 2016; Vanga et al., 2016; Zhang et al., 2016). Roasted peanuts have a high resistance for digestion than raw and boiled peanuts, and the roasting of peanut imparts trypsin inhibition activity (Maleki et al., 2014; Maleki & Hurlburt, 2004; Petersen et al., 2014). Roasting also induces the glycation of amino acids (Maillard reaction) that can produce various complex structures of molecules and form intermediate products, which may lead to the formation of new/unidentified allergenics (neo-allergen; Maleki & Hurlburt, 2004; Mueller et al., 2013; Tian et al., 2018; Verma et al., 2012; Vissers et al., 2011).

Chemical modifications

Calculated chemical modification of food is a traditional method to alter the properties and functions of proteins. This process has been widely used by industries for its diverse applications. Chemical treatments have shown great potential in reducing or eradicating the immunoreactivity of peanut allergens. Table 3 summarizes the effect of different chemical modifications and their effect on IgE binding to peanut. Different chemical treatments result in the reduction of the peanut allergen levels in soluble extracts and potentially their immunoreactivity is differently, which will be thoroughly discussed in this section. Figure 3 represents the mechanism of allergenicity alteration by chemical treatment.

Covalent modification. Some of the important covalent modifications reported for the reduction of immunogenicity are: acylation, alkylation, and reduction of disulfide bonds. Acylation blocks the positively charged amino acid groups and the remaining negatively charged groups which make the protein more negative, thus possibly masking the epitope and altering the solubility and digestibility of allergens. Acylation can be performed by anhydrates such as succinic acid or acetic acid. A few studies report the application of acylation on peas (pea protein solution) stating that the process was able to significantly reduce the immunoreactivity (91% to 99% and 79% to 97% by succinylation and acetylation, respectively) of albumin and legumin proteins by using 0.2 g of succinic and acetic anhydrides, separately, at a constant pH 8 (Szymkiewicz & Jędrychowski, 2008, 2009). The reduction in the immunoreactivity depends on the degree of acylation and the type of anhydrous compound used, which requires further study on specific parameters. Whereas in the case of peanut allergens Ara h 2 and Ara h 6 (purified allergen solution), the study showed that sequential treatment by reduction with dithiothreitol followed by alkylation with iodoacetamide had successfully reduced their IgE binding (Apostolovic et al., 2013). The sequential treatment first reduced all the disulfide bridges and then the proteins were alkylated, resulting in major tertiary and secondary structural alterations due to the loss of the α-helix and increases in the β-sheets (Apostolovic et al., 2013). However, a major concern is how this non-food grade chemical can be employed in the form of a food or can be removed from food, which is also highlighted by another researcher (Yu, 2016). Another study conducted on cashew nut allergens reports the reduction by sodium sulfite treatment caused a significant reduction in the IgE binding potency to cashew nut protein extract among allergenic people (Mattison et al., 2014).

Noncovalent modification. Interactions of noncovalent compounds with food allergens produce insoluble compounds that lower the soluble allergenic compounds and also reduce the digestibility; as a consequence, their allergenic properties may be lowered. The insoluble compounds hinder the digestive enzyme action to expose the allergenic epitopes that may reduce its recognition by IgE. For example, phytic acid treatment of peanut extract results in the formation of a complex with Ara h 1 and Ara h 2, which reduces the solubility in acidic and neutral conditions and ultimately reduces its IgE binding of the soluble material up to sixfold (Chung & Champagne, 2007). The concern regarding the efficacy of this modification was presented by a researcher (Yu, 2016) who suggested that the reduction in IgE binding by ELISA may be because of the analysis of soluble proteins only rather than the whole matrix because the phytic acid may precipitate the allergen that may reduce its content, not the immunoreactivity. Another study reported the irreversible modification of peanut allergens Ara h 1 and Ara h 2 from roasted peanut extracts and

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liquid peanut butter treated by phenolic compounds at a concentration between 50 and 100 mM (Chung & Champagne, 2009). Western blot and ciELISA showed the precipitation of these allergens by phenolic compounds and the reduction in the IgE binding was 10- to 16-fold lower. This analysis was also conducted with the supernatant in the posttreated sample and the allergenicity of newly released polyphenol-allergen complexes is unknown. Another similar report mentioned the reduction of IgE binding by using the tannic acid treatment on peanut butter extract, which forms an insoluble complex with the protein. It was suggested that these complexes were able to mask allergenic epitopes, which might reduce their allergenic potency (Chung & Reed, 2012). The resultant complex was found to be resistant toward gastric digestion (pH 2) and intestinal digestion (pH 8) (Chung & Reed, 2012). This method produces an indigestible complex which may cause stomach discomfort, thus, further *in-vivo* and clinical studies need to be explored. In another study, polyphenol fortified peanut matrices reduced the IgE binding of the major peanut allergens Ara h 1, Ara h 2, Ara h 3, and Ara h 6 (Plundrich et al., 2014). Polyphenol fortified matrices caused significantly lower basophil degranulation in *ex vivo* assays with allergic patient sera and reduced mast cell degranulation in mouse models. Furthermore, when the matrices were subjected to pepsin digestion, they showed that Ara h 2 and the basic subunits of Ara h 3, which were fortified with cranberry and green tea polyphenol complexes, were more prone to digestion and less immunoreactive than un-complexed peanut flour. This study suggests that when the polyphenol-protein complex undergoes the digestion process the IgE binding is reduced. Furthermore, the attenuated total reflection FT-IR analysis shows the changes occurred in the secondary structure of the protein, that is, α-helix and β-sheet. Thus, the reduced allergenicity is due to the change in its structure, which may alter conformational epitopes and mask the linear epitopes through complex formation. Moreover, most studies conducted on the covalent modifications on peanut allergens lack *in vivo* and clinical model studies.

