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Review Article

Crustacean Allergens and Impact of different Processing Techniques on its Allergenicity: A Review

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Abstract

Allergenicity to crustaceans is a global food safety concern in spite of acceptance of delicacies like shrimps, crabs and lobsters. They are also the leading causative of food induced anaphylaxis. Major percentage of allergic reactions are triggered by allergic protein, tropomyosin and the others such as arginine kinase, sarcoplasmic calcium-binding protein, myosin light chain, troponin C, hemocyanin are also involved. Prevalence of crustacean allergenicty, allergens reported in different crustaceans and their cross reactivities are discussed in this reviews. Besides the effect of various thermal and non-thermal processing techniques in managing the crustacean allergen is also discussed. In comparison to the enhanced allergenicity effects reported by thermal techniques, novel non-thermal techniques like high pressure processing, gamma irradiation, enzymic hydrolysis, high intensity ultrasound, pulsed ultraviolet light etc have promising effects on allergenicity reduction by structural modifications in the proteins. The advantages of combined treatments in hurdle technology approaches can make effective mitigation of crustacean allergenicity and can be suitably optimized for hypoallergic food development.

Keywords: Crustacean allergenicity, tropomyosin, thermal processing, non-thermal technique, hurdle technology

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Introduction

Seafood encompasses fishes and shellfishes which provide good amount of easily digestible proteins, polyunsaturated fatty acids and vitamins to the consumers. Global acceptance and increased frequency of consumption of seafood is linked to its nutritional advantages. But among seafood, fish and shellfish are one of the eight major food allergens as per Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO, 2001) owing to their allergic responses in sensitive individuals (Fuller et al., 2006). Food allergy is an important public health concern globally not only in developed nations but also in developing nations and is the abnormal immunological response to certain food proteins by sensitive individuals which is mainly mediated by immunoglobulin E (IgE). The quantity of protein or threshold for eliciting an allergic reaction varies from person to person as well as from allergic protein to protein. Globally, around 170 different foods are linked with allergic reactions (Boyce et al., 2010) and food allergy impacts 1–2% of adults and 3–6% of children living in developed countries (Ketnawa & Liceaga, 2017). The crustacean allergy affects more than 2% of the global population (Lopata et al., 2010; Rahman et al., 2010; Faisal et al., 2019) and USA, European Union (EU) and Japan insist mandatory labeling of food allergens including crustaceans (Bucchini et al., 2016). The most important and useful management strategy for allergic responses is the avoidance of allergic food. The crustaceans causing allergy are shrimps, crabs and lobsters in the order of incidence (Woo & Bahna, 2011). There are many food processing technologies to ensure the safety of food, modification of texture, enhancement of taste or colour and to make it digestible. In addition to the traditional practice of

thermal processing methods, many non-thermal methods are also evolved to minimize the nutritional quality loss. Crustaceans are also processed in different ways and the allergic protein components can be altered by the modifications in the structural components and hence the allergenicity . The epitope, the stretch of amino acids in an allergen binding with the IgE leading to allergic reactions, can be linear or conformational epitopes, depending on the primary structure of the protein. Processing can alter immunodominant epitopes and can potentially affect allergenic properties either by destruction of existing epitopes or by formation of new epitopes or neoallergens. Conformational epitopes are more vulnerable to processing induced destruction, while the linear epitopes are more likely to be altered by hydrolysis, chemical modifications or by mutations (Sathe et al., 2005). The structural changes of protein and allergenicity can vary with the processing method and also on the type food material (Vanga et al., 2017). Many researchers have attempted to reduce or eliminate the allergenicity of crustaceans by different processing methods. This review presents the global prevalence of allergy associated with consumption of crustaceans, major crustacean allergens and literature reports on effect of different thermal and non-thermal processing methods on crustacean allergens.

