



Review

Effects of nonthermal food processing technologies on food allergens: A review of recent research advances

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ARTICLE INFO

Keywords:

Food allergy
Nonthermal technologies
Allergen
Allergenicity
Immunoreactivity

ABSTRACT

Background: The increasing prevalence of food allergy cases is an issue of global concern. As a result, scientific innovations have been taking place to induce chemical modifications for achieving attenuation of allergenic responses in sensitive individuals or for manufacturing hypoallergenic foods using food processing technologies. Conventional processing techniques involving heat treatment are usually exploited, but may be accompanied by dramatic changes in food quality attributes due to high temperature. Therefore, alternative nonthermal technologies may be a new direction for attaining hypoallergenicity.

Scope and approach: This review presents the current knowledge and recent findings on the possibility of engaging nonthermal technologies including pulsed light, high pressure processing, irradiation, cold plasma, ultrasound and pulsed electric field, for the elimination of allergenic proteins in foods and their underlying mechanisms. In contrast to heat-based methods, nonthermal treatments can adequately retain sensorial and nutritional quality of the food products, while altering allergenicity. Food allergy classification, clinical manifestations, epitope characterization and detection methods are also presented.

Key findings and conclusions: To date, studies show that nonthermal processes have complicated effects on food allergens. Much further research efforts should be made for using nonthermal processing technologies as alternatives to replace traditional techniques. Nonetheless, it is expected that in the near future, susceptible individuals will benefit from different hypoallergenic products processed with novel nonthermal technologies at an affordable cost.

1. Introduction

Food allergy is an abnormal immunological response or allergic reaction that occurs when certain foods are consumed. It is considered a major public health concern, affecting 1–2% of adults and 3–6% of children living in developed countries (Jerschow, Lin, Scaperotti, & McGinn, 2014; Ketnawa & Liceaga, 2017; Rona et al., 2007). A survey conducted in 2013 by the Centers for Disease Control and Prevention, revealed that the number of children allergic to certain foods increased by 50% between 1997 and 2011 (Gupta, Holdford, Bilaver, Dyer, & Meltzer, 2013). Besides, over 150 million Europeans suffer from food allergy and it is speculated that an astounding 44% of adults living in Britain have at least one form of allergy or the other, growing by around 2 million from 2008 to 2009 alone (Food matters, 2010). In addition,

between 1995 and 2007, up to five-times increase in food allergy cases was reported among children living in Australia (Mullins, Dear, & Tang, 2009). Generally, over 170 foods can provoke allergic reactions, but 90% of incidences are believed to be caused by allergenic proteins present in milk, egg, soy, crustacean/shellfish, fish, tree nut, peanut, and wheat (Shriver & Yang, 2011; Thompson, Kane, & Hager, 2006), but a large number of potentially allergenic individuals are unaware of their allergic reactions to certain foods until after consumption. Typically, clinical symptoms includes respiratory issues (e.g. conjunctivitis, rhinitis), skin reactions (e.g. urticarial, eczema and angioedema), cardiovascular disorders (e.g. vascular collapse, hypotension) and problems with the gastrointestinal (GI) tract (e.g. vomiting, diarrhea, and abdominal pain) (Cianferoni & Spergel, 2009). Meanwhile, clinical manifestations associated with non-IgE-mediated food allergies include

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food protein induced enterocolitis (FPIES), weight depreciation, proctocolitis syndrome, pulmonary hemosiderosis and celiac disease (Cianferoni & Spergel, 2009; Henderson et al., 2012). Other symptoms of food allergy includes the life threatening anaphylaxis, with a detailed epidemiological survey conducted in the united states revealing that annually, about 125000 cases of emergency and 53700 cases of anaphylaxis is caused by food allergies, leading to hospitalization of 2000 patients and in acute cases, 200 deaths (Sicherer & Sampson, 2010). Unfortunately, the current best practice to prevent food allergy is to avoid consumption of allergenic foods.

The characteristic nature of antigenic proteins is basically glycoproteins with molecular masses of 10–70 kDa, which contain protein sites called epitopes. These epitopes are recognizable and interact with allergen receptors based on their three-dimensional conformation. Considering their molecular configuration and structure, any process that induces changes in protein conformation can also affect structural features of epitopes, thereby influencing antibody binding ability. In fact, some treatments such as heating induce formation of new allergenic compounds, otherwise called neo-allergens via interactions among constituents (Kumar, Verma, Das, & Dwivedi, 2012).

Conventionally, food raw materials are processed to enhance sensorial and nutritional quality as well as for preservation and detoxification. Similar to cooling (Desmond, Kenny, Ward, et al., 2000; McDonald, Sun, & Kenny, 2001; Sun, 1997; Sun & Eames, 1996; Sun & Zheng, 2006; Wang & Sun, 2001; Wang & Sun, 2004) and freezing (Cheng, Sun, & Pu, 2016; Cheng, Sun, Zhu, & Zhang, 2017; Kiani, Sun, Delgado, & Zhang, 2012; Kiani, Zhang, Delgado, Sun, 2011; Ma et al., 2015; Pu, Sun, Ma, & Cheng, 2015; Xie, Sun, Xu, & Zhu, 2015; Xie, Sun, Zhu, & Pu, 2016), techniques are usually employed to achieve these objectives (Delgado & Sun, 2002; Ma, Sun, Qu, & Pu, 2017; Pu & Sun, 2016; Pu & Sun, 2017; Qu, Sun, Cheng, & Pu, 2017; Sun, 1999; Sun & Woods, 1993; Yang, Sun, & Cheng, 2017). For proteins, as temperature increases, a gradual loss of its native structure occurs. This may influence allergenicity either positively, negatively or sometimes have no effect at all. Generally, heat treatment modifies proteins via denaturation, restructuring of disulfide bonds and generation of new intra/inter molecular bonds. Consequently, epitopes are rendered unrecognizable by immunoglobulin E (IgE) antibodies. For example, severe processing conditions of boiling and autoclaving for 60 and 30 min can reduce the immunoreactivity of allergenic proteins in lentil and chickpea, respectively (Cuadrado et al., 2009). This implies that, obtaining positive results requires extreme temperatures that can provoke losses in organoleptic quality, nutritional profile or even techno-functionality.

With the developments of nonthermal processing techniques, such as pulsed light, high pressure processing (HPP), irradiation, cold plasma, ultrasound and pulsed electric field (PEF), it is possible that nonthermal processing techniques can also induce changes in protein conformation and mitigate the allergenicity of certain proteins, while retaining the original characteristics of the food materials. Therefore, researchers have explored the impact of non-heat based processing technologies on allergenic proteins and the possibility of producing hypoallergenic foods using such approaches. For instance, pulsed light was discovered to effectively decrease gluten immunoreactivity through structural rearrangement and the formation of a broad range of molecular weight proteins, following partial depolymerization and unfolding of oligomeric and monomeric fractions respectively (Panozzo, Manzocco, Lippe, & Nicoli, 2016). Also, the allergenicity of α -lactalbumin (α -la) remarkably declined when irradiated at doses of 1–10 kGy due to extensive protein denaturation and aggregation (Meng et al., 2016).

On this basis, a few authors have summarized research findings focused on exploiting both thermal and nonthermal processing techniques to modify food allergenicity (Chung & Reed, 2014; Jimenez-Saiz, Benede, Molina, & Lopez-Exposito, 2015; Rahaman, Vasiljevic, & Ramchandran, 2016; Shriver & Yang, 2011; Vanga, Singh, & Raghavan, 2017). However, detailed descriptions on the effects of nonthermal

treatments on allergenic proteins are not available, and thus the current review intends to provide a comprehensive and up-to-date overview on the control of food allergens using nonthermal processing technologies. Various types of allergies and clinical spectrum are also presented.

2. Food allergens and their properties

Food allergy is generally classified into immunoglobulin (IgE)-mediated, non-IgE mediated and mixed-reaction types. The IgE-mediated type of allergic reactions occur when IgE antibodies are produced in patients, due to permeation of peptides or proteins from food into the respiratory lining, gut or skin (Mansouri, 2015). As described in Fig. 1, an antigen-presenting cell processes the antigen and releases it to the T cells in a major complex-restricted manner of histocompatibility, thereby activating T cells. Subsequently, food-specific IgE antibodies are produced, followed by attachment to IgE receptors present on basophil receptors before regaining exposure to the food allergen, causing a very swift response transpiration, which provokes degranulation of effectors cells and liberation of pre-formed granules such as histamine-like mediators, and prompts the immediate manifestation of allergic reactions (Cianferoni & Spergel, 2009). The non-IgE mediated type of food allergy is associated with delayed onset of symptoms (either sub-acute or chronic in nature) predominantly confined to the gastrointestinal (GI) tract (Henderson et al., 2012). On the other hand, the mixed-reaction type comprises of an overlap between delayed or chronic onset, presenting itself as either atopic dermatitis (AD) and as eosinophilic gastroenteropathy (Mansouri, 2015).

2.1. Characteristics of food allergens

Most food allergens are primarily water-soluble glycoproteins with molecular weight varying from 10 to 70 kDa, which are stable to proteolytic reactions, and heat and acid treatments (Sicherer & Sampson, 2010). However a disparity from these attributes is possible since allergenic proteins can undergo modifications especially during processing. Moreover, food allergens like vicilins that are oligomeric or those containing a reoccurring sequence of peptide units (e.g., tropomyosins) can have higher immunogenicity due to increased valency of their IgE epitope. Likewise, proteins such as lipid transfer proteins (LTPs) and 2S albumins that contain extra intramolecular disulfide bonds may bestow more allergenicity and impact greater stability against thermal or acid denaturation. Binding of ligands such as metals ions, lipids and steroids to allergenic proteins (e.g., caseins, parvalbumins, β -lactoglobulin (Lg), and Bet v 1-related food proteins respectively), may stabilize their structural conformation and enhance their resistance to proteolytic enzymes; most proteases require flexibility in substrate proteins for their action (Breiteneder & Mills, 2005; Sathe, Liu, & Zaffran, 2016). It is important to mention that ligands, which can bind to a specific protein, do it in a unique pathway involving a series of complex interactions. For instance, caseins (α_{s1} -, α_{s2} -, and β -caseins) typically contain clusters of phosphate groups *i.e.*, phosphoserine or phosphothreonine residues that can chelate metal ions, including calcium, thereby forming microstructures called nanoclusters. These nanoclusters combine into bigger structures comprising of about 1000 nanoclusters that correspond to the casein micelles present in milk (Breiteneder & Mills, 2005). Meanwhile, parvalbumin contains 3 EF-hand motifs or domains, among which two are capable of binding to calcium, while the third domain remains silent but forms a cap covering the hydrophobic surface. Loss of the protein-bound calcium in parvalbumin can induce a significant change in conformation and an accompanying loss of conformation-dependent IgE epitopes (Bugajskaschretter et al., 2000). This is why the use of antacid by patients allergic to parvalbumin is frowned upon because acid neutralization decreases the removal of calcium ions and may contribute to the resistance of parvalbumin to pepsin hydrolysis and increase the vulnerability of sensitive individuals (Untersmayr et al., 2003).

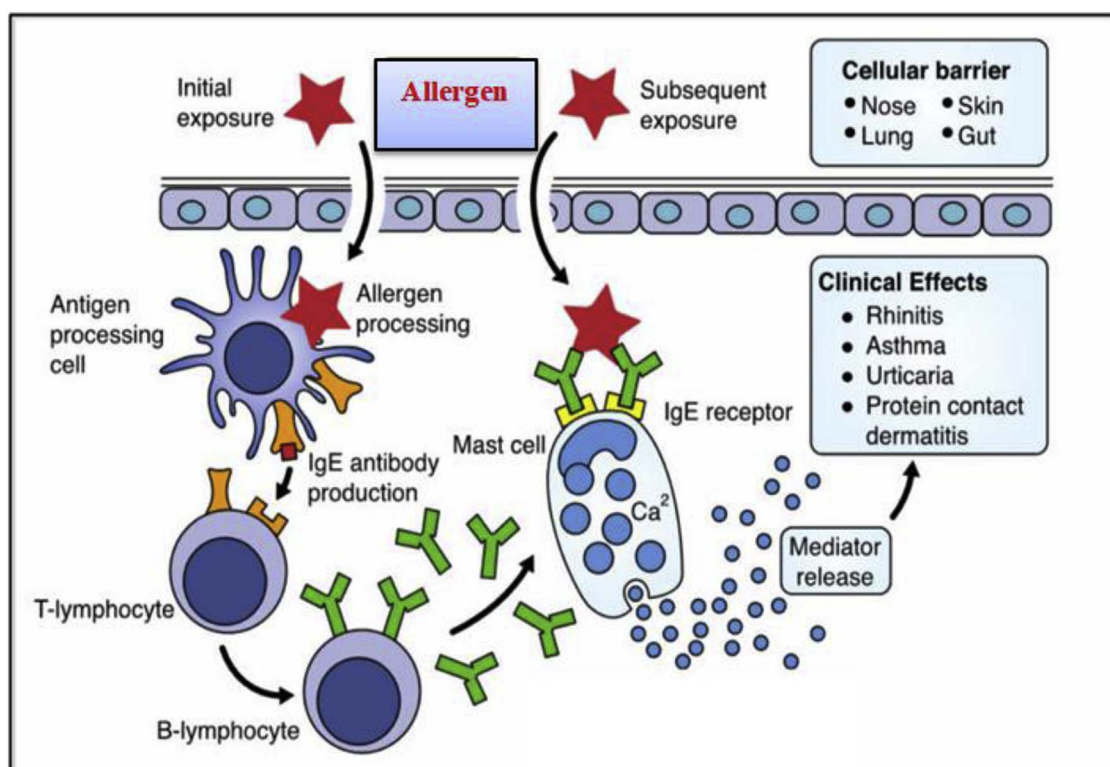


Fig. 1. Schematic representation of Type I IgE-mediated hypersensitivity reaction.