Enzymatic cross-linking and hydrolysis

Enzymatic hydrolysis seems to be one of the most promising approaches toward the reduction of peanut immunogenicity by the disruption of its IgE binding backbone (conformational and linear epitopes) completely. The enzymatic approach, which uses food-grade enzymes, is believed to be one of the safest methods of chemical modification, which requires low energy inputs. The immunoreactivity of hydrolyzed peptides depends on the enzymes used and the condition of hydrolysis (Fritsché, 2009). Enzymatic treatment affects the allergenic protein in two ways, one is the cross-linking of an enzymatic protein that buries the IgE binding epitopes, and the other uses the proteolysis of allergenic proteins into fragments, which disrupts its native structure and alters its physicochemical properties. The residual allergenicity and the type of peptide produced depend on the nature of the allergenic protein (for example, digestibility and bond strength), process conditions (for example, pH, temperature, and treatment duration), penetration of food matrices by the enzymes, and the enzyme–substrate ratio, which are discussed in detail in this section. It is also important to note that the texture and flavor of foods can be significantly altered upon enzymatic hydrolysis and may or may not be useful for use in marketed foods, but may still be useful in developing immunotherapeutic products with reduced side effects.

It has been observed that subjecting peanut allergens to some other physical treatment prior to enzymatic hydrolysis facilitates enzyme penetration and makes the proteolysis more effective. Among different peanut allergens, Ara h 2 showed the highest stability when subjected to pepsin treatment at pH 1.2 for >60 min (Astwood, Leach, & Fuchs, 1996), and Ara h 1 also showed high resistance to enzymatic hydrolysis against pepsin hydrolysis (Becker, 1997). Ara h 6 is also highly resistant to digestion, and the degree of hydrolysis of some peanut allergens is strongly influenced by disulfide bridges, which stabilize the structure (secondary or tertiary; Hazebrouck et al., 2012). One study reported a 100-fold reduction in the IgE binding ability of peanut allergens from roasted peanut as an effect of hydrolysis by using immobilized digestive assays (simulated digestive fluid: crystalline porcine pepsin, trypsin, chymotrypsin, and crude intestinal mucosa peptidases; Burks, Williams, Thresher, et al., 1992) and another study using *in vitro* assays showed similar results (Vieths, Reindl, Müller, Hoffmann, & Haustein, 1999). Another study conducted using a higher enzyme (pepsin)–substrate ratios of 1:3 and varying the treatment between 24 and 48 hr on crude peanut extracts completely reduced the IgE-binding activity of peanut proteins observed via western blot analysis with five peanut allergic patient's sera (Hong, Gabriel Michael, Fehringer, & Leung, 1999). However, it is important to note that many studies use western blot analysis to assess for IgE binding following enzymatic digestion. Standard SDS-PAGE gels have a molecular weight cutoff of approximately 4 to 10 kDas, which allows for any fragments smaller than that to run off of the bottom of the gel, which are then undetectable in a western blot by any antibody. As an example, one study reported that pepsin digested peanut preparations were reported with no IgE binding activity and active T-cell proliferation using *in vitro* stimulation with pooled serum PBMC (peripheral blood mononuclear cell) analysis among seven of 10 patients with peanut allergy symptoms. In this study, the molecular weight cutoff for the SDS-PAGE was about 10 kDa, so IgE binding to smaller fragments could not possibly be measured by an IgE western blot. However, they did show that exposure of hydrolyzed extracts to peanut allergic patients showed lowered Th-2 type cytokines production and lower T-cell proliferation when compared to exposure to native peanut protein extracts (Hong et al., 1999). Th-2 plays a significant role in inducing an IgE response and in modulating the magnitude of the allergenic response (Hong et al., 1999). T-cell proliferation is required for immunotherapy (Tao et al., 2016), thus, maintaining T-cell proliferation and diminishing IgE binding ability is a positive indication for immunotherapy treatment.

In a later study, researchers showed that enzymatic hydrolysis was a great tool to eradicate the peanut immunoreactivity (Yu et al., 2011). The report suggests that the enzymatic hydrolysis could significantly reduce the IgE binding activity to diminishing levels from roasted peanut kernels. Treatment to roasted peanut kernels by chymotrypsin and trypsin at a concentration range of 0.1% to 0.15% for 3 hr showed significant reduction in Ara h 1 and Ara h 2 content in the soluble material up to 95% and 93%, respectively. In addition, blanching prior to roasting enhanced the enzymatic activity as there was no Ara h 1 and Ara h 2 found in the soluble material by SDS-PAGE. This study also reports that in the case of raw peanut kernel samples, this enzymatic treatment has a slightly lower efficacy (Yu et al., 2011), most likely because the proteins from raw peanuts are more soluble. Although this study reports a reduction in the levels of Ara h 1 and 2, it is a perfect example of miscalculation of the allergenic potency by assessing only the soluble material following a treatment. Assessing the insoluble and soluble material at a later date by the same laboratory

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(Mikiashvili & Yu, 2018) clearly demonstrated that the allergenic potency significantly less affected than claimed by Yu et al. (2011) (also confirmed by unpublished observation by Maleki's laboratory). Another study reported the treatment to roasted peanuts with alcalase for 30 min significantly lowered the IgE binding of Ara h 1, Ara h 2, and Ara h 3, while treatment with flavourzyme individually caused an increase in the immunoreactivity observed by ELISA. Furthermore, the treatment of roasted peanut protein extracts with flavourzyme for 300 min decreased the IgE reactivity of those allergens by 65%. However, the treatment with alcalase for 90 min or the combined treatment of alcalase and flavourzyme sequentially reduced the IgE reactivity by 100% (Cabanillas et al., 2011). Another study reported that the hydrolysis of allergens from kidney bean, black gram, and peanut extracts using enzymes (alcalase and flavourzyme) attributed to the significant reduction in IgE binding observed by ELISA assays. The reductions in IgE binding were up to 62.2%, 87.1%, and 91.8%, for kidney bean, black gram, and peanut, respectively (Kasera et al., 2015). Furthermore, the *ex vivo* stripped basophil histamine release assay reported a lowering of histamine release (Kasera et al., 2015). Most recently, a study reported that the hydrolytic enzyme treatment to raw peanut kernel reduced the IgE binding to the major peanut allergens Ara h 1 (up to 99% to100%), Ara h 2 (up to 95% to 99%), and Ara h 6 (up to 85% to 88%) and significantly reduced the Ara h 3 content by 35% to 46% in the soluble material as measured by ELISA. In addition, the roasting of peanut followed by enzymatic treatment reduced the soluble proteins for Ara h 1 and Ara h 3; it had little effect on Ara h 2 solubility (Mikiashvili & Yu, 2018).