Prevalence of crustacean allergy

The variation in reporting of food allergies and absence of information gathering system make it more prevalent than actual. Seafood allergy can persist throughout the life in 90% of the patients (Zotova et al., 2019). Overall, the incidence of shellfish allergy is more in Asian countries than other developed nations. The occurrence of crustacean allergy in USA is two times that of peanut allergy, affecting mostly adults and children above 6 years (Liu et al., 2008; Faisal et al., 2019; Ayuso et al., 2008). The prevalence of fish allergy in the general population ranges between 0.2% and 2.3% (Sharp et al., 2014) while a higher incidence (10.3%) is reported in the Italian population and is about 4.8 to 7.0% among the European population (Burney et al., 2010). Sicherer & Sampson (2010) reported 0.1% and 2% allergenicity among the children and adults respectively in North America. In Thailand merely 0.9% children responded to oral food challenges while 3.3% of children were found to have sensitivity to crustaceans (Lao-araya & Trakultivakorn, 2012). In Australia around 85% of seafood allergy responses are due to crustaceans (Turner et al., 2011). Khora (2016) reported a marginal incidence (0.5 to 2.5%) among the general population and it is more in coastal nations of Asia where shellfish is the major seafood consumed. Huge consumption pattern of seafood and early exposure to life from childhood in Asia make shellfish as the most common food allergen especially in older children and adults (Chiang et al., 2007). Shellfishes are the primary foods causing anaphylaxis in South-East Asia (Hsin et al., 2011) and mild oral symptoms like itching and lip swelling are also reported in Asian population (Jirapongsananuruk et al., 2008). Teenagers of Singapore and the Philippines reported history of shellfish allergy at 5.2 and 5.1% respectively (Shek et al., 2010).

Crustacean allergens and cross reactivity

Allergic proteins identified from crustacean sources are tropomyosin, arginine kinase, sarcoplasmic calcium-binding protein, myosin light chain, troponin C and hemocyanin. Among these allergens the most commonly reported one is tropomyosin (Table 1).

Tropomyosin

Among shrimp allergies, 80% are triggered due to tropomyosin (Chapman et al., 2006; Troiano, 2016). Although tropomyosin from invertebrates is reported as major allergen, tropomyosin from vertebrate sources is known as non-allergic (Reese et al., 1999). Sensitization to tropomyosin varies from 23% to 83% based on the geographic locations whereas IgE in opposition to whole shrimp extract was shown in 94% indicating presence of other minor allergic proteins (DeWitt et al., 2004). Tropomyosin is a myofibrillar protein or salt soluble protein with high heat stability. It has two homo dimers of α-helix with coiled-coil structure having 276–284 amino acids with molecular weight in the range of 34–38 kDa (Rahman et al., 2010). It represents approximately 20% of shrimp total protein (Dual et al., 1994) both in muscle and non-muscle cells and is involved in muscle contraction along with actin and myosin (Leung et al., 2014). It is also present in non-decapods like mantis shrimp, barnacle, and krill.

Heat stable antigen II component from cooked shrimp with IgE binding ability was confirmed as tropomyosin by Hoffman et al. (1981). The high amino acid sequence homology of 86% between allergen Sa-II or Pen i 1 from *Penaeus indicus* and

tropomyosin from fruit fly *Drosophila melanogaster* was reported by Shanti et al. (1993). Tropmyosin identified in black tiger shrimp, *Penaeus monodon* and pacific white shrimp, *Litopenaeus vannamei* are referred to as Pen m 1 and Lit v 1 respectively. Met e 1, major allergen from *Metapenaeus ensis* was identified based on cloning and screening of cDNA library of shrimp using sera from patients with shellfish sensitivity (Leung et al., 1994) and is a 34-kDa protein having 281 amino acids. A 36-kDa Pen a 1 reported from Northern brown shrimp *Penaeus aztecus* contains 312 amino acid residues (Daul et al., 1994) and was further confirmed as tropomyosin based on molecular cloning and nucleotide sequencing (Reese et al., 1997). Similarity of amino acid sequences of Sa-II, Pen a 1, and Met e 1 revealed that they are identical. Motoyama et al. (2007) classified tropomyosin into three categories such as fast, slow-twitch and slow-tonic depending upon muscle fiber and majority are of fast type. The protein tropomyosin of 37 kDa was identified as the major allergen in flower tail shrimp, *Metapenaues dobsoni* (Laly et al. 2019a). *Metapenaeus affinis* tropomyosin mRNA, partial cds of 455 base pair is sequenced (Gene bank accession number MT231194).

