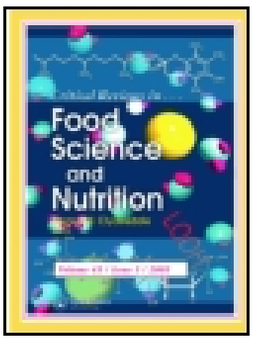

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Potential efficacy of processing technologies for mitigating crustacean allergenicity

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ABSTRACT

Crustacean allergy has become a growing food safety concern at a global scale. In the past decades, various food processing approaches have been employed to develop food products with reduced allergenic potential. Thermal treatment can dramatically influence the allergenicity of crustaceans by either reducing or enhancing their allergenic potential. Maillard reaction, enzymatic and acid treatments have shown to be promising in mitigating crustacean allergenicity. Recently, novel processing technologies, namely high-pressure processing, high-intensity ultrasound, irradiation, pulsed ultraviolet light and hurdle technology have attracted special attention from the researchers and the food industry professionals owing to their benefits over the conventional methods. In this context, this review paper provides an updated overview of the current knowledge on how different food processing methods induce structural changes of crustacean allergens and, subsequently, influence their allergenic potential. Data on prevalence and clinical relevance of crustacean allergy are presented, as well as, the molecular characterization of crustacean allergens and the main analytical methods for their detection in processed foods.

KEYWORDS

Crustacean allergens; heat treatment; enzymatic treatment; high-pressure; ultrasound; irradiation

1. Introduction

Seafood plays a crucial role in human nutrition, being considered a rich source of highly assimilated proteins, polyunsaturated fatty acids (omega-3) and vitamins. Omega-3 fatty acids offer significant health benefits, including prevention of cancer, cardiovascular diseases and improvement of glycemic control (Fernandes et al. 2015; Larsen, Eilertsen, and Elvevoll 2011; Sharp and Lopata 2014). The popularity and frequency of seafood product consumption have increased across the globe due to the referred well-established nutritional advantages. However, the increasing production and consumption of seafood have been accompanied by more frequent reports of severe adverse immunological responses among a significant part of food allergic individuals (Jeebhay and Lopata 2012; Lopata, Kleine-Tebbe, and Kamath 2016; Lopata and Jeebhay 2013; Madsen et al. 2012).

By definition, seafood encompasses all kind of edible marine organisms, which mainly includes the groups of fish and shellfish. The name shellfish is commonly attributed to crustaceans and mollusks since they possess shells or shell-like exoskeletons (Khora 2016). The most important crustaceans belong to the Decapoda order (shrimp, crab, lobster and prawn) (Figure 1), which are also considered a common cause of food allergy due to their widespread consumption, especially in coastal countries (Fernandes et al. 2015; Woo and Bahna 2011). Crustaceans are part of the eight major sources of food allergens declared by the Food and Agriculture Organization (FAO) of the United Nations and World Health Organization (FAO/WHO 2001). Therefore, the labeling of food products containing crustaceans has already become mandatory in many countries, including the USA, European Union (EU) and Japan (Allen et al. 2014; Bucchini et al. 2016). The avoidance of the allergenic food is the most useful management strategy for sensitized individuals. However, total eviction of crustaceans can be extremely difficult to accomplish and, consequently, it may cause a number of nutritional deficiency syndromes. Although oral and subcutaneous immunotherapies have been practiced, their efficacies are not always satisfactory and they are still not capable of offering a true cure for food allergy (Kobernick and Burks 2016; MacGinnite 2017; Wood 2016). Thus, there is a need to seek alternative strategies such as selective processing for minimizing the allergenic potential of crustaceans.