The enzymatic hydrolysis process on whole peanut kernels is promising as it may also be applicable to other food products. The combination of sequential hydrolysis with prior and post heat/novel processing–treatments seem to be an effective approach to reducing the allergenic potency of peanuts. Most of the above mentioned studies are based on *in vitro* allergenicity analysis, thus the results may vary in *in vivo* studies and in relation to clinical relevance, which must be evaluated.

Novel processing techniques

Currently, novel processing technologies are preferred over traditional processing techniques in order to maintain the freshness of food. More modern methods require less treatment with high heat, which may reduce the loss of important nutrients and bioactive ingredients, provide quality food products, and be more ecofriendly. Thermal processing may have the ability to reduce immunogenicity of some foods but the extensive thermal treatment may also affect the nutritional properties negatively or lead to the production of neo-allergens (Gupta et al., 2018; Okolie, Aryee, & Udenigwe, 2018). Selection of appropriate processing techniques has been shown to influence the nutritional, sensory, and rheological properties of peanut (Wang, 2018), thus this is also significant in maintaining a high-value product with a reduced allergenic potency. In an approach to look for safe and more efficient alternative strategies for mitigating peanut immunogenicity, some novel approaches of processing such as HPP, high-intensity ultrasound, gamma irradiation, PUV, and pulsed-electric field treatment (PEF) have been explored. Out of which, some applications either as a unit operation or as hurdle technology (combined processing) show a potential promise toward diminishing peanut immunogenicity, even though most of these studies are at immature stages and many reports lack *in vivo* and clinical analysis. The published effects of various novel processing techniques on peanut IgE reactivity and allergenicity and their pros and cons are summarized in Table 4.

Table 4–Effect of novel processing techniques on the immunoreactivity of peanut allergens.

Processing method	Effect on immunoreactivity	Target peanut allergen studied	Mechanism	Advantage or disadvantage	Reference
HPP	Significant reduction in IgE binding potential at very high pressure (400 to 600 MPa)	Ara h 2	Alteration of secondary structure (partial degradation); some allergen dissolved in water	Big and expensive equipment setup is required; change in food texture occurs	Cabanillas et al. (2012); Hu et al. (2011); Huang, Yang, et al. (2014); Johnson et al. (2010); Long et al. (2016)
Pulsed ultraviolet light	Significant alteration in IgE binding activity of peanut allergen in soluble material	Ara h 1, 2 & 3	Formation of insoluble protein aggregates; reduced solubility of allergens	Low processing time; allow food to be easily used in various forms such as whole intact form, extracts, and powder; may cause photo oxidation to lipid molecules	Chung et al. (2008); Yang et al. (2012)
Gamma radiation	Significant/complete loss of IgE binding	Ara h 6 and whole peanut extract	Loss in structure (α-helix)	Need to assay the other nutritional components after treatment; restricted application for food	Luo et al. (2013); Oh et al. (2009)
Ultrasonication	Reduction in IgE binding potency was reported, but needs further study.	Ara h 1 & 2	Increased solubility and structural alteration; degree of structural alteration in peanut allergenic proteins are not clear	Easy processing operation without introducing chemicals; high quality end product	Li et al. (2013); Yu et al. (2012); Yu et al. (2016)
DIC (instant controlled pressure drop)	Significant reduction in allergenic protein content and IgE binding	Ara h 1, 2 & 3	----	Short processing time; high quality end product	Cuadrado et al. (2011)

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Effect of HPP. HPP is a mode of technology that provides an excellent option for food safety by reducing microbial loads and increases shelf lives of food, which is especially applicable to perishable food like fruits, vegetables, and milk. HPP involves the pressure ranges between 100 and 800 MPa to deactivate the microorganisms and increase the shelf life of the food products. Application of HPP at ambient temperature has a negligible effect on product chemistry thus maintaining its color, flavor, and other bioactive compounds (Huang, Hsu, Yang, & Wang, 2014; Pottier, Villamonte, & de Lamballerie, 2017). The application of HPP in food processing caught significant attention due to its ability to protect the most of bioactive ingredients from food. The working mechanism of HPP on food materials relies on three key parameters, namely pressure, temperature, and exposure times, which permit various processing treatment ranges. HPP predominantly affects noncovalent bonds, thus it may not have an impact on the primary structure of proteins, which comprises amino acid chains structured by covalent bonding (Huang, Hsu et al., 2014). The secondary structure of proteins consists of polypeptide chains, which form α-helix and β-sheet/strand structures due to inter/intramolecular hydrogen bonds, metal ion legation, and salt bridges and along with the seconday, tertiary, and quaternary structure of proteins, comprised mostly of noncovalent bonding interactions are more likely to be effected by HPP (He et al., 2014; Huang, Hsu et al., 2014). Therefore, subjecting protein molecules to HPP leads to compression that alters and distorts the secondary, tertiary, and quaternary structures, which is how volume changes in the food system may occur. Recently, two studies explained the effect of HPP on the immunoreactivity of allergenic proteins which vary with an alteration in processing condition (Long et al., 2016; Yang et al., 2017). The study reports that pressure above 300 MPa (400, 500, and 600 MPa) could significantly reduce the immunoreactivity of purified peanut allergen Ara h 2 in extracts (Long et al., 2016). Moreover, the report indicated that the pressure treatment of 100–200 MPa had no effect on the immunoreactivity of Ara h 2 while 300 MPa slightly reduced its immunoreactivity (Long et al., 2016). At higher pressures above 300 MPa, the immunoreactivity of peanut allergens was significantly altered by the unfolding of their protein structures. Protein unfolding at very high pressure cause the reduction in SH group, most likely because of the formation of disulfide bonds due to oxidation, especially at alkaline pH (Chizoba Ekezie, Cheng, & Sun, 2018). The modification series and structural alteration lead to protein denaturation causing the aggregation of proteins and gel formation that results in the reduction of IgE binding (Huang, Hsu et al., 2014).