The immunoreactivity is mainly determined by epitopes, *i.e.*, the part of a protein recognized by antibodies (Fig. 2) which can be classified into linear (sequential) and conformational (discontinuous), with T-cell-binding epitopes being exclusively linear. Linear epitopes consist of continuous short peptide segment, while conformational epitopes consist of three-dimensional motif formed by spatially adjacent amino acids. Several techniques are employed to map T-cells and IgE-binding epitope in order to supply vital information for the design of peptide and/or recombinant protein-based immunotherapy for food allergy. Specifically, peptide microarrays and a sequence of overlapping peptides fabricated on a nitrocellulose membrane (SPOT membrane) are often used to ascertain IgE-binding linear epitopes (Martínez-Botas et al., 2013), whilst complex techniques such as nuclear magnetic resonance, mutant generation, x-ray crystallography, and silicon analysis are required for mapping conformational epitopes (Sharma, Gaur, & Arora, 2013). Alternatively, hydrogen/deuterium exchange footprint analysis may be employed but cannot be used for the identification of

conformational epitopes recognized by human polyclonal IgE antibodies. For helper T-cells and regulatory T-cells, which play a role in the sensitization, desensitization, and tolerance induction of food allergy, their epitopes can be mapped using peptides of 10–20 residues that overlay the complete AA sequence of the target allergen and allergen-specific T-cell lines. In addition, silico analysis can be used to identify T-cell epitope sequences, although several synthetic peptides need to be tested alongside T-cells from patients in order to substantiate the biological reactivity of the predicted epitopes (Nielsen, Lund, Buus, & Lundegaard, 2010). Table 1 summarizes the most common allergenic foods with their associated main allergens.

3. Thermal processing and food allergenicity

As mentioned previously, thermal processing is one of the most important and extensively utilized techniques for the food industry. Generally speaking, thermal treatment of food is accompanied by

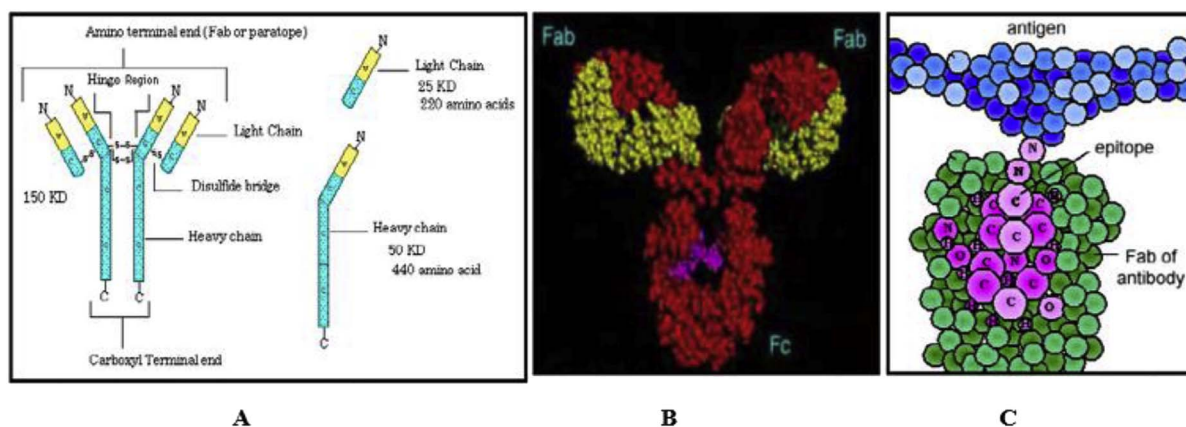


Fig. 2. A. Structure of an immunoglobulin, B. The tri-dimensional molecular model of an IgE molecule showing the antigen binding site (Fab) C. Diagram illustrating antigen bound to fab of the antibody (Yang, Mwakatage, Goodrich-Schneider, Krishnamurthy, & Rababah, 2012).

Table 1
Common sources of food allergens.

Type of Food	Food source	Protein name	IUIS Name	Molecular Weight (kDa)
Dairy products	Milk	β-Lactalbumin	Bos d 4	14.2
		β-Lactoglobulin	Bos d 5	18.3
		Serum albumin	Bos d 6	67
		Immunoglobulin	Bos d 7	160
		Caseins	Bos d 8	20–30
		α _s 1-Casein	Bos d 9	23.6
		α _s 2-Casein	Bos d 10	25.2
		β-Casein	Bos d 11	24
		k-Casein	Bos d 12	–
	Egg	Ovomucoid	Gal d 1	28
		Ovalbumin	Gal d 2	44
		Ovotransferrin	Gal d 3	77
		Lysozyme	Gal d 4	14
		Serum albumin	Gal d 5	69
Seafood	Crustaceans	Tropomyosin	Cha f 1, Cra c 1, Hom a 1, Lit v 1, Mac r 1, Mel l 1, Met e 1, Pan b1, Pan s1, Pen a1, Pen i1, Pen m1, Por p1; 3	34–39
		Myosin light chain 1	Art fr 5, Cra c 5	17.5
		Myosin light chain 2	Hom a 3, Lit v 3, Pen m 3	20–23
		Troponin C	Cra c 6, Hom a 6, Pen m 6	20–21
		Arginine kinase	Cra c 2, Lit v 2, Pen m 2	40–45
		Triosephosphate isomerase	Arc s 8, Cra c 8	28
		Troponin I	Pon 1 7	30
	Fin Fish	β-Parvalbumin	Clu h 1, Cyp c 1, Gad c 1, Gad m 1, Lat c 1, Lep w 1, Onc m 1, Sal s 1, Sar sa 1, Seb m 1, Thu a 1, Xip g 1	11–12
		β-Enolase	Gad m 2, Sal s 2, Thu a 2; 47	3–50
		Aldolase A	Gad m 3, Sal s 3, Thu a 3	40
		Vitellogenin	Onc k 5	18
		Tropomyosin	Ore m 4	33
	Tree nut	Cashew	7S globulin, 11S globulin and 2S albumin respectively	–
		Almonds	–	–
		Brazil nut	–	–
		Pecan	–	–
		Chest nut	–	–
		Walnut	–	–
		Pistachio	–	–
Legume	Hazel nut	–	–	–
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		–	–	–
	Peanut	Cupin (Vicillin-type, 7S globulin)	Ara h 1	64
		Conglutin (2S albumin)	Ara h 2	17.5
		–	Ara h 6	15
		–	Ara h 7	16.17
		Cupin (Legumin-type, 11S globulin, Glycinin)	Ara h 3	60
		Profilin	Ara h 5	14
		Pathogenesis-related protein 10 (PR-10)	Ara h 8	17
		Non-specific lipid-transfer protein type 1	Ara h 9	9.1
		Oleosin	Ara h 10	18
		–	Ara h 11	14
		Defensin	Ara h 12	5.2
		–	Ara h 13	8.4
	Soybean	Non-specific lipid transfer protein	Gly m1	18–71
		Defensin	Gly m2	8
		Pathogen related protein (PR-10)	Gly m3	17
		Profilin	Gly m3	14
		Cupin (7S vicilin like globulin)	Gly m Bd28K	28
		Thiol protease of the papain superfamily	Gly m Bd30K	34
		Lectin, SBA (agglutinin)	Gly m Lectin	14.5
		Cupin (7S vicilin like globulin)	Gly m Bd 60 K	63–67
		Glycinin, 11S seed storage protein	Gly m glycinin G1	40
		Glycinin, 11S seed storage protein	Gly m glycinin G2	22
		Glycinin, 11S seed storage protein	Gly m glycinin G4	61.2
		Kunitz trypsin inhibitor	Gly m TI	20
	Lentil	Vicilin	Len c1	48
		Seed biotinylated protein	Lenc2	66
		Non-specific lipid transfer protein 1	Len c 3	9
	Lupin	Conglutin beta (7S seed storage globulin, vicilin)	Lup an1	55–61
		Cupin	Lup a vicilin	66
		Cupin	Lup a 11S globulin	20

(continued on next page)

Table 1 (continued)

Type of Food	Food source	Protein name	IUIS Name	Molecular Weight (kDa)
Cereals	Pea	Vicilin	Pis s 1	44
		Convicilin	Pis s 2	63
	Chickpea	Prolamin (2S albumin)	Cic a 2S Albumin	10–12
		Cupin (Legumin type 11S, Glycinin)	Cic a 11S Globulin	–
	Blackgram	Homology with Rho-specific inhibitor of transcription termination	Vig m	28
	Green gram	Pathogen related protein (PR-10)	Vig r1	16
		Cupin (8S globulin beta isoform precursor)	Vig r2	52
		Cupin (38S globulin alpha isoform precursor)	Vig r3	50
		Cupin (seed albumin)	Vig r4	30
	French Bean	Non-specific lipid transfer protein 1	pha v3	8.8–9
	Red gram	Cupin superfamily (β -conglycinin α chain of soybean)	Caj c1	66
		Cupin superfamily (β -conglycinin α prime subunit of soybean)	Caj c2	45
		Cupin superfamily (β -conglycinin α prime subunit of soybean)	Caj c3	45
		Cupin superfamily (β -conglycinin α prime subunit of soybean)	Caj c4	45
		Cupin superfamily (β -conglycinin α prime subunit of soybean)	Caj c5	30
	Wheat	Profilin	Tri a 12	14
		Non-specific LTP	Tri a 14	9
		2S albumin	Fag e 2, Fag t 2	16
		7S vicilin	Fag e 3	19
		α -Amylase inhibitor	AAI, Tri a 15, Tri a	28–30
		Agglutinin	Tri a 18	21.2
		Thioredoxin	Tri a 25	13–14
		ω -5 gliadin	Tri a 19	65
		γ -Gliadin	Tri a 20	35–38
		High molecular weight glutenin	Tri a 26	88
		Low molecular weight glutenin	Tri a 36	40
Seeds	Oriental Mustard	2S-Albumin	Bra j 1	14.6
	Yellow Mustard	2S-Albumin	Sin a 1	14.2
	Rapeseed	2S-Albumin	Bra n 1	15
	Sesame	2S-Albumin	Ses i 1	10

Note: IUIS = International Union of Immunological Studies.

different modification reactions such as denaturation, hydrolysis of peptide bonds, restructuring of disulphide bonds and interaction with other components including carbohydrates and lipids. For proteins, thermal processing produces loss of secondary and tertiary structures and non-covalent interactions. Consequently, these arrays of reactions may affect allergenicity by either destroying or exposing conformational epitopes, *i.e.*, decreasing or enhancing immunoreactivity. For example, Bu, Luo, Zhang, and Chen (2010) revealed conformational changes in epitopes when β -Lg was subjected to 90 °C heat treatment, thereby enhancing its susceptibility to proteolysis while increasing allergenicity. Successful trials towards producing hypoallergenic food products using various forms of heat treatment have also been reported (Lechevalier et al., 2017; Sudha, Soumya, & Prabhasankar, 2016; Xu, Shi, Yao, Jiang, & Luo, 2016). In addition, under austere conditions, thermal processing may also be used to modify allergenic proteins via interactions with other food components. A typical example of such reaction is Maillard reaction, involving condensation of amino acids with carbonyl groups from sugars to form glycosamines, followed by the formation of advanced glycation end products (AGEs). This approach was successfully used to modify linear epitopes of β -Lg and reduce its IgE binding capacity (Taheri-Kafrani et al., 2009), however excessive glycation could reduce the proteolytic action on β -Lg, leading to increased IgE reactivity irrespective of the type of reducing sugars (Corzo-Martínez, Soria, Belloque, Villamiel, & Moreno, 2010). In general, using glycation to achieve positive outcomes depends on the reaction conditions, degree of Maillard reaction, and the amount and molecular weight of the saccharides. Additionally, heat processing may also alter the gastrointestinal digestion of food allergens by influencing their survival ability, inside the harsh acidic and proteolytic environment of the stomach. Most food allergens are associated with high stability and resistance to digestion by gastrointestinal enzymes (Astwood, Leach, & Fuchs, 1996).

Despite the effectiveness of thermal processing for altering food allergenic properties, thermal processes become less appealing when

attenuation can only be achieved at extreme temperatures, which can negatively affect organoleptic properties and nutrient composition. Moreover, some food allergens *e.g.*, tropomyosin, can withstand heat-treatment due to their exceptionally stable α -helical coiled-coil secondary structure or the presence of intra/inter molecular cystine that defines their structural integrity (Besler, Steinhart, & Paschke, 2001; Kamath, Abdel Rahman, Komoda, & Lopata, 2013; Reese, Ayuso, & Lehrer, 1999; Usui et al., 2013). Thus, it becomes imperative to seek out alternative methods to render food materials hypoallergenic, leading to the research and developments of nonthermal food processing methods such as pulsed light, HPP, irradiation, cold plasma, ultrasound and PEF for controlling food allergy.