Crustaceans are processed in diverse ways before consumption in order to improve functional, nutritional and sensorial properties, as well as for detoxification and preservation purposes. Processing techniques such as heat, enzymatic and acidic treatments, as well as other novel approaches such as high-pressure processing (HPP), irradiation, high-intensity ultrasound, pulsed ultraviolet (PUV) light and/or the combination of these technologies have been employed, being capable of altering the structure of food proteins in different ways. The possible structural modifications include unfolding,

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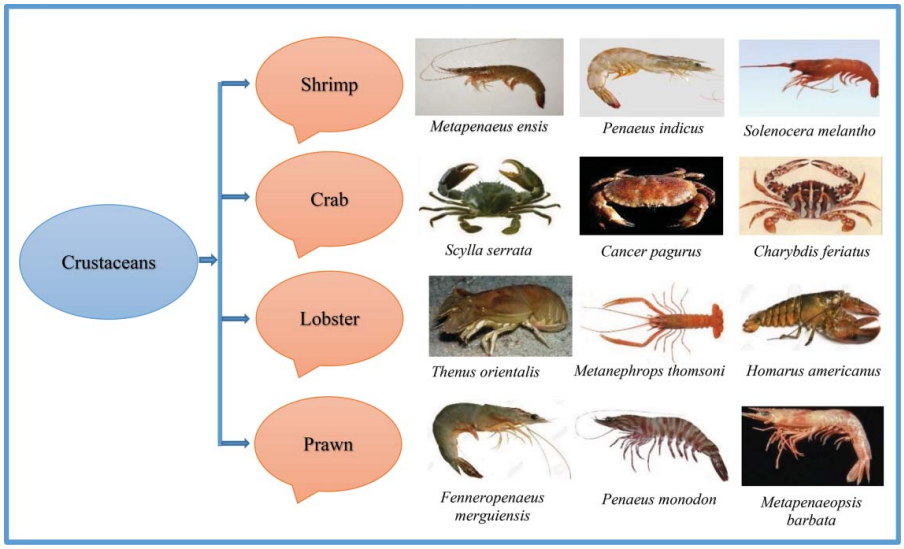

Figure 1. A schematic presentation of common crustacean species.

aggregation, crosslinking between the ingredients and chemical modifications, such as oxidation and glycation (Harder, Arthur, and Harder 2017; Lepski and Brockmeyer 2013; Nayak et al. 2017; Rahaman, Vasiljevic, and Ramchandran 2015; Yuan et al. 2017; Zhang, Deng, and Zhao 2017), which may influence allergenicity. Conformational epitopes can be exposed or hidden by unfolding or aggregation of proteins, respectively (Rahaman, Vasiljevic, and Ramchandran 2015), whereas sequential epitopes can be affected by acidic or enzymatic hydrolysis (Kasera et al. 2015) and Maillard reactions (Toda et al. 2014). Accordingly, processing alters the physicochemical properties of food proteins, affecting their gastrointestinal digestibility with subsequent influence on allergenicity (Rahaman, Vasiljevic, and Ramchandran 2016). The degree of structural changes and allergenicity depend on the type and conditions of processing, as well as, on the food matrix (Rahaman, Vasiljevic, and Ramchandran 2016; Vanga, Singh, and Raghavan 2017). Recently, various approaches have been frequently explored for reducing crustacean allergy and to protect/improve the quality of life of the sensitized/allergic individuals. For selecting appropriate processing methods, it is very important to understand how these procedures alter the structure of food proteins, both at microscopic and macroscopic levels, and subsequent gastrointestinal digestibility since all of these are known to influence food allergenicity.

The present work provides an updated overview on the current knowledge about novel food processing technologies and their effect on crustacean allergenicity. Data on prevalence and clinical relevance of crustacean allergy are presented, as well as, the molecular characterization of crustacean allergens and the main analytical methods for their detection in processed foods.