Cross-reactivity, an adaptive immune response to a specific antigen, causes reactivity to other antigens with structural similarity to the inducer (Bonds et al., 2008). The cross reactivity occurs due to shared/common or frequent epitopes on antigens and the conformational resemblance of epitopes to which the antibody is binding. Tropomyosin is a major cross-reactive allergen (Shanthi et al., 1993) showing cross reactivity among different crustacean species. Monoclonal antibodies against Pen a 1 exhibited related sensitivity towards crayfish, crab, and lobster proteins (Daul et al., 1992). Positive immunoblotting results of shrimp sensitive sera showed reactivity against muscle bivalves, gastropods and cephalopods (Leung et al. (1994). Crespo et al. (1995) explained the cross reactivity of shrimp (*Pandalus borealis*) and German cockroach (*Blattellager manica*) allergic proteins. Cross reactivity between the allergic proteins of arthropod groups Crustacea, Arachnida (mites) and Insects have also been recognized (Witteman et al., 1994). However, it is important to note that the IgE against shellfish tropomyosin does not cross-react with vertebrate ones. The allergen is greatly conserved among arthropods and mollusks, with a homology of 61% (Leung et al., 2014). Species-specificity can be due to lower sequence homology among allergens as well as the presence of species-specific allergen (Piboonpocanun et al., 2011).

Arginine kinase

Arigine kinase (AK) is the second major allergen after tropomyosin in shrimps having a molecular weight of 40 to 42 kDa and is a water soluble protein (Garcia-Orozco et al., 2007). AK is a monomeric phosphagen ATP phospho transferase present in myosinogen, performing the reversible transfer of high energy phosphoryl group of adenosine triphosphate (ATP) to arginine, yielding phosphoarginine and adenosine diphosphate (Kang et al., 2011). Arginine kinase from black tiger shrimp, Pen m 2 is reported to have 96% similarity to Lit v 2, reported in Pacific white shrimp and has cross-reactivity among shrimp, lobster, crab and crawfish (Yu et al., 2003; Srinroch et al., 2015). Shrimp arginine kinase is also interpreted as cross-reactive marker and is identified in 75% of patients who were atopic to house dust mite and cockroach. It is highly abundant in invertebrate muscle and it cannot withstand thermal and acid base treatment. But IgE binding to arginine kinase in thermally processed shrimps is associated with the intact IgE epitopes after aggregation (Kamath et al., 2014). Alergic reactions similar to AK is observed in interaction with house dust mite (*Dermatophagoides farinae*) (Bi & Chew 2004), Indian-meal moth (*Plodia interpunctella*) (Binder et al., 2001) and silkworm larvae (*Bombyx mori*) (Liu et al., 2009). Crustacean arginine kinase along with tropomyosin caused sensitivity in crab processing workers through respiration (Abdel Rahman et al., 2011).

Sarcoplasmic calcium binding protein

Sarcoplasmic calcium binding protein is an invertebrate EF-hand (helix-loop-helix structural domain or motif) calcium buffering protein which can act similarly as vertebrate major allergen, parvalbumin. These acidic cytosolic proteins with a molecular weight of 20-22 kDa, have four potential EF hand calcium binding sites (Hermann & Cox 1995). It is a thermostable protein, stable to acidic and alkaline treatments and accounts for 10 to 15% of sensitizations along with argine kinase (Giuffrida et al., 2014). This protein is also reported in kuruma shrimp *(Penaeus japonicus)*, American lobster *(Homarus americanus)*, pink shrimp *(Pandalus eous)*, king crab *(Paralithodes camtschaticus),* snow crab *(Chionoecetes opilio)* etc. and shows a significant amino acid sequence homology of 80 to 98% with

crustaceans (taxonomically related species) but only 15 to 21% in other instances (Mita et al., 2013). Sarcoplasmic calcium binding protein was identified in around 74% of children and 10% of adults with shrimp allergy, indicating as a major trigger in pediatric population (Ayuso et al., 2009).

Myosin light chain proteins

Myosin light chain, a protein involved in muscle contraction along with actin, tropomyosin and troponin belongs to EF hand domain super family which encompasses both food and inhalant allergens. The myosin light chains, Lit v 3 isolated from *Litopenaeus vannamei* (Ayuso et al., 2008) was reported to have exclusive sensitivity in some patients. With similar molecular weight (20kDa) and isoelectric point (4.2) to sarcoplasmic calcium binding protein, it is difficult to recognize this protein, responsible for allergic reactions, by standard laboratory methods (Ayuso et al., 2008) and the information on immunological cross-reactivity of myosin light chain is lacking.