4. Non-thermal technologies for food allergy control

4.1. Pulsed light

Pulsed light (PL) consists of a sequence of extremely short and high-power pulses of white light with broad spectrum, containing about 54% ultraviolet (UV) light, 20% infrared and 26% visible light radiation (Shriver & Yang, 2011). It is considered as an effective alternative for microbial inactivation during processing, production and handling of food products, in contrast to conventional or continuous UV light, owing to its high penetration power and rapid dissipation of high-energy pulses. In a typical PL emission process, high voltage is utilized to excite an inert gas, *e.g.*, xenon from a ground state to an excited state. Subsequently, the xenon returns to the ground state, energy is liberated in photons and absorbed by the food molecules, leading to series of photo-thermal, photo-physical, and photo-chemical reactions occurring in the food material (Shriver, Yang, Chung, & Percival, 2011). These photo-effects are accountable for how PL interacts with food molecules with benefits such as reduced energy consumption, less time consumption, absence of residues and easy adaptability.

One of the characteristics of photo-reactive food biomolecules is the

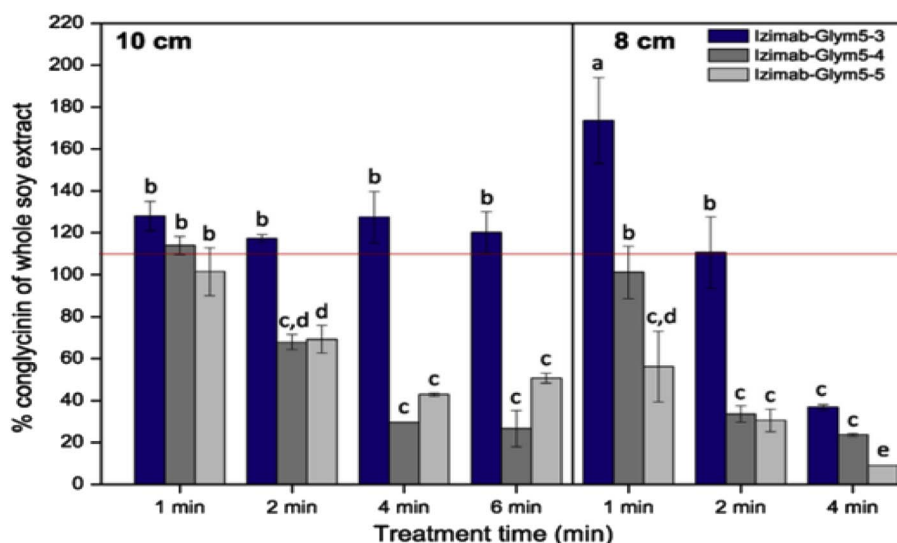


Fig. 3. Residual immunoreactivity of SPI dispersions treated with PUV light (8 and 10 cm) as measured by sandwich ELISA (Meinlschmidt et al., 2016).

existence of chromophores within the molecule and extensive exposure of food to UV light causes depolymerization of starch, peroxidation of unsaturated fatty acids and formation of insoluble complexes. Proteins are prone to photoreactions because they contain efficient chromophores, e.g., prosthetic groups and amino acid chains. Absorption of light by protein chromophores causes side-chain oxidation, protein cross-linking and aggregation, formation of insoluble proteins and backbone fragmentation, at high energy levels, and as a result, it may cause alteration in IgE binding to allergens (Li, Yu, Ahmedna, & Goktepe, 2013a). For instance, Ara h1, Ara h2 and Ara h3, the major allergens found in raw and roasted peanut extracts can be significantly decreased by PL treatment (Yang et al., 2012). The technology was also evidenced to decrease the IgE binding capacity of allergens in almond (Li et al., 2013a), peanut (Zhao et al., 2014), wheat (Nooji, 2011) and shrimp (Shriver et al., 2011).

Furthermore, a recent investigation confirmed a decline in immunoreactivity of soy Gly m5 and Gly m6 after treatment by pulsed ultraviolet light (PUV), emitted from a distance of 8–10 cm from the food material under varying treatment times. SDS-PAGE analysis revealed that after 2 min, bands of Gly m5 subunits completely disappeared while Gly m6 persisted until after 6 min. The disappearance of bands on the SDS-PAGE could be attributed to protein damage and precipitation. The presence of smeared bands signified the occurrence of cross-linkages between soy proteins and formation of aggregates due to exposure of hydrophobic and aliphatic side chains which could lead to protein-protein interactions (Meinlschmidt et al., 2016). In addition, a sandwich ELISA confirmed a significant reduction in immunoreactivity of all the samples. Notably, samples placed at 8 cm distance from the PUV lamp for 4 min showed up to 91% reduction in immunoreactivity (Fig. 3), owing to higher energy dose that could better modify conformational epitopes. Although the exact mechanical pathway needs to be thoroughly elucidated, the photo-effects from PUV could be summarized as the putative mechanism responsible for the reduction in antibody-binding ability of the soy proteins, as evident results reported by Li et al. (2013a) and Zhao et al. (2014). Generally, aromatic amino acids like phenylalanine and tyrosine present in food proteins can readily absorb UV light, accompanied by photo-oxidation and dissociation before recombining to form covalent cross-links in proteins, leading to formation of larger aggregates. These bands of larger soluble macro-aggregates (150–250 kDa) are observed when the process is accompanied heating or photo-thermal effects at a certain protein concentration (Mills, Huang, Noel, Gunning, & Morris, 2001). Actually, a higher concentration renders proteins resistant to photo-effects, since their environment will favour conformations that are less

susceptible to structural rearrangements (Manzocco, Panozzo, & Nicoli, 2013). Therefore, it is said that protein photoreactivity will likely transpire at concentrations lower than the concentrations required for macromolecular crowding. With that said, photo-oxidation of amino acid chromophores have been reported in several food proteins including egg white proteins, β -Lg and polyphenol oxidase (Fernández et al., 2012; Manzocco et al., 2013). In addition, light alter protein integrity, inducing oxidation of disulphide bonds (S-S) accompanied by various products including sulfhydryl groups (Pattison, Rahmanto, & Davies, 2012). Disruption of the S-S would directly affect the structure of the protein matrix, contributing to its rearrangement. The locations of both inter- and intramolecular S-S bonds are vital for the level of aggregation of high and low molecular weight proteins. Most allergens could be affected by PUV treatment, but the energy levels required to achieve attenuation effect is peculiar to each allergen and its matrice composition. Other factors which determine the effect of PL on allergens include the protein nature, processing conditions and hydration level. Further investigation involving animal models and *in vivo* clinical trials such as skin prick test (SPT) or oral challenge should be conducted to validate the allergenic responses of PL-treated samples (see Fig. 4).

4.2. High pressure processing

High pressure processing (HPP) is a nonthermal technology that offers excellent opportunities for food safety by microbial decontamination of food products and shelf-life extension with minimal changes to sensorial and nutritional profile (Hogan, Kelly, & Sun, 2005). In HPP, there are three key parameters (*i.e.*, temperature, pressure, and exposure time) that permit adaptability of process design. Typically, pressures in the range of 100–800 MPa is distributed rapidly in a uniform manner throughout the food product, irrespective of the size and geometry of the food material, in contrast to thermal processing known for its gradual dissipation of heat (Bañuelos et al., 2016; Yi et al., 2016).

Besides applications for food safety and shelf-life extension, HPP may create the possibilities of product innovation, by delivering conditions that can alter the structure and functionality of macromolecules. Due to poor compressibility of covalent bonds, flavor compounds, vitamins and minerals are seldom affected by HPP (Barba, Terefe, Buckow, Knorr, & Orlie, 2015), thus pressure treatment predominantly affects non-covalent bonds like hydrophobic, hydrogen, and ionic bonds and the effect of high pressure is mainly associated with their cleavage. In relation to proteins, since the primary structure of proteins consist of amino acid sequence linked by covalent bonds,

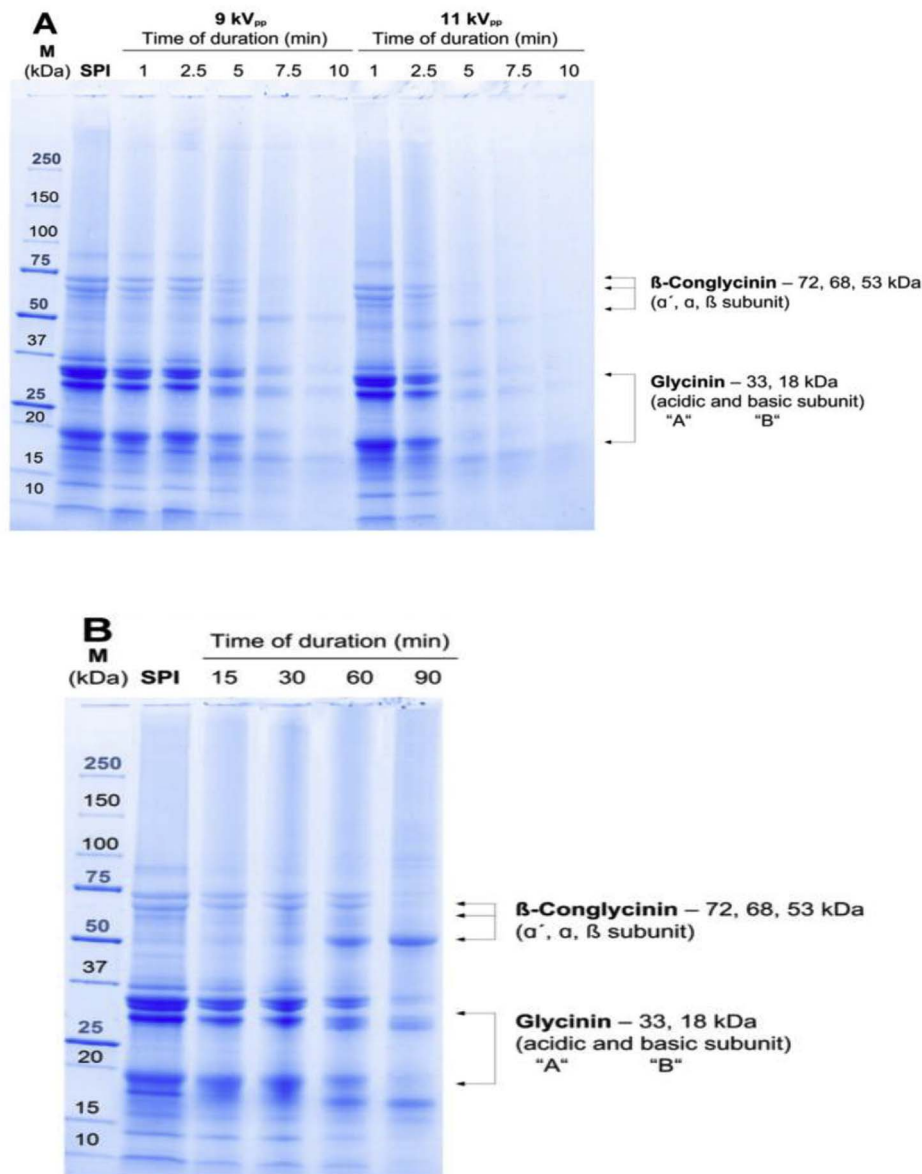


Fig. 4. SDS-PAGE profiles of soy protein isolates dispersions indicating the disappearance and formation of new protein bands after plasma treatment (A) SPI treated with direct plasma up to 10 min (B) electrophoretic pattern of samples treated with remote plasma for up to 90 min (Meinlschmidt et al., 2016).

they are unaffected by very high pressures (Huang, Yang, & Wang, 2014). Whereas, secondary protein structures consisting of polypeptide chain which forms α -helices or β -sheets/strands by intra- or inter-molecular hydrogen bonds, are affected by HPP due to fragmentation of hydrogen bonds, metal-ion ligations and salt bridges. HPP also immensely affect the tertiary and quaternary structures of the protein, because they are maintained by non-covalent bonds. In general, a triple-effect pathway has been observed when pressure acts on protein molecules, contingent on the amplitude of pressure applied (Huang et al., 2014). Firstly, when low pressure is employed, primary and secondary bonds undergo reversible distortions. Although the volume change in the system due to compression of primary bonds is considered inconsequential, the compression arising from hydrogen bonds leads to conformational changes, which may diminish the dimension of the internal cavities in the biomolecule. Yang et al. (2017) explained that alterations in tertiary structure and other intermolecular interactions occurs at 200 MPa, as a result of an amplified molecule surface inclined to dense hydration layer in the monomer state and by the cavities at the internal surface of the aggregates due to improper packing. The quaternary structures dissociates at pressure levels of

150–200 MPa. Increasing the pressure further to 500 MPa could unfold the protein, which was evidenced by a disordered structure peculiar to bio-macromolecules. At this pressure, protein unfolding allows the SH groups to be exposed and the number of unmasked SH groups decreases as the treatment progresses, probably due to the formation of disulfide bonds by oxidation, especially at alkaline pH where thiolate anions are more reactive. These series of modifications i.e., reversible or irreversible structural modifications in the proteins lead to decrease in protein solubility or aggregation, protein denaturation or fragmentation and gel formation, which alter conformational epitopes and their IgE binding capacity, rendering them unrecognizable by IgE antibodies (Huang et al., 2014).