A previous study found that there was no alteration in peanut allergen when purified Ara h 2 allergen subjected to HPP treatment, while apple allergen Mal d 3 was significantly altered when subjected to HPP and heat (Johnson et al., 2010). Another study (Huang, Yang et al., 2014) illustrated a significant reduction in peanut immunoreactivity ($P < 0.05$) with HPP treatment of ground peanut at the pressures 400, 600, and 800 MPa for 10 min, which reduced the immunoreactivity of the soluble peanut material in an ELISA up to 64.3%, 69.2%, and 73.3%, respectively, most likely due to reduction in the solubility of the proteins. As a consequence, HPP at 800 MPa decreased the total amino acid composition significantly (Huang, Yang et al., 2014).

Another study using the high-pressure microfluidization (HPM) treatment showed a significant reduction in the immunoreactivity of purified peanut allergen Ara h 2 even at a low pressure range of 60 to 180 MPa (Hu et al., 2011). Alteration of the Ara h 2 structure, such as the loss of α-helices and increase in β-sheets as an effect of high pressure were reported. In addition, the S-H group continuously increased with an increase in pressure, which suggests the disruption (reduction) of disulfide or S-S bonds. High pressure treatment also increased the surface hydrophobicity of the protein (Hu et al., 2011), which is expected with unfolding of a protein and exposure of the hydrophobic core. It is important to mention at this point that the treatment of a purified protein in a solution will have very different effects than the treatment of an entire peanut or peanut within a food matrix or within an extract. A protein in solution, without a matrix of other proteins or ingredients, will be significantly easier to unfold, digest, and manipulate, which may explain some of the variations in the reported observations. As previously mentioned, it is also important to keep in mind that the majority of the thermal and other treatments we discuss in this review use aqueous extracts of treated material. This is important because any treatment that unfolds the proteins or disrupts the structure is likely to expose the hydrophobic core, which in turn renders them insoluble. Therefore, if the study is based on measuring the soluble material, the observations will show a reduction in IgE binding or immunoreactivity due to reduction in total protein and allergen content in solution. This does not necessarily mean that there was not a reduction in immunoreactivity of the insoluble material, but only that it is rarely determined. The science is limited by solution-based immunoassays. The structural alteration in aqueous dispersion of peanut protein isolate (PPI) disaggregated at relatively low pressure via HPM treatment was also reported previously (Gong et al., 2017). The study showed significant changes in the molecular weight distribution and improved degree of hydrolysis by using a low range of pressure (30 to 120 MPa; Gong et al., 2017). This study showed the varied effect on high and low molecular weight peptides on varying pressure ranges of HPM. HPP also showed potential to reduce the allergenic potency in the soluble extracts of other foods such as soy and walnut (Meinlschmidt et al., 2017; Yang et al., 2017). On the other hand, HPP combined with heat showed a higher potential to reduce food allergenic potency or IgE binding to proteins present in the soluble extracts in comparison to HPP treatment as a unit operation. The hurdle approach combining high pressure (500 and 600 MPa) and thermal treatment at 75 °C showed a significant reduction in IgE binding to peanut allergen Ara h 2 (Long et al., 2016). Long et al. (2016) also used a BALB/c model to assess their HPP treated peanuts, but it is important to note that they fed mice with soluble extracts of the HPP material and not the entire HPP-treated meal, which would contain the insoluble material. The study showed that the soluble extracts of high pressure and heat treated peanut (PNH) did not produce any significant adverse clinical signs while it reduced the specific IgE titers I in comparison to only high pressure or thermally treated peanut extracts fed to mice. Furthermore, it decreased cytokine levels such as IL-4, IFN-γ, and IL-10 significantly, and PNH-treated mice reduced the serum histamine level than non-treated peanuts or peanuts treated differently (Long et al., 2016). Another study used combined processing by various pressurized heating conditions using an autoclave (Cabanillas et al., 2015). This study showed that heat processed peanut in all three forms, boiled, fried, and roasted, when subsequently autoclaved showed a significant reduction in their immunogenicity and allergenic content in comparison to the heat treatment alone. The structural alterations, such as a significant loss in the α-helix structure (only 11% left) and an increase in the random coils, demonstrated significant structural changes upon being subjected to heat and autoclave

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treatment combined when compared with thermal treatment alone. Interestingly, when subjected to autoclaving (121 °C at 2.56 atm, 30 min) the roasted peanut also showed a remarkable reduction in its immunogenicity (Cabanillas et al., 2012). The reduction of allergenic potency of roasted peanut following autoclaving is likely due to the water-based processing condition, as water plays a key role in reducing the immunogenicity during heat treatment (Rao et al., 2016) as seen with boiling. It is also known in the food industry that the presence of water inhibits the Maillard reaction. However, the HPP treatment alone at very high pressures above 400 MPa has a significant effect on the immunogenicity of peanut over boiling/extended boiling while it showed diminishing effect of allergens in combined processing with heat.

Another novel technique called instant controlled pressure drop (DIC) provides a wide range of processing application to food materials and its modifications. DIC technique consists of a short heating step of 10-60 s followed by steam injection under high pressure (up to 1 MPa) to the products kept under vacuum, followed by an abrupt pressure drop creating a vacuum (Hamoud-Agha & Allag, 2019). The working mechanism is based on heating and mechanical stress where the initial vacuum allows constant heating rapidly and the instant pressure drop causes significant mechanical stress. Application of DIC technique on whole seeds of roasted and raw peanuts, soybeans, lentils, and chickpeas resulted in the significant reduction of their allergenicity (IgE binding) attributes (Cuadrado et al., 2011). According to the study, the treatment at 6 bar for 3 min showed a significant change in peanut allergenic proteins (Ara h 1, Ara h 2, Ara h 3, and other lower molecular weight proteins). This result showed a very low intensity of the soluble proteins on SDS-PAGE and IgE immunoblot results, which was also reflected by a low IgE binding, interestingly, the result was more effective for roasted peanut seeds. A similar result was observed for lentil and chickpea allergens while soluble soybean allergens were completely unobservable in the soluble extracts (Cuadrado et al., 2011).