2. Prevalence and clinical relevance of crustacean allergy

Despite the lack of firm data on prevalence, there are evidences that food allergies have been increasing to levels close to 10% (Sicherer and Sampson 2018). The diagnosis of food allergy is difficult to perform and depends on several factors. The results from diagnostic tests, often inconclusive, the inconsistencies in defining food allergies and in collecting reliable data lead to the discrepancy in reporting allergies around the world. Like for other allergenic foods, the prevalence of crustacean allergy is based on self-reported symptoms (clinical history), specific IgE blood tests and/or skin prick test sensitization, rather than open food challenges or double-blind placebo-controlled food challenges (Burks et al. 2012). In spite of this, recent data suggest that shellfish allergy is estimated at 0.5–2.5% of the general population. However, this prevalence can vary with specific geographical and cultural eating habits, degree of consumption by age and/or with the type of food processing (Woo and Bahna 2011).

In developed countries (European countries, USA and Australia), shellfish allergy is very common among children and adults, but it seems to be even more prevalent in Asian countries (Boye 2012; Lee et al. 2012). Reports show that in North America, 0.1% and 2% of the children and adults, respectively, are affected by shellfish allergy (Sicherer, Muñoz-Furlong, and Sampson 2004; Sicherer and Sampson 2010). The mean prevalence of shrimp allergy (which contributes to the major part of shellfish allergy) in some European countries and Australia was estimated in 5.4%, with Italy and France presenting the highest levels (10.2% and 7.0%, respectively). In coastal countries of Europe, where the consumption of shellfish is high, the prevalence of shrimp allergy ranged from 4.8% to 7.0%, although some countries like Portugal and Finland were not included in this prevalence survey (Burney et al. 2010). Therefore, in Europe, the prevalence of shellfish allergy is most likely underestimated. In Asian countries, seafood allergy is particularly common due to its high consumption and early exposure in life, especially to shellfish species (Lee et al. 2012; Misnan et al. 2005; Wu et al. 2012). Interestingly, the first intake of seafood seems to be very early in life in the Asian diet, with an average age of as low as 7 months. The prevalence of shellfish allergy is about 7% in case of children and adults in Southeast Asian countries (Wu et al. 2012). Similarly, in Singapore and Philippines, the prevalence of shellfish among children was about 5% (Shek et al. 2010). Data showed that 33% of adults in Singapore were sensitized to crustaceans, followed by mollusks (19%) and fish (4%) (Ross et al. 2008). Chiang et al. (2007) estimated a

prevalence of 39% of shellfish allergy among a test population of children (<16 years) in Singapore, being even more relevant for children older than 4 years.

Crustaceans are responsible for triggering hypersensitivity reactions mediated by the immunoglobulin E (IgE) in sensitized/allergic individuals (Khora 2016; Lee and Taylor 2011; Rahman et al. 2012). In general, these hypersensitivity reactions are induced by the accidental ingestion of the offending food or by the consumption of products containing undeclared crustaceans. Contrary to other food allergies, such as milk and egg, allergy to crustaceans tends to persist in adults. In general, the allergic reactions to crustaceans occur immediately (few minutes up to 2 h) after the ingestion, the manipulation or inhalation of the cooking vapors (Fernandes et al. 2015; Jeebhay and Lopata 2012; Lee and Taylor 2011; Lopata and Jeebhay 2013). Delayed allergic reactions are also likely to occur since symptoms might be developed up to 8 hours (Wang and Sampson 2007). The clinical symptoms include gastrointestinal (nausea, vomiting, diarrhea and abdominal pain), respiratory (laryngospasm, wheezing, upper airway obstruction) and/or cutaneous (angioedema, urticaria, generalized pruritus) disorders. Other manifestations associated with oral allergy syndrome (OAS), such as palate itching, lip swelling and difficulty in swallowing, can also be experienced by crustacean allergic subjects. On a rare basis, severe and systemic responses, such as anaphylactic shocks can occur upon consumption of crustaceans (Fernandes et al. 2015; Khora 2016; Lopata, O'hehir, and Lehrer 2010; Vierk et al. 2007).