Concerning the effect of high pressure treatment on allergenic proteins, a preliminary investigation posit that structural changes and reversible folding can indeed attenuate allergenicity (Peñas, Gomez, Frias, Baeza, & Vidal-Valverde, 2011). For example, the allergenicity of walnut, known to contain allergenic proteins including Jug r 1–5 could be altered by up to 46.64% at pressure levels from 550 to 650 MPa (Yang et al., 2017). In addition, Cabanillas et al. (2014) reported that HPP treatment (300–600 MPa) did not reduce the recognition of the

Table 2

An overview of studies conducted on the effect of high pressure processing on food allergens.

Food Material	Target allergen	Treatment conditions	Effect on immunoreactivity	References
Walnut	NS	650 MPa, 100 °C, 15 min	Decrease	Yang et al. (2017)
Cow milk	α -casein	200-MPa, 5 min	Decrease	Hu et al. (2016)
Peach	Pru p3	600 MPa, 5 min	Increase or Decrease	Lavilla et al. (2016)
Egg	Ovalbumin	400 MPa, 40 °C, 30 min	No effect	Ma, Lozano-Ojalvo, Chen, Lopez-Fandiño, and Molina (2015)
Squid	Tropomyosin	400 and 600 MPa, 15 min	Decrease	Jin et al. (2015)
Peanuts	Ara h 1 and Ara h 2	800 MPa for 10 min	Decrease	Huang et al. (2014)
Almond	Amandin	450 and 600 MPa, 90 °C, 300 s	Decrease	Dhakal et al. (2014)
Walnut	Jug r 4	300–600 MPa	No effect	Cabanillas et al. (2014)
Hazelnut	NS	300–600, 20 °C, 15 min	No effect	Prieto et al. (2014)
Sesame protein	NS	100–500 MPa, 10 min	Decrease	Achouri and Boye (2013)
		1M NaCl (pH 7.0 and pH 10.0)		
Almond	NS	300 MPa, 40 °C, 15 min	No effect	Li et al. (2013b)
Soy protein isolates	NS	300 MPa, 15 min	Decrease	Li, Zhu, Zhou, and Peng (2012)
Apple	Mal d 3	700 MPa, 20 °C	No effect	Husband et al. (2011)
Celeriac	Api g 1	700 MPa, 20 °C	No effect	Husband et al. (2011)
Soybean sprouts	NS	300 MPa, 40 °C, 15 min	No effect	Peñas et al. (2011)

acidic or basic subunits of Jug r 4 in walnuts. Similar pressure levels were not effective in altering the immunoreactivity of hazelnut proteins, but could induce changes in protein solubility after HHP processing (Prieto et al., 2014). Whilst ELISA response of protein solutions from sesame seeds at various pHs showed reduced antigenic properties (Achouri & Boye, 2013). On the other hand, Hu, Zheng, Liu, Deng, and Zhao (2016) reported that increasing the magnitude of pressures corresponded with decline in immunoreactivity, especially at pH 7.0 and 10.0. Similarly, Huang et al. (2014) indicated that HPP at 600 MPa could attenuate the antigenicity of α -casein in milk, while a 73.3% decrease in peanut allergenicity was observed at 800 MPa for 10 min. However, results obtained from the *in vitro* (ELISA) and *in vivo* studies (skin prick test) on the effect of HPP on Pru p 3 in peach, exhibited a two way effect at some point, i.e., either increase or decrease, when 600 MPa for 6 min was applied (Lavilla, Orcajo, Díaz-Perales, & Gamboa, 2016). The presumption was that HPP may be able to release another epitope usually enclosed in Pru p 3 structure or to influence the complex matrixes surrounding Pru p 3, and HPP in extracts could make the protein more resistant to denaturation due to the presence of intermolecular disulphide bonds (Brenna et al., 2000; Lavilla et al., 2016). Notwithstanding, with careful examination of data available in literature, it may be inferred that HPP may be potentially beneficial for actualizing hypoallergenization of various food products, under appropriate processing conditions. Though in some of these studies reported, reduction of residual immunoreactivity was actually achieved and maximized by treatment of the food with HHP combined with thermal conditions above 80 °C (Cabanillas et al., 2014; Husband et al., 2011; Yang et al., 2017). Table 2 provides a summary of recent studies conducted on the effect of HPP on food allergens.

4.3. Gamma irradiation

Irradiation entails subjecting food materials to either electrons or x rays generated using a machine electron accelerator or γ rays produced from a radioisotope source such as cobalt 60 or cesium 137. Generally, an intermediate dose (1–10 kGy) is used to achieve pasteurization of perishables such as fruit and vegetables, while elevated doses ranging from 10 to 50 kGy are utilized to sterilize low-moisture food materials such as spices. γ rays penetrates materials readily and are usually applied during bulk food processing while electron beams are used for surface irradiation (Castagna, Dall'Asta, Chiavaro, Galaverna, & Ranieri, 2014; Moriyama et al., 2013; Sung et al., 2013). Although γ -irradiation offers ample of benefits towards food safety, the technology may induce changes in functional integrity of biomolecules. For proteins, exposure to γ -rays expedites cross-linking, unfolding, fragmentation and generation of new reactive groups, which causes

modifications in protein structure. These alterations are caused by the breaking of covalent bonds either directly by photons or indirectly by reactive oxygen species (ROS) (Vaz et al., 2012). ROS reacts with the biomolecules to form secondary radicals but the major form of damage is the removal of hydrogen from side chains of amino acids, aromatic rings and disulfide bonds. These changes could also generate alterations in immunoreactivity of food proteins depending on irradiation dose, protein concentration, presence of oxygen and molecular structure. Therefore irradiation at doses of up to 10 kGy can modify antibody binding epitopes in food antigens/allergens, which can be used as a practical approach to reducing or eliminating allergenic properties of egg, peanut and pistachio nut (Noorbakhsh et al., 2010; Oh et al., 2009; Seo et al., 2007). Irradiating proteins in a solution reduces protein solubility and attenuates allergenicity (Kasera et al., 2012), due to cross-linking and intermolecular aggregation (Kaddouri et al., 2008). But these effects occur via direct interactions and are less noticeable when the protein is in a dry matrix. However, for proteins in solution, indirect effects involving free radicals generated by water radiolysis are predominant (Meng et al., 2016). As such, moisture content during irradiation of the samples ought to be considered, when piloting analogous experiment. Therefore, Kasera et al. (2012) established that the combination of irradiation (25 kGy) and boiling generate pronounced effect on legume proteins in reducing protein solubility and allergenic potency, than irradiation alone.

Recently, Meng et al. (2016) used γ -irradiation of 1–10 kGy to attenuate bovine α -lactalbumin (α -La), which is characteristically known for its high conformational stability owing to the presence of four disulfide bridges and calcium, and observed a dose-dependent decrease in IgG and IgE binding properties of bovine α -La. With the increase in the irradiation doses, more hydrophobic groups were exposed, mainly ascribable to structural unfolding. Since the linear and conformational epitopes of the native protein buried inside the matrix are usually hydrophobic amino acid residues, exposure of IgG and IgE epitopes becomes auspicious on the molecule surface, leading to austere destruction (Meng et al., 2016). Correspondingly, an irradiation dose of 10 kGy could reduce the antigenicity of both purified Ara h6 and peanut protein extract, with only 5% residual IgG binding ability lingering after treatment (Luo et al., 2013). In contrast, Gomaa and Boye (2015) reported no significant decrease in antigenicity of bread dough and pasta made from wheat as well as egg proteins (ovalbumin and ovomucoid), following treatment with γ -irradiation at a dose of 10 kGy. Similar findings by Kaddouri et al. (2008) indicated the absence of attenuation in antigenicity of β -Lg in whey and liquid milk treated with γ -irradiation, and instead an increase in antigenicity was noted, probably due to different experimental conditions employed. Besides, the antigenicity of protein in processed foods can be affected by processing

Table 3
Summary of recent studies on the effect of gamma irradiation on food allergens.

Food matrix/Target allergen	Irradiation dose	Effect on IgE binding capacity and protein structure	References
Purified α -lactalbumin	1–10 kGy	Decrease and causing extensive protein denaturation and aggregation	Meng et al. (2016)
Purified Ara h 6 and whole peanut protein extract.	10 kGy	Decrease and inducing significant changes in the secondary and tertiary structures	Luo et al. (2013)
Soybean (β -conglycinin, Gly m Bd 30K and Gly m 4)	30kGy	No effect	Moriyama et al. (2013)
Purified lectin	25 kGy	Decrease and rendering lectin innocuous by irreversibly compromising their molecular structure.	Vaz et al. (2013)
Purified Agglutinin	> 10 kGy.	Decrease with loss of intrinsic activity and the formation of insoluble amorphous aggregates	Vaz et al. (2012)
Purified lectin from Lectin <i>Sebastiania jacobinensis</i>	> 1 kGy	Decrease and promoting polypeptide fragmentation alongside hydrophobic surface modification	Vaz et al. (2011)

techniques, food matrix, type of allergen and extraction procedure. Table 3 summarizes recent studies about the effects of γ irradiation on food allergens.

4.4. Cold plasma technology

In the past decade, plasma technology has gained considerable attention from researchers as a potent technology suitable for effective microbial decontamination of food products. The ionized gas commonly called the fourth state of matter is composed of a variety of reactive species, i.e., UV photons, ions, electrons, free radicals, molecules, excited atoms, etc., which are generated following excitation and ionization of gas. These reactive species can induce certain interactions with proteins and change their conformational structures (Dasan & Boyaci, 2017; Tammineedi, Choudhary, Perez-Alvarado, & Watson, 2013; Tolouie, Hashemi, Mohammadifar, & Ghomi, 2017). Presumptively, food allergens are proteins, cold plasma can thus initiate similar reactions with food allergens through reactive species (Tolouie et al., 2017). Although the specific pathway remains uncertain, it is currently hypothetical that multiple mechanisms can affect food allergen reactivity and the primary concept is always the alteration of conformational and linear epitopes. Firstly, conformational epitopes can be altered by formation of insoluble aggregates when protein solubility decreases or protein crosslinking occurs, while fragmentation alters linear epitopes. Besides, reactive species may cleave peptide bonds and amino acids are oxidized leading to significant effects on protein integrity (Surowsky, Fischer, Schlueter, & Knorr, 2013). Disulfide bonds occurring within a peptide are also cleaved, because of a dissociative addition of a single hydroxyl radical to form RSH and RSO $\cdot$. In addition, ROS attack amino acids via oxidation, thereby incapacitating the binding sites of antibodies (Meinlschmidt et al., 2016).

Meinlschmidt et al. (2016) applied cold plasma directly or and remotely to soy allergens, i.e., β -conglycinin and glycinin, and found out that cold plasma caused the corresponding protein bands to become less intensive, followed by formation of new protein bands at 50 kDa (Fig. 3). In addition, the formation of insoluble aggregates after treatment led to the disappearance of protein bands in SDS-PAGE profile, and up to 89–100% decrease in immunoreactivity of soy protein samples was noted during ELISA analysis (Meinlschmidt et al., 2016). A decrease in protein solubility and consequent formation of aggregates or generation of new proteins by cross-linkage of free amino acids, led to the loss of proteins bands in SDS-PAGE. This trait of reduced protein solubility have previously been documented in a study where proteins from *Pisum sativum* were subjected to plasma treatment (Bußler, Steins, Ehlbeck, & Schlüter, 2015). A similar investigation targeted at tropomyosin, which is the major allergen present in shrimp, showed a 76% decrease in allergenicity under 5 min of direct dielectric discharge plasma (DBD) at a voltage of 30 kV and a frequency of 60 Hz (Shriver, 2011). Likewise, wheat allergenicity could be reduced by 37% using DBD plasma (Nooji, 2011). However, contrarily to the above results, Tammineedi et al. (2013) used cold plasma to treat milk allergens

(casein, β -Lg and α -La) and their SDS-PAGE results showed no noticeable changes in gel band intensities with no significant difference in the IgE binding values between the control and treated samples. Conceivably, the poor plasma efficiency associated with remote plasmas might be responsible for the absence of attenuation in immunoreactivity. More studies are still required to validate the potency of cold plasma towards controlling food allergy.

4.5. Ultrasonication

Ultrasonication is a versatile technology with broad food applications including drying, sterilization, enzyme inactivation, extraction, filtration, homogenization, meat tenderization etc. (Onwude et al., 2017). It is widely applauded over conventional techniques for being safe, eco-friendly with low processing time and economic cost. High power ultrasonic waves can induce modifications in materials by physical disintegration or acceleration of certain chemical reactions by building up pressure and temperature gradient in the material where the waves are propagated. Interestingly, the waves generated can be absorbed, causing a manifold of changes to the characteristic nature of the food. These effects arise from acoustic cavitation generated during sonication, i.e., the production, multiplication and sudden collapse of bubbles present in the material. As a consequence, high level of pressure and local heating are accumulated. For proteins, these effects induce a manifold of changes in native structure, e.g., conformational changes, damage to secondary structure, generation of other intra/inter molecular interactions, re-structuring of disulfide bond. Accordingly, sonication can influence allergenic features. For example, using power ultrasound operating at 50 °C for 1.5 h can positively alter the allergenicity of shrimp extracts and Pen a 1, the major allergen present in raw shrimps (Li, Cao, Lin, & Jamil, 2006). Essentially, the generation of local reactive species allows the target protein to undergo covalent bond reformation causing the formation of disulfide-bridged dimers, trimers, and higher oligomerized species. In the case of lactoglobulin, its β -variants are more susceptible to dimerization following sonication treatment than its α -variants (Stanic-Vucinic et al., 2012).