Troponin C

Troponin C is identified in shrimps and also a cockroach allergen, Blag 6 by Hindley et al. (2006). It is also an EF hand calcium binding protein of 20 kDa, and its thermal stability is not clearly reported. IgE binding frequency to troponin C is 15% lower as that of tropomyosin, arginine kinase and sarcoplasmic calcium binding protein. Troponin C identified from Northern sea shrimp, *Crangon crangon* is denoted as Cra c 6 which is the first crustacean troponin C to which IgE binding is reported (Bauermeistera et al., 2011). The protein is reported to have three subunits such as troponin C, troponin T and troponin I which bind to Ca^{2+}, tropomyosin act in respectively, preventing actin-myosin reaction (Pedrosa et al., 2015). Troponin C along with triose-phosphate isomerase (28 kDa glycolytic enzyme) and fatty acid binding protein (15–20 kDa transport protein) is responsible for sensitivity in 10–23% of allergic individuals (Ayuso et al., 2011).

Triosephosphate isomerase

Triosephosphate isomerase is reported as an allergen in Northern sea shrimp (Cra c 8), cray fish (Arc s 8), and cockroach (Bla g TPI). This protein has a molecular weight of 28 kDa and is heat sensitive (Bauermeister et al., 2011). This allergen is also reported from octopus (*Octopus fangsiao*) (Yang et al., 2017) and its cross-reactivity nature is not well understood.

Hemocyanin

Hemocyanin is also a thermally stable crustacean allergen, from hemolymph which has cross-reactivity to snail and house dust mite (Faber et al., 2017; Khanaruksombat et al., 2014). It transports oxygen and constitutes 75–95% of the total protein. It is a hexamer or multi hexamer with 75 kDa subunits in its natural state and it varies with species (Hodgson and Spicer 2001). Hemocyanin isolated from giant fresh water shrimp (*Macrobrachium rosenbergii*), is reported to have 62.5% to 100% of sequence homology with other crustaceans (Piboonpocanun et al., 2011) and is also a thermally stable allergen.

Processing methods and crustacean allergenicity

Food materials are processed for enhancing palatability, digestion and microbial safety as well as to improve organoleptic properties and increase preference for consumption. Food allergenicity is influenced by the type and conditions of the processing method as well as the characteristics of the allergen (Jimenez-Saiz et al., 2015). Besides, sensitization pattern of allergic patient's population is also important (Maleki, 2004). Application of thermal and non-thermal processing methodology can modify physical and chemical characteristics of proteins leading to the change of allergenic epitopes (Khan et al., 2019). Although complete removal of allergenic ability by processing is rather difficult to achieve, reduction in the allergic threshold could be accomplished by the appropriate conditions (Fei et al., 2016).

The immunoreactivity of allergens is based on the retention of structural epitopes and its recognition by IgG or IgE (Verhoeckx et al., 2015). The disruption of epitope can prevent the binding of IgE antibody to antigen. The conformational epitopes are susceptible to processing treatments in comparison to linear epitopes. Antibodies identify these epitopes by their three-dimensional structure and can be damaged by severe food processing methods such as enzymatic hydrolysis. Linear epitopes, based on amino acid sequence are recognized by their primary structure and hence denaturing is not fully effective. The amino acid sequence of the epitope needs to be cut or disrupted to decrease the IgE binding capacity (Albrecht et al., 2009). The