Effect of PUV. PUV is an emerging technology providing a great alternative to thermal and chemical processing methods to hinder the microbial growth, inactivate enzymes, and modify the structural properties of food. PUV has potential for different food processing applications (Abuagela et al., 2018; Cao, Fang, Liu, Min, & Liu, 2018; Pellicer & Gómez-López, 2017; Zhang, Wang, Zeng, Han, & Brennan, 2018). PUV is a nonthermal technology that consists of a broad spectrum of white light of wavelengths ranging from 200 nm (UV) to 1,000 nm (near-infrared; Rowan et al., 1999). The intensity of pulsed light may be up to 20,000 times more intense than sunlight (Abida, Rayees, & Masoodi, 2014). Upon treatment of food products to high-intensity pulse, the molecules are excited and while returning to their ground state, they release the energy in the form of heat or photons, which mediate the photochemical and photothermal reactions (Heinrich, Zunabovic, Varzakas, Bergmair, & Kneifel, 2016; Shriver & Yang, 2011). Proteins contain adequate chromophores, that is, amino acid chains and prosthetic groups, thus PUV can induce side-chain oxidation. The photon absorption by protein molecules causes cross-linking and aggregation, fragmentation, and formation of insoluble protein, which can alter immunoreactivity. PUV treatment of peanut products inhibits the IgE binding and reduces the allergenic content of the soluble material to variable degrees. One study reported that PUV treated peanut extract and peanut butter lowered the IgE binding specifically to the high molecular weight allergen Ara h 1 (63 kDa). The immunoreactivity appeared lower than the thermally treated (boiled) peanut, and it did not show any change in the IgE binding to the low molecular weight, 18 to 20 kDa, allergens (Chung, Yang, & Krishnamurthy, 2008). The treatment was applied using a Xenon RS-3000C for 3 pulses/s, 14.6 cm from the central axis of the lamp, for 4 min (peanut extract) or 3 min (liquid peanut butter). The solubility of Ara h 1 was reduced and the formation of insoluble aggregates took place that suggests the reason for the missing of 63 kDa protein band in the soluble fraction. Ara h 1 has also been shown to become less soluble with thermal treatments (Schmitt, Nesbit et al., 2010). According to the study, IgE binding was reduced up to sevenfold when compared to the untreated sample (Chung et al., 2008). The reduction in allergenic content and IgE binding is most likely due to the alteration of protein solubility and formation of precipitate of allergenic proteins in response to the PUV light (Chung et al., 2008). The reduction of the lower molecular weight allergenic band was also achieved in a later study via PUV treatment on raw and roasted peanut protein extracts and peanut butter slurry that was achieved by applying slightly longer duration of treatment (Yang et al., 2012). All the allergenic proteins bands (Ara h 1, 2, 3, and others) from peanut had disappeared from the soluble fraction on the SDS-PAGE gels after PUV treatment for 4 to 6 min. Even the most stable and potent allergen Ara h 2 solubility and hence IgE binding were reduced significantly when PUV was applied to raw and roasted peanut extracts for 4 to 6 min or to peanut butter for 1 to 3 min. Peanut extracts and peanut butter slurry after PUV treatment showed a significant reduction in IgE binding up to 12.9- and 6.7-folds, respectively. The intensity of allergenic bands lowered with increasing the treatment duration (Yang et al., 2012). A study reported that upon subjecting proteins to ultraviolet light, the amino acid side chains absorb UV light and cross-link together to form larger aggregates (Gennadios, Rhim, Handa, Weller, & Hanna, 2008). Historically, induction of protein and protein–DNA cross-linking via exposure to UV has been used for a variety of molecular biological methods (Greenberg, 1979; Sperling & Sperling, 1978). One concern regarding the application of the PUV treatment is the high unsaturated fat content of peanut can increase the lipid oxidation kinetics. Although being at an immature stage, these studies represent that PUV treatment may have a future role in mitigating peanut and other food allergies. Hence, further studies are required on PUV treatment considering *in vivo* and clinical relevance reports.

Effect of irradiation. Irradiation has a wide application in the food industry and research. It is a fascinating tool for food preservation having minimum alteration of food constituents while maintaining its sensorial properties. It is also applied widely as a cold pasteurization method for the preservation of food and is adopted worldwide in around 60 countries (Mostafavi, Mirmajlessi, & Fathollahi, 2012). Irradiation processing involves the treatment of food material with electrons or X-rays produced by an electron accelerator machine or γ rays produced by a radioisotope source. The intensity of irradiation dose for food application lies around 1 to 10 kGy (intermediate dose) and elevated dose range lies between 10 and 50 kGy for low moisture food (Fellows, 2016). The treatment of food with this high energy radiation leads to structural changes in protein molecules by fragmentation, aggregation, cross-linking, amino acid modification, and generation of new reactive groups. These alterations are provoked due to the disruption of covalent bonds either by direct application of photons or by the application of reactive species. All these processes may attenuate the immunogenicity of food based on the irradiation dose, protein concentration, and structural stability of allergenic proteins (Harder, Arthur, & Harder, 2017; Luo et al., 2013).