Recently, Yang, Gao, Yang, and Chen (2015) treated soybean seeds with ultrasound at different power levels (0–300 W), followed by germination for 5 days. At peak power, approximately 51.39% reduction in IgE-binding capacity was recorded for sprouted soybean protein. Apparently, the allergenic proteins were modified by disruption or elimination of epitopes. Specifically, local heating and sonication effect induces the disruption of some forces (hydrogen interactions between the polar groups and interactions of nonpolar groups) responsible for maintaining the stability of the secondary and tertiary structures (Stanic-Vucinic et al., 2012). A similar investigation targeted at various peanut cultivars germinated for 3 days showed complete degradation of allergenic proteins when a high-intensity ultrasonic wave of 100 kHz was employed (Yu, Liu, Shi, Liu, & Wang, 2016). This implies that the efficacy of the process depends on treatment conditions such as ultrasound intensity, treatment duration, characteristics of the protein and

environmental conditions. In addition, a sequential ultrasonication and enzymatic digestion with trypsin and chymotrypsin, reduces the immunoreactivity of peanut allergens Ara h1 and Ara h2 by up to 50% (Li et al., 2013a). This combined approach also enabled significant reduction in IgE binding properties of pastes made from treated cashew and pistachio nuts (Cuadrado et al., 2018). At present, scanty literature on the effect of ultrasound on food allergens exist but the technique could be a powerful approach for allergen mitigation in food products, and further studies are thus needed.

4.6. Pulsed electric field (PEF)

Pulsed electric field (PEF) is an excellent nonthermal technique for food preservation that employs short pulses of electricity for the permeabilization and disintegration of microbial cells, accompanied by minimal changes to the quality attributes of the food product. Usually, high voltage pulses of about 20–80 kV is applied to the electroconductive food positioned between the generating electrodes. Albeit being used predominantly for microbial decontamination, PEF can also be used for enzyme inactivation, enhancement of mass transport during drying operations (Onwude et al., 2017), and possibly allergen reduction. With regards to allergen mitigation, PEF can induce changes in the structural characteristics of food allergens. For instance, Toshiko and Takayuki (2004) observed conformational changes in ovalbumin following PEF treatment, which lowered allergen-antibody interaction. PEF was also shown to induce glycosylation, alteration in secondary structure with a decrease in heat-induced aggregation in whey proteins (Sun, Yu, Zeng, Yang, & Jia, 2011). Conversely, Johnson et al. (2010) applied PEF with various electric field strengths (0–35 kV/cm) at a frequency of 2 Hz to peanut and apple allergens, but no significant structural modifications for the plant allergens was observed. Therefore, no conclusions can be made based on these limited studies until more investigations focusing on other allergenic proteins under similar PEF conditions or further studies relating to the effect of PEF on a variety of food allergens are available.

4.7. Fermentation and enzymatic hydrolysis

Fermentation takes place when microorganisms act on food substrates such as sugars, under anaerobic conditions to yield end products e.g., alcohol, carbon dioxide and organic acids. The specific products liberated is determined by the type of microorganism and process conditions i.e., temperature, pH, substrate concentration etc. During fermentation, hydrolysis occurs and induces changes in food proteins by modification or destruction of linear and conformational epitopes which affects (Zhou et al., 2016) allergenicity. As an illustration, whey milk fermented by a single lactic acid bacterium (LAB) significantly decreases the allergenicity of β -Lg and α -La. This positive effect can be further amplified when two separate LAB strains (*Lactobacillus bulgaricus* and *Lactobacillus helveticus*) is simultaneously utilized to ferment skim milk, compared to using each strain alone (Bu et al., 2010). This trait also applies to fermented soy product fed to mice subjects and after consumption, allergic symptoms (dermatitis and skin severity) declined significantly in a time-dependent manner (Matsuda et al., 2012). Though, a slight increase occurred at some point due to cleavage of oligopeptides by peptidase which causes unmasking of hidden epitopes. Fermentation of reconstituted milk by *L. casei* 1134 for 12 h could also significantly reduce the antigenicity and allergenicity of different proteins namely, β -Lg, α -La, α -caseins (mixture of α_{s1} - and α_{s2} -casein), and β -casein, by 68.07%, 59.88%, 67.69% and 37.02%, respectively (Yao, Xu, Luo, Shi, & Li, 2015). Although the mechanisms by which they exert changes in antigenicity are not fully understood, the decrease in antigenicity was simply ascribed to the breakdown action of proteolytic enzymes in milk produced by *L. casei* 1134. The production of lactic acid with resulting decrease in pH was speculated to cause modifications in protein structure, such as the aggregation and cross-linking of

proteins and hydrogen bond rearrangement (Kleber, Weyrich, & Hinrichs, 2006; Yao et al., 2015). For casein proteins, the consequent effect of processing on casein micelles can influence the allergenic potential of α_{s1} -, α_{s2} -, and β -caseins, although it has been shown that dephosphorylation modifies IgE binding to caseins (Bernard, Meisel, Creminon, & Wal, 2000).

Nonetheless, an earlier investigation by Gänzle, Loponen, and Gobetti (2008) showed that sourdough fermentation decreases the disulphide bonds in gluten network which influence its digestibility and may alleviate celiac disease in people with gluten sensitivity. When fermented sourdough is digested *in vitro*, peptides which provokes allergic reactions (celiac disease) via pre-proteolytic reactions of LAB strains were completely eliminated, leading to up to 80% rise in gluten tolerance in susceptible individuals (Di Cagno et al., 2004). More recently, a new strain of yeast has been created to reduce wine allergy (Lockwood, 2012). The causative agents, responsible for wine allergies are biogenic amines like tyramines and histamines generated by bacteria during fermentation. The new genetically engineered yeast is designed to specifically degrade the biogenic amines, thereby yielding a hypoallergenic wine devoid of biogenic amines (Lockwood, 2012). The intake of probiotics have also be found to influence the release of cytokines that alters allergen-antibody interaction by an immune-modulatory pathway (Bu et al., 2010). Therefore, Han et al. (2012) concluded that intake of one serving per day of probiotics (*Lactobacillus plantarum*) can diminish the levels of eosinophils and interleukin-4 (IL-4) and ameliorate eczema in children. These studies suggest that consumption of fermented foods instead of their raw forms may be a soft-option for food allergy sufferers.

Furthermore, with respect to enzymatic hydrolysis, this approach mainly destroys the linear epitopes by partially or extensively hydrolyzing the allergens with proteolytic or cellular enzymes like proteases into peptides and/or amino acids. The residual antigenicity and type of peptides produced is determined by process conditions such as pH, temperature, the extent of hydrolysis and enzyme-substrate ratio. Kulis et al. (2012) has demonstrated that cashew proteins digested by pepsin exhibited a reduced allergenic activity in mice. In another study, mice orally sensitized with trypsin-digested β -Lg from milk showed decreased anaphylaxis (Duan et al., 2014). Even hydrolysis of peanut allergens by trypsin have shown effective reduction of allergenicity (Hazebrouck et al., 2012; Yu, Ahmedna, Goktepe, Cheng, & Maleki, 2011). In the case of cereals, alkaline protease was found quite effective in reducing allergenicity of buckwheat protein and outcomes of immunoblotting showed that the rabbit antiserum to buckwheat protein no longer recognized it as an antigen (Sung et al., 2014). Allergenic proteins in rice can also be attenuated by 15 min of hydrolysis, resulting in a remarkable 92% inhibition of mast cell degranulation (Knipping et al., 2012). Employing multiple enzymes may be more efficient than using a single enzyme. For instance, extracts containing lentil protein hydrolyzed by the sequential action of alcalase and flavourzyme, results in higher destruction of IgE-binding epitopes, compared with both enzymes tested individually (Cabanillas et al., 2010). The authors observed that after 3 h, a 24% degree of hydrolysis was recorded by alcalase; the addition of flavourzyme, increased the degree of hydrolysis to 56% after 8 h. It is possible that the alcalase enzyme produced an initial digestion that increased the number of target sites for the action of the flavourzyme. Hydrolysis of extracts from kidney bean, black gram and peanut using similar enzymes (alcalase and flavourzyme), also resulted in reduction of IgE binding in ELISA by 62.2, 87.1, and 91.8, respectively ($p < 0.01$) (Kasera, Singh, Lavasa, Prasad, & Arora, 2015). In contrast, peptic hydrolysis of soybean 2S protein slightly reduces the allergenicity but subsequent chymotrypsin hydrolysis, increases allergenicity (Sung et al., 2014). This suggests that sequential digestion by multiple enzymes ensure maximum hydrolysis, allergenic epitopes may not be fully destroyed even after multiple enzyme action due to exposure of some hidden epitopes and may require supplementary treatments. Besides, if both enzymes are added simultaneously

they may suppress the activity of each other. Notwithstanding, when hydrolysis is accompanied with heat treatment known to induce modifications in proteins alongside exposure of cleavage sites for enzymes, proteolysis can indeed be enhanced. For instance, enzymatic hydrolysis after heat treatment improves hydrolysis of milk proteins (Peyron, Mouécoucou, Frémont, Sanchez, & Gontard, 2006). Likewise, the addition of microwave irradiation during enzymatic hydrolysis decreases IgE-binding properties of whey proteins in contrast to proteolysis alone (El Mecherfi et al., 2011). At the moment, numerous hypoallergenic foods especially milk-based products are available in the market as hydrolyzed whey or casein. These products present no risk of abnormal immune response and can be consumed by children with milk allergy (Terheggen-Lagro, Khouw, Schaafsma, & Wauters, 2002).

4.8. Other nonthermal techniques

In addition to the nonthermal technologies mentioned thus far, other nonthermal methods such as RNA interference (RNAi) and the use of magnetic beads have also been exploited to eliminate or modify allergenic proteins in foods. RNA interference is a genetic modification tool for influencing gene expression and has been successfully used to silence allergenic proteins including Ara h 2 in peanuts (Dodo, Konan, Chen, Egnin, & Viquez, 2008). Dubois et al. (2015) confirmed the potency of RNA interference towards modification of Mal d 1 allergenicity in apple. In their investigation, reverse transcription-PCR revealed an immediate silencing of Mal d 1 gene expression, and clinical responses indicated that nearly half of the apple-allergic subjects assayed showed no symptoms after consumption of the treated apples, although speculations regarding possible health risks of such foods remain an issue of concern if suppression is done half way or silencing is unexpressed. So far gene silencing, it has been possible to achieve reduced or block expression of genes that code for particular food allergens in rice, soybean, tomato, carrot, and peanut (Gallo & Sayre, 2009). Also some products labeled hypoallergenic (HA) infant formulas, treated with enzymatic protein hydrolysis and heat treatment are available (Fritsché, 2009). Finally, allergenic protein could be recovered by using a magnet, which confines the bead-protein complex to expedite the elimination process. Chung and Champagne (2009) employed this technique successfully to attenuate peanut allergen and decrease their IgE binding abilities, by conjugating magnetic bead to phenolic compounds such as ferric ions, caffeic acid or a hydrophobic compound that could bind easily with proteins.

5. Advantages and limitations of nonthermal techniques for food allergen mitigation

So far, each of these processing approaches discussed in this review affect physicochemical properties of food proteins in diverse ways and in turn influences their gastrointestinal digestion, bioavailability and allergenicity. In thermal processing, the heat produced reduces allergenic potential by changing protein structure, alteration of IgE binding conformational epitopes and increased digestibility but may have undesirable outcomes on quality attributes. On the other hand, the core advantage of most non-thermal methods is that they induce minimal changes to food quality attributes and can extend the shelf-life of food. Also, some of these non-thermal techniques are relatively cheap and chemical free, rendering them environmentally friendly and sustainable. However, the hypoallergenic foods currently marketed in food stores are primarily manufactured using enzymatic hydrolysis but the proteolysis process may leave negative impact on organoleptic properties, making them unacceptable by consumers. In addition, changes in the allergenicity of food allergens by processing methods are affected by many complex factors like other food constituents, nature of the allergen, processing conditions and the binding interaction of the allergen with antibodies. For instance, under varying conditions of pH and ionic strength, proteins show different tolerance to high pressure

treatments. All these factors cumulatively are also responsible for inconsistent results in the application of non-thermal treatment for mitigation of food allergen. Besides, for potential application in industrial food production especially in processes like ultrasonication, less control of critical factors would reduce efficiency; therefore a combination of ultrasound with other methods may be more beneficial for commercial applications. In reality, the additional cost to be incurred by manufacturers for acquisition of these novel techniques ought to be considered. Moreover, studies showing any toxicological and safety risks as a consequence of allergen modification are relatively scarce, so these methods may not be adopted any time soon until safety is established. Notwithstanding, the main challenge associated with on non-thermal processes that requires urgent attention is standardization. Thorough understanding of these technologies and how they influence allergenic proteins is a crucial point for their implementation and future adoption.