3. Molecular characterization of crustacean allergens

So far, some families of proteins such as tropomyosin (TM), arginine kinase (AK), sarcoplasmic calcium-binding protein (SCP), myosin light chain (MLC), troponin C and hemocyanin have been classified as crustacean allergens, although the most representative one corresponds to TM. Table 1 summarizes the crustacean allergens described to date (ALLERGEN 2017; ALLERGOME, 2017; Encyclopedia of Life 2017) and Figure 2 exemplifies the structures of TM, AK, SCP, MLC and troponin C.

3.1. Tropomyosin

In the early 1990s, a number of researchers elucidated the molecular identity of shrimp allergens. Shanti et al. (1993) reported the identification of a 34-kDa heat-stable protein containing 300 amino acid residues, which was designated as Pen i 1 (*Penaeus indicus*). This protein presented 86% of sequence homology with TM from the fruit fly (*Drosophila melanogaster*). Leung et al. (1994) expressed a recombinant TM from *Metapenaeus ensis* (Met e 1) with a molecular weight (MW) of 34 kDa and 281 amino acids, which shared a high similarity with the fruit fly TM. Daul et al. (1994) first reported another allergen (Pen a 1) in Northern brown shrimp (*Penaeus aztecus*), which exhibited an open reading frame of 312 amino acids with an MW of 36 kDa. Later on, Reese et al. (1997) also expressed this recombinant allergen. The assessment of the amino acid sequences of Pen i 1, Met e 1 and Pen a 1 revealed that they are similar allergens. All these molecules were further classified as major allergens in shrimp because they presented IgE-reactivity with more than 50% of the sera from allergic patients.

Table 2 lists the allergenic TM of shrimp, lobster, crab, prawn and other crustaceans. Tropomyosin is present in muscle, as well as in some non-muscle cells, and interferes with the regulatory progression of muscle contraction, together with actin and myosin (Leung et al. 2014; Wai et al. 2014). Biochemically, TM is a 34–38 kDa protein with an alpha-helical coiled-coil secondary protein structure. Their epitopes comprise various amino acids, namely glutamic acid, arginine, tyrosine, serine and phenylalanine (Leung et al. 2014). The IgE-binding epitopes of TM of various crustacean species differ and exhibit specific serological IgE-reactivity in sensitized individuals (Wai et al. 2014).

Tropomyosin is recognized as the major cross-reactive shellfish panallergen, mainly attributed to the high resemblance in amino acid sequences among various species. Similarly, there is a high structural homology among the crustacean and mollusk TM, accounting 93.8% and 77.2%, respectively. The contact with the crustacean or mollusk species through ingestion, or even by manipulation or inhalation, can lead to cross-reactivity in the majority of the shellfish-allergic individuals due to their high homology. About 75% of shellfish allergic individuals are at risk of cross-reacting to other species (Chu, Wong, and Leung 2000; Lee and Taylor 2011; Leung et al. 2014; Lv et al. 2017a; Reese, Ayuso, and Lehrer 1999; Tsabouri et al. 2012). Tropomyosin can be unfolded up to a limited extent during food processing because it is thermally stable and can regain its original structure upon

Table 1. List of identified and characterized crustacean allergens.

Biochemical names	MW (kDa)	Function	Heat Resistance	Allergens (Examples)	References
TM	34–38	Muscle contraction	Yes	Pen a 1 Hal-m-1	Reese et al. (1997)
AK	40	Metabolic role (regulation and transport)	No	Pen m 2 Lit v 2	Yu et al. (2003) García-Orozco et al. (2007)
SCP	20–22	Regulate muscle contraction	Yes	Pen m 4 Lit v 4	Shiomi et al. (2008) Mita et al. (2013)
MLC	20	Muscle contraction	Yes	Lit v 3 Cra c 5	Ayuso et al. (2008) Bauermeister et al. (2011)
Troponin C	21	Muscle contraction/relaxation		Hom a 6	Rahman et al. (2013)
Hemocyanin	75	Oxygen transport	Yes	Mac ro 2	Piboonpocanun et al. (2011)
Paramyosin	100	Muscle contraction	No		Suzuki et al. (2011)
MHC	225	Muscle contraction			Khanaruksombat et al. (2014)
Triose Phosphate Isomerase	28	Glycolysis	No	Cra c 8	Bauermeister et al. (2011)
α-actin	31–42	Muscle contraction	No		Rahman et al. (2011)
SERCA	113	Enzyme			Rahman et al. (2011)
GADPH	37	Enzyme			Rahman et al. (2013)