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Research suggests the dose range of 10 kGy can alter IgE binding epitopes greatly. Applying a high dose of gamma irradiation on peanut extracts indicated the reduction of its allergenicity attributes in terms of Th-2 lymphocyte activity (Oh et al., 2009). Irradiation leads to the reduction in protein solubility, which is one of the reasons given for attenuation of immunogenicity (Kasera et al., 2012). Another study reported by Luo and co-workers suggests that allergenic potency of purified peanut allergen Ara h 6 and whole peanut extracts decreased with an increase in gamma irradiation doses of 1, 3, 5, or 10 kGy. After the high dose treatment of 10 kGy, Ara h 6 was undetectable by SDS-PAGE and only had a 5% IgG binding ability (Luo et al., 2013). Moreover, CD spectra revealed a significant alteration in the secondary structure of Ara h 6 including a clear loss in α-helix, β-turn, and random coils (Luo et al., 2013). An *in vivo* study using irradiated peanut in peanut-sensitized mice revealed that lowering of T-cell proliferation activity, suppressed production of Th-2 cytokines IL4, and induced production of Th-1 cytokine IFN-γ (Oh et al., 2009).

A major part of the population avoids consuming irradiated food marked with the "Radura" symbol out of fear of consuming radioactive material. However, it has been suggested that irradiated food with a dose limit up to 10 kGy is safe for consumption with no hazards (Kebede, Simachew, Disassa, Kabeta, & Zenebe, 2015), Some researchers disagree and present their concern regarding some of the negative health impacts of irradiated food material, and this kind of quality perception effects consumer acceptance toward irradiated food (Bearth & Siegrist, 2019; Harrell, Djonov, Fellabaum, & Volarevic, 2018).

Effect of ultrasonication. Ultrasonication technology is an emerging novel processing technique with versatile application in food processing industries such as sterilization, enzyme inactivation, homogenization, filtration, rapturing of cells and biomolecules. As a safe and feasible processing technique, ultrasound processing has been applauded over conventional food processing methods. High-intensity ultrasound applies high energy waves (20-100 kHz), induces sonicating bubbles, and collapses the cavity in the food matrices which subsequently causes the formation of a localized region surrounded by these cavities. As a result, the conformation of protein molecules may be altered in terms of its native form, secondary structure, other inter/intramolecular interactions, and susceptibility to cleavage of its peptide bonds in the environment of collapsed cavities and due to the sheer force (Corzo-Martínez, Villamiel, & Moreno, 2017; Rodríguez et al., 2018). The cavities increase with the increase in pressure and frequency, initiating diverse chemical effects such as generating free radicals and accelerating the chemical reactions because of the effect of heat and pressure gradients. The collapse of the bubbles surround those cavities causing a pressure and heat gradient to occur, leading to mechanical and chemical changes to the protein. These effects may ultimately disrupt the protein structure in terms of hydrogen bonds and Van der Waals interactions, leading to a reduction in immunogenicity (Corzo-Martínez et al., 2017). The modification in the secondary structure of the milk allergen β-lactoglobulin has been previously reported with the formation of a random coil of β-variants and α-helices due to the dimerization effect of ultrasonication with the slight reduction in immunogenicity (Stanic-Vucinic et al., 2012).

According to a recent study, a remarkable reduction (51.39%) in the IgE binding of a soybean allergen was reported upon treatment of soybean with ultrasound (300 W power) prior to germination (Yang, Gao, Yang, & Chen, 2015). The increased amino acid content of soybean in post ultrasound treated soybean indicates a disruption of the peptide bonds, which can cause the release of amino acids from the peptide chain. Moreover, the ultrasound treatment of roasted peanut kernels increased the protein solubility and caused the cleavage of peptide bonds as an impact of the shear force that significantly decreased Ara h 1 levels, while the Ara h 2 concentration was not significantly altered (Li et al., 2013). The effect of the combination of ultrasound and subsequent enzymatic hydrolysis (hurdle approach) by trypsin and chymotrypsin on ultrasound treated whole peanut kernels was also analyzed by the researchers (Li et al., 2013). This treatment reduced the immunoreactivity of soluble peanut allergens Ara h 1 and 2 by approximately 50%. A previous report (Yu et al., 2012) suggested the change in the native structure of peanut proteins and cleavage of peptide bonds from the shearing force that takes place by the surface cavities generated by ultrasound. Thus, this disruption/loosening of peptides facilitates the penetration of the enzyme into the kernel and enhance contact with the protein molecules (Li et al., 2013). Recently, another combined approach using ultrasonication and germination of different peanut cultivars were reported, subjecting a high-intensity ultrasonic wave of 100 kHz prior to germination for 3 days showed completely diminished extraction of allergenic proteins (Yu et al., 2016). This study signifies that the efficiency of the process depends on the treatment conditions such as ultrasound intensity, treatment duration, and the characteristics of the protein. Although in the case of allergen treatment, especially peanut allergens, ultrasonication seems to be at a proof-of-concept stage, which has not been studied much.

Effect of pulsed electric field. Pulsed electric field (PEF) technology is emerging as a way toward minimal processing of food preservation and maintains the nutritional and sensorial quality. PEF technology was recently considered suitable for industrial application in food science, although its first application to milk was presented in 1919 (Anderson & Finkelstein, 1919). PEF employs very high voltage pulses (1 to 80 KV/cm) for fractions of a second such as millisecond or microsecond; the voltage and the treatment duration can be adjusted according to the requirements. Besides microbial inactivation, PEF is also applied for alternative purposes such as enzyme inactivation (Onwude et al., 2017), and to increase extraction yield (Puértolas & Martínez De Marañón, 2015; Yan, He, & Xi, 2017). In relation to proteins, PEF has been noted to alter the structural features of proteins; for example, a PEF treatment of 35 KV/cm for 40 μs induces vibrational changes on the bonded side of amino acid chains, altering β-sheets and β-turns suggesting the denaturation of the protein (Liu, Zeng, Deng, Yu, & Yamasaki, 2011). Another recent study reports that the PEF treatment at 25 KV has altered the ovalbumin protein structure by reducing its α-helix content by 66.02% and increasing its β-sheet content by 41.30% and therefore disruption of the native conformation (Qian, Ma, Wang, & Jiang, 2016).