6. Conclusion and future trends

Food allergy is a global health concern. Processing techniques, especially nonthermal methods such as pulsed light, HPP, irradiation, cold plasma, ultrasound and PEF can be beneficial for reducing allergenicity of food proteins by altering conformational epitopes through promoting the aggregation or crosslinking of proteins or by modifying linear epitopes through fragmentation or alteration in amino acid sequence. Although complete elimination of food allergens by processing may not be possible at the moment, attenuation of elicitation threshold could be achieved through controlling and optimizing process conditions. Since a significant number of investigations shows that non-thermal techniques can be a successful approach towards eliminating allergenic proteins, there remains a need to develop robust, thoroughly evaluated and validated methods.

In order to improve hypoallergenic potency, application of “hurdle technology” concept could be more effective. This may involve applying mild heat treatment as a preceding operation. In the future, more studies are needed focusing on the effects of nonthermal techniques on the functional properties of the modified allergenic proteins following treatments. Moreover, finding the best conditions and treatment dosages is important, if the desired techno-functionality and applicability are to be maintained afterwards. Also, as the specific biochemical mechanism responsible for protein polymerization or depolymerization is still unclear, future detailed studies are thus required.

In addition, processed food products are more complex systems. Efficient detection and identification methods of allergen from processed foods requires attention, i.e., robust techniques that can identify multiple allergens even in very minute amounts should be beneficial for advancing studies on processed foods. More importantly, animal and human tests should be carried out to confirm results and ensure the safe consumption of the supposed hypoallergenic products. These future investigations should provide valuable support for the development of the hypoallergenic products. Unfortunately, avoidance of the allergenic food remains the current best therapeutic strategy for susceptible individuals.

Acknowledgement

The authors are grateful to the National Key R&D Program of China (2017YFD0400404) for its support. This research was also supported by the Collaborative Innovation Major Special Projects of Guangzhou City (201604020007, 201604020057), the Guangdong Provincial Science and Technology Plan Projects (2015A020209016, 2016A040403040), the National Key Technologies R&D Program (2015BAD19B03), the Natural Science Foundation of Guangdong Province (2017A030310558), the China Postdoctoral Science Foundation (2017M612672), the Fundamental Research Funds for the Central Universities (2017MS067, 2017MS075), the Agricultural Development and Rural Work of Guangdong Province (2017LM4173),

the S&T Project of Guangdong Province (2017B020207002), the Pearl River S&T Nova Program of Guangzhou (201610010104), the International and Hong Kong – Macau – Taiwan Collaborative Innovation Platform of Guangdong Province on Intelligent Food Quality Control and Process Technology & Equipment (2015KGJH2001), the Guangdong Provincial R & D Centre for the Modern Agricultural Industry on Non-destructive Detection and Intensive Processing of Agricultural Products, the Common Technical Innovation Team of Guangdong Province on Preservation and Logistics of Agricultural Products (2016LM2154) and the Innovation Centre of Guangdong Province for Modern Agricultural Science and Technology on Intelligent Sensing and Precision Control of Agricultural Product Qualities. Flora-Glad Chizoba Ekezie is in receipt of financial support from China Scholarship Council for her PhD study (2016GXZ300).

References

- Achouri, A., & Boye, J. I. (2013). Thermal processing, salt and high pressure treatment effects on molecular structure and antigenicity of sesame protein isolate. *Food Research International*, 53(1), 240–251.
- Astwood, J. D., Leach, J. N., & Fuchs, R. L. (1996). Stability of food allergens to digestion *in vitro*. *Nature Biotechnology*, 14(10), 1269–1273.
- Bañuelos, M. A., Loira, I., Escott, C., Del Fresno, J. M., Morata, A., Sanz, P. D., et al. (2016). Grape processing by high hydrostatic pressure: Effect on use of non-Saccharomyces in must fermentation. *Food and Bioprocess Technology*, 9(10), 1769–1778.
- Barba, F. J., Terefe, N. S., Buckow, R., Knorr, D., & Orlén, V. (2015). New opportunities and perspectives of high pressure treatment to improve health and safety attributes of foods. A review. *Food Research International*, 77, 725–742.
- Bernard, H., Meisel, H., Creminon, C., & Wal, J. M. (2000). Post-translational phosphorylation affects the IgE binding capacity of caseins. *FEBS Letters*, 467(2), 239–244.
- Besler, M., Steinhart, H., & Paschke, A. (2001). Stability of food allergens and allergenicity of processed foods. *Journal of Chromatography B: Biomedical Sciences and Applications*, 756(1), 207–228.
- Breiteneder, H., & Mills, E. N. C. (2005). Molecular properties of food allergens. *The Journal of Allergy and Clinical Immunology*, 115(1), 14–23.
- Brenna, O. V., Pompei, C., Ortolani, C., Pravettoni, V., Farioli, L., & Pastorello, E. A. (2000). Technological processes to decrease the allergenicity of peach juice and nectar. *Journal of Agricultural and Food Chemistry*, 48(2), 493–497.
- Bugajskaschretter, A., Grote, M., Vangelista, L., Valent, P., Sperr, W. R., Rumpold, H., et al. (2000). Purification, biochemical, and immunological characterisation of a major food allergen: different immunoglobulin E recognition of the apo- and calcium-bound forms of carp parvalbumin. *Gut*, 46(5), 661–669.
- Bu, G., Luo, Y., Zhang, Y., & Chen, F. (2010). Effects of fermentation by lactic acid bacteria on the antigenicity of bovine whey proteins. *Journal of the Science of Food and Agriculture*, 90(12), 2015–2020.
- Bußler, S., Steins, V., Ehlbeck, J., & Schlüter, O. (2015). Impact of thermal treatment versus cold atmospheric plasma processing on the techno-functional protein properties from *Pisum sativum* 'Salamanca'. *Journal of Food Engineering*, 167, 166–174.
- Cabanillas, B., Maleki, S. J., Rodríguez, J., Cheng, H., Teuber, S. S., Wallowitz, M. L., et al. (2014). Allergenic properties and differential response of walnut subjected to processing treatments. *Food Chemistry*, 157, 141–147.
- Cabanillas, B., Pedrosa, M. M., Rodríguez, J., González, Á., Muzquiz, M., Cuadrado, C., et al. (2010). Effects of enzymatic hydrolysis on lentil allergenicity. *Molecular Nutrition & Food Research*, 54(9), 1266–1272.
- Castagna, A., Dall'Asta, C., Chiavaro, E., Galaverna, G., & Ranieri, A. (2014). Effect of post-harvest UV-B irradiation on polyphenol profile and antioxidant activity in flesh and peel of tomato fruits. *Food and Bioprocess Technology*, 7(8), 2241–2250.
- Cheng, Jun-Hu, Sun, Da-Wen, & Pu, Hongbin (2016). Combining the genetic algorithm and successive projection algorithm for the selection of feature wavelengths to evaluate exudative characteristics in frozen-thawed fish muscle. *Food Chemistry*, 197, 855–863.
- Cheng, Lina, Sun, Da-Wen, Zhu, Zhiwei, & Zhang, Zi (2017). Emerging techniques for assisting and accelerating food freezing processes: A review of recent research progresses. *Critical Reviews in Food Science and Nutrition*, 57, 769–781.
- Chung, S. Y., & Champagne, E. T. (2009). Reducing the allergenic capacity of peanut extracts and liquid peanut butter by phenolic compounds. *Food Chemistry*, 115(4), 1345–1349.
- Chung, S. Y., & Reed, S. (2014). Reducing food allergy: Is there promise for food applications? *Current Pharmaceutical Design*, 20(6), 924–930.
- Cianferoni, A., & Spergel, J. M. (2009). Food allergy: Review, classification and diagnosis. *Allergy International*, 58(4), 457–466.
- Corzo-Martínez, M., Soria, A. C., Belloque, J., Villamiel, M., & Moreno, F. J. (2010). Effect of glycation on the gastrointestinal digestibility and immunoreactivity of bovine β -lactoglobulin. *International Dairy Journal*, 20(11), 742–752.
- Cuadrado, C., Cabanillas, B., Pedrosa, M. M., Varela, A., Guillaumon, E., Muzquiz, M., et al. (2009). Influence of thermal processing on IgE reactivity to lentil and chickpea proteins. *Molecular Nutrition & Food Research*, 53(11), 1462–1468.
- Cuadrado, C., Cheng, H., Sanchiz, A., Ballesteros, I., Easson, M., Grimm, C. C., et al. (2018). Influence of enzymatic hydrolysis on the allergenic reactivity of processed cashew and pistachio. *Food Chemistry*, 24, 372–379.
- Dasan, B. G., & Boyaci, I. H. (2017). Effect of cold atmospheric plasma on inactivation of *Escherichia coli* and physicochemical properties of apple, orange, tomato juices, and sour cherry nectar. *Food and Bioprocess Technology*, 1–10.
- Delgado, A. E., & Sun, D.-W. (2002). Desorption isotherms and glass transition temperature for chicken meat. *Journal of Food Engineering*, 55(Pp), 1–8.
- Desmond, E. M., Kenny, T. A., Ward, P., et al. (2000). Effect of rapid and conventional cooling methods on the quality of cooked ham joints. *Meat Science*, 56, 271–277.
- Dhakal, S., Liu, C., Zhang, Y., Roux, K. H., Sathe, S. K., & Balasubramanian, V. M. (2014). Effect of high pressure processing on the immunoreactivity of almond milk. *Food Research International*, 62, 215–222.
- Di Cagno, R., De Angelis, M., Auricchio, S., Greco, L., Clarke, C., De Vincenzi, M., et al. (2004). Sourdough bread made from wheat and nontoxic flours and started with selected lactobacilli is tolerated in celiac sprue patients. *Applied and Environmental Microbiology*, 70(2), 1088–1096.
- Dodo, H. W., Konan, K. N., Chen, F. C., Egnin, M., & Viquez, O. M. (2008). Alleviating peanut allergy using genetic engineering: The silencing of the immunodominant allergen Ara h 2 leads to its significant reduction and a decrease in peanut allergenicity. *Plant Biotechnology Journal*, 6(2), 135–145.
- Duan, C., Li, A., Yang, L., Zhao, R., Fan, W., & Huo, G. (2014). Comparison of immunomodulating properties of Beta-lactoglobulin and its hydrolysates. *Iranian Journal of Allergy, Asthma and Immunology*, 13(1), 26–32.
- Dubois, A. E., Pagliarini, G., Brouwer, R. M., Kollen, B. J., Dragsted, L. O., Eriksen, F. D., et al. (2015). First successful reduction of clinical allergenicity of food by genetic modification: Mal d 1-silenced apples cause fewer allergy symptoms than the wild-type cultivar. *Allergy*, 70(11), 1406–1412.
- El Mecherfi, K. E., Saidi, D., Kheroua, O., Boudraa, G., Touhami, M., et al. (2011). Combined microwave and enzymatic treatments for β -lactoglobulin and bovine whey proteins and their effect on the IgE immunoreactivity. *European Food Research and Technology*, 233(5), 859.
- Fernández, E., Artiguez, M. L., Martínez de Marañón, I., Villate, M., Blanco, F. J., & Arboleya, J.-C. (2012). Effect of pulsed-light processing on the surface and foaming properties of β -lactoglobulin. *Food Hydrocolloids*, 27(1), 154–160.
- Foods Matter (2010). *Mintel's allergy and allergy remedies UK*. Retrieved 27th November, 2017, from http://www.foodsmatter.com/allergy_intolerance/miscellaneous/articles/mintel_allergy_report_2010.html.
- Fritsché, R. (2009). Utility of animal models for evaluating hypoallergenicity. *Molecular Nutrition & Food Research*, 53(8), 979–983.
- Gallo, M., & Sayre, R. (2009). Removing allergens and reducing toxins from food crops. *Current Opinion in Biotechnology*, 20(2), 191–196.
- Gänzle, M. G., Loponen, J., & Gobetti, M. (2008). Proteolysis in sourdough fermentations: Mechanisms and potential for improved bread quality. *Trends in Food Science & Technology*, 19(10), 513–521.
- Gomaa, A., & Boye, J. (2015). Impact of irradiation and thermal processing on the immunochemical detection of milk and egg allergens in foods. *Food Research International*, 74, 275–283.
- Gupta, R. S., Holdford, D. A., Bilaver, L. A., Dyer, A. A., & Meltzer, D. O. (2013). The high economic burden of childhood food allergy in the United States. *The Journal of Allergy and Clinical Immunology*, 131(2), AB223.
- Han, Y., Kim, B., Ban, J., Lee, J., Kim, B. J., Choi, B. S., et al. (2012). A randomized trial of *Lactobacillus plantarum* CJLP133 for the treatment of atopic dermatitis. *Pediatric Allergy & Immunology*, 23(7), 667–673.
- Hazebrouck, S., Guillon, B., Drumare, M., Paty, E., Wal, J., & Bernard, H. (2012). Trypsin resistance of the major peanut allergen Ara h 6 and allergenicity of the digestion products are abolished after selective disruption of disulfide bonds. *Molecular Nutrition & Food Research*, 56(4), 548–557.
- Henderson, C. J., Abonia, J. P., King, E. C., Putnam, P. E., Collins, M. H., Franciosi, J. P., et al. (2012). Comparative dietary therapy effectiveness in remission of pediatric eosinophilic esophagitis. *The Journal of Allergy and Clinical Immunology*, 129(6), 1570–1578.
- Hogan, Eamonn, Kelly, Alan L., & Sun, Da-Wen (2005). High pressure processing of foods: An overview. In D.-W. Sun (Ed.). *Emerging technologies for food processing book series: Food science and technology-international series* (pp. 3–32).
- Huang, H. W., Yang, B. B., & Wang, C. Y. (2014). Effects of high pressure processing on immunoreactivity and microbiological safety of crushed peanuts. *Food Control*, 42, 290–295.
- Husband, F. A., Aldick, T., Van der Plancken, I., Grauwet, T., Hendrickx, M., Skypala, I., et al. (2011). High-pressure treatment reduces the immunoreactivity of the major allergens in apple and celeriac. *Molecular Nutrition & Food Research*, 55(7), 1087–1095.
- Hu, G., Zheng, Y., Liu, Z., Deng, Y., & Zhao, Y. (2016). Structure and IgE-binding properties of alpha-casein treated by high hydrostatic pressure, UV-C, and far-IR radiations. *Food Chemistry*, 204, 46–55.
- Jerschow, E., Lin, R. Y., Scaperotti, M. M., & McGinn, A. P. (2014). Fatal anaphylaxis in the United States, 1999–2010: Temporal patterns and demographic associations. *The Journal of Allergy and Clinical Immunology*, 134(6), 1318.
- Jimenez-Saiz, R., Benede, S., Molina, E., & Lopez-Exposito, I. (2015). Effect of processing technologies on the allergenicity of food products. *Critical Reviews in Food Science and Nutrition*, 55(13), 1902–1917.
- Jin, Y., Deng, Y., Qian, B., Zhang, Y., Liu, Z., & Zhao, Y. (2015). Allergenic response to squid (*Todarodes pacificus*) tropomyosin Tod p1 structure modifications induced by high hydrostatic pressure. *Food and Chemical Toxicology*, 76, 86–93.
- Johnson, P. E., Van der Plancken, I., Balasa, A., Husband, F. A., Grauwet, T., Hendrickx, M., et al. (2010). High pressure, thermal and pulsed electric-field-induced structural changes in selected food allergens. *Molecular Nutrition & Food Research*, 54(12),