Table 1. Crustacean allergens

Crustacean	Tropomyosin	Arginine kinase	Myosin light chain	Sarcoplasmic binding protein	Troponin C	Triosephosphate isomerase
Shrimps						
Northern sea shrimp (*Crangon crangon)*	Cra c 1	Cra c 2	Cra c 5	Cra c 4	Cra c 6	Cra c 8
Whiteleg shrimp *(Litopenaeus vannamei)*	Lit v 1	Lit v 2	Lit v 3	Lit v 4		
Brown Shrimp (*Penaeus aztecus)*	Pen a 1			Pen a 4		
Indian white prawn (*Penaeus indicus)*	Pen i 1			Pen i 4		
Black tiger shrimp (*Penaeus monodon)*	Pen m 1	Pen m 2	Pen m 3	Pen m 4	Pen m 6	
Sand shrimp (*Metapenaeus ensis)*	Met e 1			Cra c 4		
Narrow-clawed crayfish (*Pontastacus leptodactylus)*	Pon i 1			Pon i 4		
Crabs						
Crucifix crab (*Charybdis feriatus)*	Cha f 1	Cha f 2		Cha f 4		
Giant mud crab (*Scylla serrata*)	Scy s 1	Scy s 2				
Green mud crab (*Scylla paramamosain*)	Scy pa 1	Scy pa 2		Scy pa 4		
Blue swimming crab (*Portunus pelagicus*)	Por p 1	Por p 2		Por p 4		
Lobsters						
American lobster (*Homarus americanus)*	Hom a 1		Hom a 3	Hom a 4	Hom a 6	
Green lobster (*Panulirus stimpsoni*)	Pan s 1					
Scalloped spiny lobster (*Panulirus homarus*)	Pan h 1					
European lobster (*Homarus gammarus*)	Hom g 1	Hom g 2				
Spiny lobster (*Panulirus stimpsonii*)	Pen s 1					

*Source – Lopata et al. (2016); Khan et al. (2019)

relationship between the number and nature of epitopes, and severity of the allergic reaction need to be investigated for each allergen (Sathe et al., 2005).

Effect of thermal processing

Thermal treatment can result in hydrolysis of peptide bonds, aggregation by disulfide and non-covalent bonds, denaturation and reactions with other food components (Khan et al., 2019) leading to loss of conformational epitopes or formation of new ones (Mejrhit et al. 2017).

Most crustacean allergens are heat stable in nature and heat treatment is observed to retain allergenicity of tropomyosin (Lasekan & Nayak, 2016; Liu et al., 2010; Lopata et al., 2010) and high immunoreactivity of heat-treated tropomyosin is reported (Yu et al., 2011). Kamath et al. (2013) reported an increase in monoclonal antibody binding to boiled shrimp extracts than the raw extracts. The collapse of helical structure of tropomyosin by heating at 80°C, can be regained to natural conformation on cooling to 25°C (Khan et al., 2019). Boiling of shrimp extract for 4 min did not change the tropomyosin band intensity (Shriver et al., 2011) and more intense bands of tropomyosin were observed in thermal processed shrimp extracts (Lasekan & Nayak (2016). Sockalingam et al. (2017) reported prominent heat stable bands of 32 to 38 kDa in the extracts of boiled, steamed and fried giant river prawn. Similarly, other major shrimp allergen, myosin light chain (Lit v 3) exhibited high resistance to boiling (Ayuso et al., 2008). Elevation in IgE-reactivity of extracts from boiled crab and prawn based on ELISA, western blot and basophil activation test is also reported (Abramovitch et al., 2013). The recognition of allergenic properties of tropomyosin during extended boiling was reported in flower tail shrimp (laly et al., 2019b).

Frying is a faster and high temperature cooking in oil. Lehrer et al. (2010) reported shrimp allergenic activity in the cooking oil regardless of shrimp in breaded or non-breaded form. Hence the usage of same cooking oil containing shrimp allergen can transfer the allergen to other non-allergic food materials. The high temperature during frying elevated the antigenicity by 6 to 8 times in comparison to control. Frying process can alter the allergenicity by removal of existing protein epitopes to reduce allergenicity or may create new epitopes to increase allergenicity (Phiriyangkul et al., 2015). The increase in the band intensity of tropomyosin in the shrimp extracts prepared after frying was reported (Lasekan, 2017) and the percentage IgE inhibition of tropomyosin of the fried shrimp was comparable to that of raw shrimp. Faisal et al. (2019) while evaluating the impact of frying temperature in reducing the allergenicity of banana prawn reported comparatively lesser sharp protein bands in the SDS electrophoretic band pattern and increase in the intensity of tropomyosin band with the increase in frying temperature in immunoblotting analysis.