The effect of PEF treatment in peanut protein has also been explored recently. FT-IR spectra showed significant alteration in protein structure (α-helixes, β-sheets, β-turns, and random coils) as an effect of PEF treatment (Vanga et al., 2016). Thus, in general, PEF can also induce alterations in the structure and immunogenicity of food allergens. Very few attempts have been made to evaluate the effect of PEF treatment on allergenic proteins. One study demonstrated that PEF treatment had minimal success toward mitigating the immunogenicity of peanut as this treatment did not show high impact on the structural alteration of peanut (Ara h 2, Ara h 6) or apple (Mal d 3, Mal d 1b) allergens (Johnson et al., 2010). However, more extensive research with varying

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conditions are required to assess its effect on allergenicity as a single treatment or in combination with other processing techniques and in different allergenic food matrices. Therefore, no clear interpretations can be derived based on these limited studies until more detailed research focusing on different allergenic proteins can be conducted. Ohmic heating, the other electric field induced mechanism, has not been studied for the applicability to alter allergenic proteins. Ohmic heating of food causes thermal and electrical effects that may alter the allergenicity attributes by altering the allergenic protein in food (Jaeger et al., 2016); however, more research is necessary to fully understand the effects of Ohmic heating on allergenic proteins.

Hurdle technology

With a demand for nutritious food material and to achieve the desired modification of food products, a combined processing effect has the potential for various applications. Hurdle technology is described as a combination of two or more processing techniques, which has been successfully used or attempted to effectively preserve food (Pottier et al., 2017). Hurdle technology promises safe food while maintaining its nutritive and sensory properties.

Table 5 summarizes the effect of hurdle techniques studied for attenuating peanut allergenicity. One study combined high pressure and heat, causing the complete reducing of Ara h 2 IgE reactivity of peanut protein extracts that indicated immunoreactivity of peanut may be eliminated (Long et al., 2016). Furthermore, this study was also conducted in a mouse model for clinical relevance. Results indicated that the IgE and serum histamine levels were significantly reduced, and there were no or very low levels of cytokines among mice fed with pressure and heat treated peanut extracts (Long et al., 2016). Ara h 2 has already been shown to be the most structurally stable allergen in peanut toward processing than any other allergenic proteins, thus, this study of combined processes may provide a future hope to mitigate peanut allergenicity through processing.

As previously discussed in section "Effect of ultra-sonication," another study combined ultrasound and enzymatic hydrolysis (Li et al., 2013), which sequentially treated roasted peanut kernels via ultrasound-trypsin-alpha chymotrypsin. This study suggests that the sequential enzymatic and ultrasound treatment of a whole roasted peanut kernel may reduce the Ara h 2 and Ara h 1 solubility up to 98% and >99%, respectively. Ultrasound treatment prior to enzymatic treatment allow easier penetration of enzyme into food matrices and may facilitate enzymatic digestion of proteins (Yu et al., 2012). More recently, another significant work with post enzyme treatment roasting on raw peanut kernels reports that there was a reduction of 99% to 100%, 95% to 99%, 35% to 46%, and 85% to 88% of the major peanut allergens Ara h 1, Ara h 2, Ara h 3, and Ara h 6, in soluble extracts, respectively (Mikiashvili & Yu, 2018). Subsequently enzymatically treated roasted peanut kernels reduced the content of Ara h 3 and Ara h 6, slightly increased the content of Ara h 1, and did not alter the content of Ara h 2 (discussed in section "Enzymatic cross-linking and hydrolysis" in detail). Another recent study reported the effect of a combined processing approach on cashew and pistachio nuts allergen (Cuadrado et al., 2018). This study reports that the combined treatments of heat (using an autoclave) and enzymatic hydrolysis under sonication significantly reduced or even diminished the IgE binding activity of cashew and pistachio allergens in nuts pastes. Researchers (Cuadrado et al., 2018) suggest that the combination of heat with enzymatic hydrolysis was necessary to obtain such a degree of reduction that might not be achieved by simple unit operation processes previously.

Other studies using combined processing approaches have been discussed in previous sections of this review; however, studies surrounding hurdle approaches are rare. More extensive hurdle studies with different treatment aspects are required, which combine physical–physical and physical–chemical processing, toward diminishing the allergenicity of peanut.

Interpreting Remarks & Future Prospective

The prevalence of IgE mediated food and peanut allergy among the globe, sensitization to peanut, and severity of reaction have increased greatly and continue to increase. Thus, there is need for finding a way to improve the quality of life for allergic individuals. The lack of effective controlling strategies and reliable therapies make the issue crucial. Immunotherapies, especially OIT in clinical trials, showed promising results in desensitization of affected individuals. In the meantime, OIT is facing concerns due to the fear of adverse reactions during the course of therapy, and many other questions such as persistency of desensitization among other questions that need more research. The persistence of peanut allergy is one of the main reasons for severe and deadly anaphylaxis, particularly because of accidental ingestions. The current management for sensitized individuals only relies on the awareness and avoidance of peanut and potentially cross-reactive foods, which is being addressed by labeling legislation on processed foods. ELISA is the most commonly adopted tool for the detection of peanut contaminants in food but genomic (PCR) and proteomic (MS) approaches have gained some appeal and may take over in the future. However, most cases of reactions from peanut are due to accidental ingestion, and undeclared allergens are also responsible for the highest food recall from the market. The development of high throughput and highly sensitive detection and quantification

Table 5–Effect of hurdle technology on the immunoreactivity of peanut allergens.

Hurdle approach	Peanut allergen	Effect of processing	Mechanism	Reference
HPP and heat	Ara h 2	IgE binding level was altered to an almost diminished stage. The effect was also confirmed by *in vivo* study.	High structural alteration might have occurred.	Long et al. (2016)
Ultrasonication and enzymatic hydrolysis	Ara h 1 & 2	IgE reactivity level was reduced to 50%.	The structural alteration by ultrasound treatment may largely facilitate enzymatic activity.	Li et al. (2013)
Ultrasonication and germination	–	IgE reactivity was completely diminished.	–	Yu et al. (2016)
Roasting and enzymatic hydrolysis	Ara h 1, 2, 3 & 6	IgE reactivity of all major peanut allergens were remarkably reduced (up to >90%).	–	Mikiashvili and Yu (2018)
Roasting and ultrasound	Ara h 1 & 2	IgE binding ability and allergen content were significantly reduced.	Alteration in the protein structures occurred, thus, epitopes were altered.	Li et al. (2013)

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methods with high specificity for these allergens that can easily be adopted by processing industries are very important. Therefore, enforcement of the labeling regulations with most reliable approach may improve safety and minimize accidental ingestions and food recalls.