- 1701–1710.
- Kaddouri, H., Mimoun, S., El-Mecherfi, K., Chekroun, A., Kheroua, O., & Saidi, D. (2008). Impact of γ -radiation on antigenic properties of cow's milk β -lactoglobulin. *Journal of Food Protection*, 71(6), 1270–1272.
- Kamath, S. D., Abdel Rahman, A. M., Komoda, T., & Lopata, A. L. (2013). Impact of heat processing on the detection of the major shellfish allergen tropomyosin in crustaceans and molluscs using specific monoclonal antibodies. *Food Chemistry*, 141(4), 4031–4039.
- Kasera, R., Singh, A. B., Kumar, R., Lavasa, S., Prasad, K. N., & Arora, N. (2012). Effect of thermal processing and γ -irradiation on allergenicity of legume proteins. *Food and Chemical Toxicology*, 50(10), 3456–3461.
- Kasera, R., Singh, A. B., Lavasa, S., Prasad, K. N., & Arora, N. (2015). Enzymatic hydrolysis: A method in alleviating legume allergenicity. *Food and Chemical Toxicology*, 76, 54–60.
- Ketnawa, S., & Liceaga, A. M. (2017). Effect of microwave treatments on antioxidant activity and antigenicity of fish frame protein hydrolysates. *Food and Bioprocess Technology*, 10(3), 582–591.
- Kiani, Hossein, Zhang, Zhihang, Delgado, Adriana, & Sun, Da-Wen (2011). Ultrasound assisted nucleation of some liquid and solid model foods during freezing. *Food Research International*, 44, 2915–2921.
- Kiani, Hossein, Sun, Da-Wen, Delgado, Adriana, & Zhang, Zhihang (2012). Investigation of the effect of power ultrasound on the nucleation of water during freezing of agar gel samples in tubing vials. *Ultrasonics Sonochemistry*, 19, 576–581.
- Kleber, N., Weyrich, U., & Hinrichs, J. (2006). Screening for lactic acid bacteria with potential to reduce antigenic response of β -lactoglobulin in bovine skim milk and sweet whey. *Innovative Food Science & Emerging Technologies*, 7(3), 233–238.
- Knipping, K., Van Esch, B. C. A. M., Dijk, A. G. V. I., Van Hoffen, E., Van Baalen, T., Knippels, L. M. J., et al. (2012). Enzymatic treatment of whey proteins in cow's milk results in differential inhibition of IgE-mediated mast cell activation compared to T-Cell activation. *International Archives of Allergy and Immunology*, 159(3), 263–270.
- Kulis, M. D., Macqueen, I., Li, Y., Guo, R., Zhong, X. P., & Burks, A. W. (2012). Pepsinized cashew proteins are hypoallergenic and immunogenic and provide effective immunotherapy in mice with cashew allergy. *The Journal of Allergy and Clinical Immunology*, 130(3), 716–723.
- Kumar, S., Verma, A. K., Das, M., & Dwivedi, P. D. (2012). Molecular mechanisms of IgE mediated food allergy. *International Immunopharmacology*, 13(4), 432–439.
- Lavilla, M., Orcajo, J., Díaz-Perales, A., & Gamboa, P. (2016). Examining the effect of High Pressure Processing on the allergenic potential of the major allergen in peach (Pru p 3). *Innovative Food Science & Emerging Technologies*, 38, 334–341.
- Lechevalier, V., Guérin-Dubiard, C., Anton, M., Beaumont, V., David Briand, E., Gillard, A., et al. (2017). Effect of dry heat treatment of egg white powder on its functional, nutritional and allergenic properties. *Journal of Food Engineering*, 195, 40–51.
- Li, Z. X., Cao, L. M., Lin, H., & Jamil, K. (2006). Reduction of allergenic properties of shrimp (*Penaeus Vannamei*) allergens by high intensity ultrasound. *European Food Research and Technology*, 223(5), 63.
- Li, Y., Yang, W., Chung, S.-Y., Chen, H., Ye, M., Teixeira, A. A., et al. (2013b). Effect of pulsed ultraviolet light and high hydrostatic pressure on the antigenicity of almond protein extracts. *Food and Bioprocess Technology*, 6(2), 431–440.
- Li, H., Yu, J., Ahmedna, M., & Goktepe, I. (2013a). Reduction of major peanut allergens Ara h 1 and Ara h 2, in roasted peanuts by ultrasound assisted enzymatic treatment. *Food Chemistry*, 141(2), 762–768.
- Li, H. J., Zhu, K. X., Zhou, H. M., & Peng, W. (2012). Effects of high hydrostatic pressure treatment on allergenicity and structural properties of soybean protein isolate for infant formula. *Food Chemistry*, 132(2), 808–814.
- Lockwood, D. (2012). Hypoallergenic vino, wearing wine. *C&EN*, 90, 48.
- Luo, C. P., Hu, C. Q., Gao, J. Y., Li, X., Wu, Z. H., Yang, A. S., et al. (2013). A potential practical approach to reduce Ara h 6 allergenicity by gamma irradiation. *Food Chemistry*, 136(3–4), 1141–1147.
- Ma, X., Lozano-Ojalvo, D., Chen, H., Lopez-Fandiño, R., & Molina, E. (2015). Effect of high pressure-assisted crosslinking of ovalbumin and egg white by transglutaminase on their potential allergenicity. *Innovative Food Science & Emerging Technologies*, 29, 143–150.
- Ma, Ji, Pu, Hongbin, Sun, Da-Wen, Gao, Wenhong, Qu, Jia-Huan, & Ma, Kai-Yue (2015). Application of Vis-NIR hyperspectral imaging in classification between fresh and frozen-thawed pork Longissimus Dorsi muscles. *International Journal of Refrigeration-Revue Internationale Du Froid*, 50, 10–18.
- Ma, Ji, Sun, Da-Wen, Qu, Jia-Huan, & Pu, Hongbin (2017). Prediction of textural changes in grass carp fillets as affected by vacuum freeze drying using hyperspectral imaging based on integrated group wavelengths. *LWT-Food Science and Technology*, 82, 377–385.
- Mansouri, M. (2015). Food allergy: A review. *Archives of Pediatric Infectious Diseases*, 3(3), e22470.
- Manzocco, L., Panozzo, A., & Nicoli, M. C. (2013). Effect of pulsed light on selected properties of egg white. *Innovative Food Science & Emerging Technologies*, 18, 183–189.
- Martínez-Botas, J., Cerecedo, I., Zamora, J., Vlaicu, C., Dieguez, M. C., Gómez-Coronado, D., et al. (2013). Mapping of the IgE and IgG4 sequential epitopes of ovomucoid with a peptide microarray immunoassay. *International Archives of Allergy and Immunology*, 161(1), 11–20.
- Matsuda, A., Tanaka, A., Pan, W., Okamoto, N., Oida, K., Kingyo, N., et al. (2012). Supplementation of the fermented soy product ImmuBalance™ effectively reduces itching behavior of atopic NC/Tnd mice. *Journal of Dermatological Science*, 67(2), 130–139.
- McDonald, K., Sun, D.-W., & Kenny, T. (2001). The effect of injection level on the quality of a rapid vacuum cooled cooked beef product. *Journal of Food Engineering*, 47, 139–147.
- Meinlschmidt, P., Ueberham, E., Lehmann, J., Reineke, K., Schlüter, O., Schweiggert-Weisz, U., et al. (2016). The effects of pulsed ultraviolet light, cold atmospheric pressure plasma, and gamma-irradiation on the immunoreactivity of soy protein isolate. *Innovative Food Science & Emerging Technologies*, 38, 374–383.
- Meng, X. Y., Li, X., Wang, X. K., Gao, J. Y., Yang, H., & Chen, H. B. (2016). Potential allergenicity response to structural modification of irradiated bovine α -lactalbumin. *Food & Function*, 7(7), 3102–3110.
- Mills, E. N. C., Huang, L., Noel, T. R., Gunning, A. P., & Morris, V. J. (2001). Formation of thermally induced aggregates of the soya globulin β -conglycinin. *Biochimica et Biophysica Acta*, 1547(2), 339–350.
- Moriyama, T., Yano, E., Kitta, K., Kawamoto, S., Kawamura, Y., & Todoriki, S. (2013). Effect of gamma irradiation on soybean allergen levels. *Bioscience, Biotechnology and Biochemistry*, 77(12), 2371–2377.
- Mullins, R. J., Dear, K., & Tang, M. L. K. (2009). Characteristics of childhood peanut allergy in the Australian capital territory, 1995 to 2007. *The Journal of Allergy and Clinical Immunology*, 123(3), 689–693.
- Nielsen, M., Lund, O., Buus, S., & Lundegaard, C. (2010). MHC class II epitope predictive algorithms. *Immunology*, 130(3), 319–328.
- Nooji, J. K. (2011). *Reduction of wheat allergen potency by pulsed ultraviolet light, high hydrostatic pressure, and nonthermal plasma* University of Florida, M.Sc thesis. Florida, United States: University of Florida.
- Noorbakhsh, R., Mortazavi, S. A., Sankian, M., Shahidi, F., Maleki, S. J., Nasirai, L. R., et al. (2010). Influence of processing on the allergenic properties of pistachio nut assessed in vitro. *Journal of Agricultural and Food Chemistry*, 58(18), 10231–10235.
- Oh, S., Jang, D.-I., Lee, J.-W., Kim, J.-H., Byun, M.-W., & Lee, S.-Y. (2009). Evaluation of reduced allergenicity of irradiated peanut extract using splenocytes from peanut-sensitized mice. *Radiation Physics and Chemistry*, 78(7–8), 615–617.
- Onwude, D. I., Hashim, N., Janius, R., Abdan, K., Chen, G., & Oladejo, A. O. (2017). Nonthermal hybrid drying of fruits and vegetables: A review of current technologies. *Innovative Food Science & Emerging Technologies*, 43, 223–238.
- Panozzo, A., Manzocco, L., Lippe, G., & Nicoli, M. C. (2016). Effect of pulsed light on structure and immunoreactivity of gluten. *Food Chemistry*, 194, 366–372.
- Pattison, D. I., Rahmanto, A. S., & Davies, M. J. (2012). Photo-oxidation of proteins. *Photochemical and Photobiological Sciences*, 11(1), 38–53.
- Peñas, E., Gomez, R., Frias, J., Baeza, M. L., & Vidal-Valverde, C. (2011). High hydrostatic pressure effects on immunoreactivity and nutritional quality of soybean products. *Food Chemistry*, 125(2), 423–429.
- Peyron, S., Mouécoucou, J., Frémont, S., Sanchez, C., & Gontard, N. (2006). Effects of heat treatment and pectin addition on β -lactoglobulin allergenicity. *Journal of Agricultural and Food Chemistry*, 54(15), 5643–5650.
- Prieto, N., Burbano, C., Iniesto, E., Rodriguez, J., Cabanillas, B., Crespo, J. F., et al. (2014). A novel proteomic analysis of the modifications induced by high hydrostatic pressure on hazelnut water-soluble proteins. *Foods*, 3(2), 279–289.
- Pu, Yuan-Yuan, & Sun, Da-Wen (2016). Prediction of moisture content uniformity of microwave-vacuum dried mangoes as affected by different shapes using NIR hyperspectral imaging. *Innovative Food Science & Emerging Technologies*, 33, 348–356.
- Pu, Yuan-Yuan, & Sun, Da-Wen (2017). Combined hot-air and microwave-vacuum drying for improving drying uniformity of mango slices based on hyperspectral imaging visualisation of moisture content distribution. *Biosystems Engineering*, 156, 108–119.
- Pu, Hongbin, Sun, Da-Wen, Ma, Ji, & Cheng, Jun-Hu (2015). Classification of fresh and frozen-thawed pork muscles using visible and near infrared hyperspectral imaging and textural analysis. *Meat Science*, 99, 81–88.
- Qu, Jia-Huan, Sun, Da-Wen, Cheng, Jun-Hu, & Pu, Hongbin (2017). Mapping moisture contents in grass carp (*Ctenopharyngodon idella*) slices under different freeze drying periods by Vis-NIR hyperspectral imaging. *LWT-Food Science and Technology*, 75, 529–536.
- Rahaman, T., Vasiljevic, T., & Ramchandran, L. (2016). Effect of processing on conformational changes of food proteins related to allergenicity. *Trends in Food Science & Technology*, 49, 24–34.
- Reese, G., Ayuso, R., & Lehrer, S. B. (1999). Tropomyosin: An invertebrate pan-allergen. *International Archives of Allergy and Immunology*, 119(4), 247–258.
- Rona, R. J., Keil, T., Summers, C., Gislason, D., Zuidmeer, L., Sodergren, E., et al. (2007). The prevalence of food allergy: A meta-analysis. *The Journal of Allergy and Clinical Immunology*, 120(3), 638–646.
- Sathe, S. K., Liu, C., & Zaffran, V. D. (2016). Food allergy. *Annual Review of Food Science and Technology*, 7(1), 191–220.
- Seo, J. H., Lee, J. W., Kim, J. H., Byun, E. B., Lee, S. Y., Kang, I. J., et al. (2007). Reduction of allergenicity of irradiated ovalbumin in ovalbumin-allergic mice. *Radiation Physics and Chemistry*, 76(11–12), 1855–1857.
- Sharma, P., Gaur, S. N., & Arora, N. (2013). In silico identification of IgE-binding epitopes of osmotin protein. *PLoS One*, 8(1), e54755.
- Shriver, S. K. (2011). *Effect of selected nonthermal processing methods on the allergen reactivity of Atlantic white shrimp (Litopenaeus setiferus)* MSc Thesis. Florida, United States: University of Florida.
- Shriver, S. K., & Yang, W. W. (2011). Thermal and nonthermal methods for food allergen control. *Food Engineering Reviews*, 3(1), 26–43.
- Shriver, S., Yang, W., Chung, S. Y., & Percival, S. (2011). Pulsed ultraviolet light reduces immunoglobulin E binding to Atlantic white shrimp (*Litopenaeus setiferus*) extract. *International Journal of Environmental Research and Public Health*, 8(7), 2569–2583.
- Sicherer, S. H., & Sampson, H. A. (2010). Food allergy. *The Journal of Allergy and Clinical Immunology*, 125(2), S116–S125.
- Stanic-Vucinic, D., Stojadinovic, M., Atanaskovic-Markovic, M., Ognjenovic, J., Gronlund, H., van Hage, M., et al. (2012). Structural changes and allergenic properties of β -lactoglobulin upon exposure to high-intensity ultrasound. *Molecular Nutrition & Food Research*, 56(12), 1894–1905.
- Sudha, M. L., Soumya, C., & Prabhasankar, P. (2016). Use of dry-moist heat effects to improve the functionality, immunogenicity of whole wheat flour and its application