Autoclaving, a moist heating technology used for sterilization, is reported to alter the action of many food allergens. Through competitive inhibition ELISA it was shown that high pressure steaming can reduce the activity of shrimp allergen (Lasekan & Nayak 2016). The combination of pressure and heat during autoclaving could facilitate proteolysis resulting in protein fragmentation (Kulis et al., 2012). Processes with high pressure and low temperature cannot make similar effect as autoclaving (Cabanillas et al., 2014). Best reducing effect towards immune competence of shrimp allergen from *Metapenaeus ensis* was reported by Zheng et al. (2011). Significantly lighter ($p \leq 0.05$) intensity of bands in the range of 10–250 kD were reported in banana prawn protein treated at 121°C (Faisal et al., 2019).

Microwave processing can generate heat immediately and the heat generation can dependent on the nutrient components and the homogeneity of the food product. Microwaves can modify the native structure of proteins and could lead to changes in protein recognition by IgE (Jimenez-Saiz et al., 2015). A comparison of different thermal processing methods like boiling, steaming, frying, baking, grilling and microwave cooking demonstrated that microwave treatment alone is not sufficient to reduce the proteins antigenicity and has to be combined with other treatments to reduce allergenicity (Lasekan. 2017).

Non-thermal processing methods

The non-thermal processing techniques, *viz.,* high pressure processing, gamma irradiation, pulsed electric field etc. do not contribute to heating of food to make alteration in the product and hence, is expected to retain most of the nutritional benefits as well as organoleptic characteristics in comparison to thermally treated foods. Besides, chemical and enzymatic treatments can also be applied to modify

or cross link the allergic protein. During enzymatic digestion, linear epitopes can be altered via fragmentation. Hydrolysis can induce changes in food proteins by modification or destruction of linear and conformational epitopes which in turn can contribute to alteration in allergenicity (Zhou et al., 2016).

High pressure-cooking uses pressures in the range of 100–800 MPa to destroy pathogens for extending the shelf life of foods. This process, often, leads to reversible or irreversible structural modifications in proteins, resulting in denaturation, aggregation, or gelatinization. However, the original color, flavor, and nutrients of most food commodity is retained, but with limited reduction in allergenicity (Ahmed et al., 2016; Zhang et al., 2017). The process can be optimized with respect to temperature, pressure, and exposure time (Kaur et al., 2016). Pressure is applied directly and evenly in the food material, independently of the size and geometry of the food product (Balasubramaniam et al., 2015) and the possible changes involved are protein denaturation, conformational alterations leading to modulation of the allergenicity. High pressure processing of king prawns and black tiger prawns at 600 MPa and 30°C for 10 min caused significant reduction in tropomyosin allergenicity (Yohannes et al., 2008). High pressure processing at 450 MPa, for 55 min combined with enzymatic hydrolysis enabled diffusion of proteases into shrimp tissue to attenuate immunoreactivity resulting in a hypoallergenic product (Hu & Xie, 2013). Other allergenic mollusks and fish based foods showed reduction in allergenicity by high pressure processing due to structural changes in tropomyosin (Jin et al., 2015; Liu & Xue 2010). Evaluation of allergic response of thermally supported high pressure processed shrimp extract using pooled sera of shrimp sensitive individuals showed a fully IgE inhibition, leading to reduction in allergenicity of tropomyosin (Long et al., 2015). Also, the treated shrimp showed a significant decrease in specific IgE in BALB/c mouse model along with a decrease in histamine levels.

Irradiation is food processing technique with negligible changes in nutritive and sensorial properties. Gamma irradiation is widely employed for prolonging the shelf life, microbial destruction, and minimizes losses in production (Farkas, 2006). Food irradiated at a dosage of 10 kGy is considered safe for human consumption and free from biological and chemical hazards (Hackwood, 1991). Irradiation is reported to reduce the immunoreactivity of many food allergic proteins. IgE activity of shrimp tropomyosin was reduced by 20% with the increase of dosage to 10 kGy. Gamma irradiation of whole shrimp reduced the IgE-binding capacity of heat stable allergens based on dosage of radiation (Byun et al., 2000; Byun et al., 2002). Effectiveness of electron beam irradiation on the immunoreactivity of shrimp tropomyosin in frozen stage was reported by Liu et al. (2017). Shrimp protein extract and the purified allergen (Pen a 1) subjected to irradiation at different dosages showed increased IgE-binding capacity at lower dosages for shrimp allergen (Li et al., 2007; Zhenxing et al., 2007).