To date, 16 peanut proteins are registered as being allergenic and there may be more unknown proteins with allergenic attributes. Some of those allergens have been characterized fully and some are characterized partially while characteristics of many of them are completely unknown and should be studied. Characteristics of the allergenic proteins such as physico-chemical, sequential, and structural features often give us an understanding of their function, digestibility, and stability during thermal and other processing methods. Food processing is the key tool that has been shown to alter the allergen structure and properties. Peanut allergens can cause severe allergic reactions in minute quantities; thus, the future goal to provide safety to peanut sensitized individuals may not be accomplished until we can mitigate the risk of allergenicity. The allergens are typically more resistant to different processing than other proteins, which make the issue more complex. Each allergen reducing methodology reported can mitigate the IgE reactivity of peanuts to a varying degree. Since the complete elimination of allergenicity of the peanut is unlikely by most conventional processing techniques, the selection of proper conditions/approaches and combining different processes may be important in significantly reducing or eliminating IgE reactivity, which may increase the threshold dose for reactivity, reduce the severity of allergic symptoms, and ultimately increase safety for allergic individuals.

Recent studies suggest that some processing technology, either unit operation or hurdle technology, may have the potential to mitigate peanut immunoreactivity. Traditional physical methods such as boiling significantly reduce the immunogenicity of peanut to a certain degree but cannot eliminate IgE binding as a single treatment. On the contrary, roasting and other heat treatments can increase the immunogenicity and may produce new allergenic complexes or neo-allergens. The novel processing approaches such as PUV, HPP, high-intensity ultrasound, irradiation, and PEF methods demonstrated a potential replacement against traditional heat treatments with perhaps, a higher potential to reduce the immunogenicity of peanut or other food allergens depending on the processing conditions and treatments. Among the physical processing techniques, PUV and gamma radiations as a single unit operation appear to be the effective treatments toward diminishing the immunoreactivity of peanut allergens; on the other hand, PUV is also prone to cause oxidation in peanut matrices due to its high oil content and the consideration of a safe dose of gamma radiation in food is a concern. The covalent and noncovalent modification of peanut proteins or peanut derived products, especially some studies using polyphenol complexes with peanuts, to mask the allergenic epitopes, seems to be potential approaches to reduce allergen levels in solution and they require less energy. Digestibility of such soluble or insoluble complexes and behavior in a living system are mostly unknown, consequently, it will also be crucial to figure out the allergenic response to these products upon digestion in the body. Enzymatic hydrolysis, alone and/or its combination with other processing techniques, seems to be the most effective processing approach in diminishing the immunoreactivity of peanut as it has the potential to disrupt both conformational and linear epitopes of the allergenic proteins. In addition, few studies using the application of enzymatic hydrolysis in whole peanut kernels may be interesting as it may allow the whole peanut kernel to transform in any product, although its *in vivo* allergenicity behavior is unknown. In addition, Hurdle approaches studied so far, such as HPP and heat, ultrasound and enzymatic hydrolysis or germination, and enzymatic hydrolysis and roasting, may have the potential to significantly diminish immunoreactivity toward peanut allergens. Hurdle approach has advantages over other techniques as it allows the combining of different processes, which may emerge as the future, most effective tool to eliminate allergenicity attributes. Most importantly, the initial trial of immunotherapy with boiled peanut indicates that the processed peanut with very low IgE binding may serve as a better choice for immunotherapy due to reducing the risk of adverse events, while retaining the ability to desensitize the affected individuals.

The development of appropriate processing techniques to diminish peanut allergenicity and produce hypoallergenic/non-allergenic peanut products is a common goal to provide safety, perhaps reduce the development of allergy, and improve the life style of allergic individuals. In this regard, extensive research is needed to determine the suitability of techniques and optimum conditions. Most of these novel approaches and Hurdle techniques are at a proof-of-concept stage that needs further investigation. More importantly, many of the reported allergenicity reducing techniques lack the *in vivo*, *ex vivo*, and clinical relevance studies to analyze its real interactions in the body system and to fully understand the mechanisms. Allergenic proteins typically are resistant to proteolysis and, indeed, they may not be completely hydrolyzed during digestion, yet the hydrolyzed fragments may still be allergenic. Subsequently, it is also crucial to study the influence of different processing techniques on the allergenicity upon digestion. Most of the studies conducted so far are based on only some of the major peanut allergens, even though many more allergens may contribute to severe allergenic reactions. Further research using hypoallergenic peanut created by different processing techniques for safe application as immunotherapy may be quite interesting.

Peanut shares a huge number of cultivars worldwide that vary in their nutritional composition, thus, it might be interesting to find out whether or not different cultivars are lacking or have lower contents of different allergenic proteins. Furthermore, the feasibility of processing techniques in relation to peanut products such as whole peanut, peanut extracts, and powdered nuts are important considerations as well as common processing techniques that may be applied to most industrial products.

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Authors Contributions

S. Faisal researched prior studies, data collection, study design, writing, compiling, and interpreting the study. A. Shi collected references, provided guidance and suggestions, helped in revising, and helped in compiling the manuscript. *Q. Wang researched the prior studies, discovered the idea, content selection, guidance

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throughout the manuscript, revising, and helped to interpret the results. *S. J. Maleiki collaborated throughout the study and revised and edited the manuscript. J. Ashley provided critical suggestions, guidance, and revised the manuscript. C. Kronfel helped in revising and editing the manuscript. B. Adhikari and J.-C. Zhang helped in collecting the references and revising part of the manuscript.

Conflicts of Interest

The authors describe there is no conflict of interest.

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