- in bread making. *Journal of Cereal Science*, 69, 313–320.
- Sun, D.-W. (1997). Solar powered combined ejector vapour compression cycle for air conditioning and refrigeration. *Energy Conversion and Management*, 38, 479–491.
- Sun, D.-W. (1999). Comparison and selection of EMC ERH isotherm equations for rice. *Journal of Stored Products Research*, 35, 249–264.
- Sun, D.-W., & Eames, I. W. (1996). Performance characteristics of HCFC-123 ejector refrigeration cycles. *International Journal of Energy Research*, 20, 871–885.
- Sun, D.-W., & Woods, J. L. (1993). The moisture-content relative-humidity equilibrium relationship of wheat - a review. *Drying Technology*, 11, 1523–1551.
- Sun, D.-W., & Zheng, L. Y. (2006). Vacuum cooling technology for the agri-food industry: Past, present and future. *Journal of Food Engineering*, 77, 203–214.
- Sung, N. Y., Byun, E. B., Song, D. S., Jin, Y. B., Kim, J. K., Park, J. H., et al. (2013). Effect of gamma irradiation on mistletoe (*Viscum album*) lectin-mediated toxicity and immunomodulatory activity. *FEBS Open Bio*, 3, 106–111.
- Sung, D., Lee, J., Han, Y., Shon, D., Ahn, K., Oh, S., et al. (2014). Effects of enzymatic hydrolysis of buckwheat protein on antigenicity and allergenicity. *Nutrition Research and Practice*, 8(3), 278–283.
- Sun, W. W., Yu, S. J., Zeng, X.-A., Yang, X. Q., & Jia, X. (2011). Properties of whey protein isolate-dextran conjugate prepared using pulsed electric field. *Food Research International*, 44(4), 1052–1058.
- Surowsky, B., Fischer, A., Schlueter, O., & Knorr, D. (2013). Cold plasma effects on enzyme activity in a model food system. *Innovative Food Science & Emerging Technologies*, 19, 146–152.
- Taheri-Kafrani, A., Gaudin, J.-C., Rabesona, H., Nioi, C., Agarwal, D., Drouet, M., et al. (2009). Effects of heating and glycation of β -lactoglobulin on its recognition by IgE of sera from cow milk allergy patients. *Journal of Agricultural and Food Chemistry*, 57(11), 4974–4982.
- Tammineedi, C. V. R. K., Choudhary, R., Perez-Alvarado, G. C., & Watson, D. G. (2013). Determining the effect of UV-C, high intensity ultrasound and nonthermal atmospheric plasma treatments on reducing the allergenicity of α -casein and whey proteins. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology*, 54(1), 35–41.
- Terheggen-Lagro, S. W., Khouw, I. M., Schaafsma, A., & Wauters, E. A. (2002). Safety of a new extensively hydrolysed formula in children with cow's milk protein allergy: A double blind crossover study. *BMC Pediatrics*, 2(10), 1–7.
- Thompson, T., Kane, R. R., & Hager, M. H. (2006). Food allergen labeling and consumer protection act of 2004 in effect. *Journal of the American Dietetic Association*, 106(11), 1742–1744.
- Tolouie, H., Hashemi, M., Mohammadifar, M. A., & Ghomi, H. (2017). Cold atmospheric plasma manipulation of proteins in food systems. *Critical Reviews in Food Science and Nutrition*. <https://doi.org/10.1080/10408398.2017.1335689>.
- Toshiko, K., & Takayuki, O. (2004). Effect of PEF on allergen molecule in aqueous solution. *Seidenki Gakkai Koen Ronbunshu*, 2004, 85–86.
- Untersmayr, E., Scholl, I., Swoboda, I., Beil, W. J., Forsterwaldl, E., Walter, F., et al. (2003). Antacid medication inhibits digestion of dietary proteins and causes food allergy: A fish allergy model in BALB/c mice. *The Journal of Allergy and Clinical Immunology*, 112(3), 616–623.
- Usui, M., Harada, A., Ishimaru, T., Sakumichi, E., Saratani, F., Sato-Minami, C., et al. (2013). Contribution of structural reversibility to the heat stability of the tropomyosin shrimp allergen. *Bioscience, Biotechnology, and Biochemistry*, 77(5), 948–953.
- Vanga, S. K., Singh, A., & Raghavan, V. (2017). Review of conventional and novel food processing methods on food allergens. *Critical Reviews in Food Science and Nutrition*, 57(10), 2077–2094.
- Vaz, A. F. M., Costa, R. M. P. B., Coelho, L. C. B. B., Oliva, M. L. V., Santana, L. A., Melo, A. M. A., et al. (2011). Gamma irradiation as an alternative treatment to abolish allergenicity of lectins in food. *Food Chemistry*, 124(4), 1289–1295.
- Vaz, A. F. M., Souza, M. P., Carneiro-da-Cunha, M. G., Medeiros, P. L., Melo, A. M. A., Aguiar, J. S., et al. (2012). Molecular fragmentation of wheat-germ agglutinin induced by food irradiation reduces its allergenicity in sensitised mice. *Food Chemistry*, 132(2), 1033–1039.
- Vaz, A. F. M., Souza, M. P., Vieira, L. D., Aguiar, J. S., Silva, T. G., Medeiros, P. L., et al. (2013). High doses of gamma radiation suppress allergic effect induced by food lectin. *Radiation Physics and Chemistry*, 85, 218–226.
- Wang, L. J., & Sun, D.-W. (2001). Rapid cooling of porous and moisture foods by using vacuum cooling technology. *Trends in Food Science & Technology*, 12, 174–184.
- Wang, L. J., & Sun, D.-W. (2004). Effect of operating conditions of a vacuum cooler on cooling performance for large cooked meat joints. *Journal of Food Engineering*, 61, 231–240.
- Xie, Anguo, Sun, Da-Wen, Xu, Zhongyue, & Zhu, Zhiwei (2015). Rapid detection of frozen pork quality without thawing by Vis-NIR hyperspectral imaging technique. *Talanta*, 139, 208–215.
- Xie, Anguo, Sun, Da-Wen, Zhu, Zhiwei, & Pu, Hongbin (2016). nondestructive measurements of freezing parameters of frozen porcine meat by NIR hyperspectral imaging. *Food and Bioprocess Technology*, 9, 1444–1454.
- Xu, Q., Shi, J., Yao, M., Jiang, M., & Luo, Y. (2016). Effects of heat treatment on the antigenicity of four milk proteins in milk protein concentrates. *Food and Agricultural Immunology*, 27(3), 401–413.
- Yang, H., Gao, J., Yang, A., & Chen, H. (2015). The ultrasound-treated soybean seeds improve edibility and nutritional quality of soybean sprouts. *Food Research International*, 77(Part 4), 704–710.
- Yang, W. W., Mwakatage, N. R., Goodrich-Schneider, R., Krishnamurthy, K., & Rababah, T. M. (2012). Mitigation of major peanut allergens by pulsed ultraviolet light. *Food and Bioprocess Technology*, 5(7), 2728–2738.
- Yang, Qian, Sun, Da-Wen, & Cheng, Weiwei (2017). Development of simplified models for nondestructive hyperspectral imaging monitoring of TVB-N contents in cured meat during drying process. *Journal of Food Engineering*, 192, 53–60.
- Yang, X., Sun, J., Tao, J., Ma, Y., Wei, J., & Long, F. (2017). The allergenic potential of walnuts treated with high pressure and heat in a mouse model of allergy. *Innovative Food Science & Emerging Technologies*, 39, 165–170.
- Yao, M., Xu, Q., Luo, Y., Shi, J., & Li, Z. (2015). Study on reducing antigenic response and IgE-binding inhibitions of four milk proteins of *Lactobacillus casei* 1134. *Journal of the Science of Food and Agriculture*, 95(6), 1303–1312.
- Yi, J., Kebede, B. T., Grauwet, T., Van Loey, A., Hu, X., & Hendrickx, M. (2016). Comparing the impact of high-pressure processing and thermal processing on quality of "hayward" and "jintao" kiwifruit purée: Untargeted headspace fingerprinting and targeted approaches. *Food and Bioprocess Technology*, 9(12), 2059–2069.
- Yu, J., Ahmedna, M., Goktepe, I., Cheng, H., & Maleki, S. J. (2011). Enzymatic treatment of peanut kernels to reduce allergen levels. *Food Chemistry*, 127(3), 1014–1022.
- Yu, M., Liu, H., Shi, A., Liu, L., & Wang, Q. (2016). Preparation of resveratrol-enriched and poor allergic protein peanut sprout from ultrasound treated peanut seeds. *Ultrasonics Sonochemistry*, 28, 334–340.
- Zhao, X. Y., Yang, W. D., Chung, S.-Y., Sims, C. A., Otwell, S. W., & Rababah, T. M. (2014). Reduction of IgE immunoreactivity of whole peanut (*Arachis hypogaea* L.) after pulsed light illumination. *Food and Bioprocess Technology*, 7(9), 2637–2645.
- Zhou, H., Wang, C., Ye, J., Tao, R., Chen, H., & Cao, F. (2016). Effects of enzymatic hydrolysis assisted by high hydrostatic pressure processing on the hydrolysis and allergenicity of proteins from ginkgo seeds. *Food and Bioprocess Technology*, 9(5), 839–848.