Enzymic hydrolysis of allergenic proteins is a gentle and harmless method to degrade food allergens. It can be done either by cross linking to bury IgE reactive epitopes or by proteolytic hydrolysis to make proteins into small peptides (Yu, 2016). Different proteases used in food industry for cross linking are transglutaminases, peroxidases, laccases and tyrosinases which can change protein molecular weight, structure, charge, surface and biological properties (Fei et al., 2016; Yuan et al., 2017). Allergic proteins are resistant to proteases. The differentiation of food allergens from non-allergens depends upon its stability during digestion process. Larger protein fragments are potentially allergic. In comparison to natural tropomyosin, crosslinked one with tyrosinase or horseradish peroxidase can be quickly fragmented. Yuan et al. (2017) reported transglutaminase mediated glycosylation of allergen from *Metapenaeus ensis*, showing a decrease in allergenicity and correlation in structural changes. Similarly, Fei et al. (2016) reported effect of tyrosinase on arginine kinase in thermally polymerized crab (*Scylla paramamosain*). Liu et al. (2017) reported enzymatic crosslinking of IgG/IgE binding activity by horseradish peroxidase or tyrosinase, digestibility, impacting the oral tolerance to crab tropomyosin. It is reported that tyrosinase catalyzed crosslinking on the IgE binding potential and structure of tropomyosin from *M. ensis* by unfolding of the 3D structure and loss of the 2D structure after protein crosslinking (Ahmed et al., 2018). Hydrolysis can alter conformational and linear epitopes (Kasera et al., 2015; Yu, 2016).

Attempts to reduce the crustacean allergenicity by treating with acidic medium were reported by a few researchers. Perez-Macalalag et al. (2007) reported reduced immunoreactivity of extracts from shrimp by soaking in vinegar prior to boiling, as evidenced

by reduction in wheal size in the skin prick test. Changes in pH on the structural characteristics and allergenicity of tropomyosin from short-neck clam (*Ruditapes philippinarum*) indicated an increase in the α helices of the secondary structure at low pH of 1, while a shift to pH 2, the β sheets increased with corresponding decrease in α helices (Lin et al., 2015). Immunogenicity of shrimp tropomyosin after vinegar marination at varying pH (1.0 to 4.8) indicated significant reduction in IgE binding potential at pH 1.0-3.5 compared to that at higher pH (Lasekan et al., 2017). Faisal et al. (2019) also reported a significant reduction in antigenicity of *Fenneropenaeus merguinensis* after treatment with acetic acid and hydrochloric acid.

In ultrasound technique, modification of food proteins takes place by cavitation phenomenon (Mawson et al., 2011). It can also change the secondary structure of proteins leading to the formation of random coil α helices and negligible change on the IgE binding characteristics of beta-lactoglobulin (Stanic-Vucinic & Velickovic, 2012). High intensity ultrasound applies mechanical waves of elevated energy in the range of 20 to 100 kHz, which stimulate cyclic generation and the collapse

Table 2. Impact of processing techniques on crustacean allergenicity

Crustacean	Allergen	Processing method	Impact on allergenicity	Reference
		Thermal processing methods		
Shrimp	Tropomyosin	Boiling	Increase	Liu et al., 2010
Shrimp	Myosin light chain	Boiling	Increase	Ayuso et al., 2008
Lobster	Protein extract	Boiling	Increase	Carnes et al., 2007
Crab	Protein extract	Boiling	Increase	Abramovitch et al., 2013
Shrimp	Tropomyosin	Frying	Increase	Lasekan 2017; Faisal et al. (2019)
Shrimp	Tropomyosin	Autoclaving	Decrease	Zheng et al. (2011); Faisal et al. (2019)
Shrimp	Protein extract	Microwave	Unchanged	Lasekan (2017)
		Non thermal processing methods		
Shrimp	Tropomyosin	High pressure processing	Decrease	Yohannes et al., 2008
Shrimp	Tropomyosin	Gamma irradiation	Decrease	Byun et al. (2002)
Shrimp	Tropomyosin	Electron beam irradiation	Decrease	Liu et al. (2017)
Shrimp	Tropomyosin	Enzymic hydrolysis	Decrease	Yuan et al. (2017)
Shrimp	Actin Myosin heavy chain	Enzymic hydrolysis	Decrease	Liu et al. (2011)
Clam	Tropomyosin	Acid treatment	Decrease	Lin et al. (2015)
Shrimp	Tropomyosin	Acid treatment	Decrease	Lasekan et al. (2017) Faisal et al. (2019)
Shrimp	Tropomyosin	High intensity ultrasound	Decrease	Li et al. (2006)
Shrimp	Tropomyosin	Pulsed ultraviolet light	Decrease	Shriver et al. (2011)
		Hurdle technology		
Shrimp	Protein extract Tropomyosin	Gamma irradiation and Boiling	Decrease	Li. et al. (2007)
Shrimp	Tropomyosin	PUV light and Boiling	Increase	Shriver et al. (2011)
Crab	Tropomyosin	Ultrasound and Boiling	Decrease	Yu et al. (2011)
Shrimp	Tropomyosin	High pressre processing and heating	Decrease	Long et al. (2015)

of cavities (sonication bubbles) in food (Khan et al., 2019). Conformational changes and peptide bond cleavage in presence of collapsed cavities via sheer force can alter allergenicity (Rodriguez et al., 2018; Nowacka et al., 2018). Li et al. (2006) reported the effect of high intensity ultrasound on the IgE binding ability of allergen Pen a 1. It is further reported that the evaluation of allergenic and textural characteristics of ultrasound (800 W, 30 kHz) treated *L. vannamei*, both raw and boiled, indicated decline in allergenicity of the boiled prawns in comparison to raw prawns (Li et al., 2011).

Pulsed ultraviolet (PUV) light is a non-ionizing radiation introduced by instantaneous high energy short pulses (Harder et al., 2017). In presence of high intense light pulses, molecules get excited and while returning to ground state, they release energy in the form of heat or photons which alters the protein structure via aggregation, unfolding and fragmentation etc. leading to alteration in IgE binding nature of allergens (Abida et al., 2014). Yang et al. (2012) evaluated crustacean allergen stability modified by the PUV light and reported the preparation of hypoallergenic shrimp products having decreased allergenicity under human gastrointestinal conditions. Shriver et al. (2011) reported decrease of IgE-binding ability of Atlantic white shrimp (*Litopenaeus setiferus*), after PUV light exposure.

Hurdle technology methods

Processing methods can be combined successfully for effective preservation of foods. The hurdle technology approach can enhance safety and stability of food material along with the retention of nutrients and sensory properties. There are reports indicating the reduction in IgE activity of shrimp allergen by the combined effect of gamma irradiation and boiling Li et al., 2007; Yu et al., 2011. There was no impact reported on the allergenicity of shrimp tropomyosin due to the application of PUV light with heat treatement (Shriver et al., 2011) while a significant reduction in tropomyosin specific IgE as a result of high pressure processing conmbined with heat treatment (Long et al., 2015).

Conclusion

This review covers the prevalence of crustacean allergenicity, reported allergens in different crustaceans along with its cross reactivity and impact of different thermal and non-thermal processing techniques in altering the allergenicity. The prevalence of shellfish allergy is more in Asian countries compared to developed countries and ranged from 0.5 to 2.5%. The most reported major allergen in crustaceans is tropomyosin and other allergens include arginine kinase, myosin light chain, sarcoplasmic binding protein, troponin C, triosephosphate isomerase, haemocyanin etc. Allergic protein components can be altered by modifying the structural components leading to increase or decrease of allergenicity. As the immunoreactivity of allergens is based on the recognition of epitopes by IgG or IgE, the disruption of epitope can prevent the binding of IgE antibody to antigen. Among epitopes, conformational epitopes are susceptible to processing treatments in comparison to linear epitopes. Complete removal of allergenicity by processing techniques seems unlikely and selection of appropriate technique can make a significant impact in reducing the allergenicity. Heat stable nature of major crustacean allergen, topomyosin resulted an increase in allergenicity subsequent to thermal processing while a decrease in allergenicity noticed in autoclaving can be due to its combined effect of pressure and temperature. In the case of non-thermal techniques, structural modifications in allergic proteins by novel processing techniques effected decrease in allergenicity besides the retention of nutritional and organoleptic quality. Hurdle technology makes use of their advantages in reducing the allergenicity more effectively. Suitable processing technology for preparing hypoallergic food is to be optimized together with the development of analytical tools for detecting hidden allergens in trace levels which can protect sensitive individuals.

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