MW, molecular weight; TM, tropomyosin; AK, arginine kinase; SCP, sarcoplasmic calcium protein; MLC, myosin light chain; MHC, myosin heavy chain; SERCA, smooth endoplasmic reticulum Ca^{2+} ATPase; GADPH, glyceraldehyde-3-phosphate dehydrogenase.

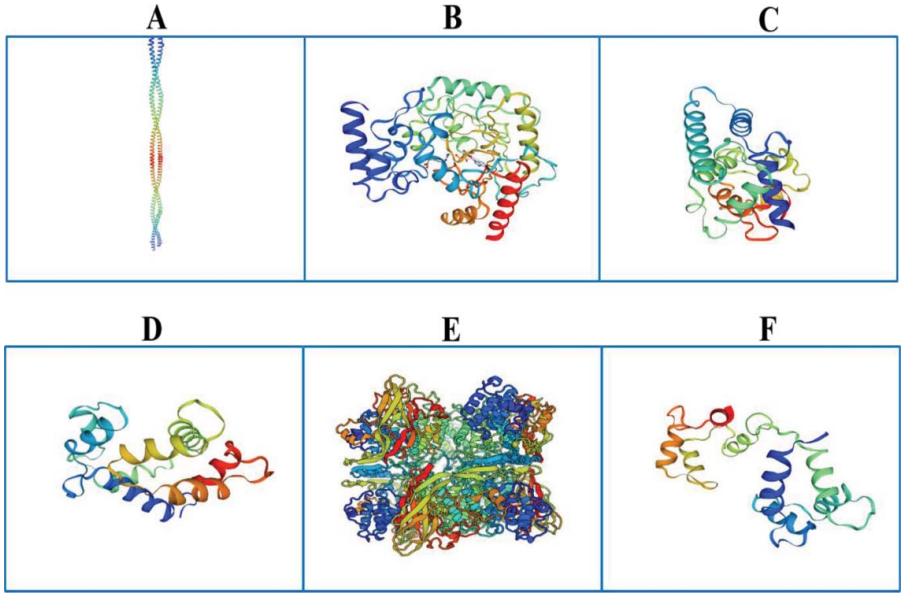

Figure 2. Secondary structure of crustacean allergens: A) tropomyosin of Northern red shrimp (*Pandalus borealis*) (UniProt accession number P86704); B) arginine kinase of Karuma prawn (*Marsupenaeus japonicas*) (UniProt accession number P51545); C) sarcoplasmic Ca^{2+}-binding protein of mud crab (*Scylla paramamosain*) (UniProt accession number I2DDG2); D) myosin light chain of giant tiger prawn (*Penaeus monodon*) (UniProt accession number E7CGC3); E) troponin C of brown shrimp (*Crangon crangon*) (UniProt accession number D7F1Q2); F) hemocyanin of white leg shrimp (*Litopenaeus vannamei*) (UniProt accession number Q26180). Retrieved from: http://www.uniprot.org/ (Accessed on 2017 December 6).

cooling. The presence of this allergenic protein in processed food, even at very low concentrations, can be the cause of severe immunological reactions in sensitized consumers (Faber et al. 2017; Kamath et al. 2014; Lasekan et al. 2017; Leung et al. 2014). However, various approaches can be employed to diminish the allergenic potential of TM, as discussed in below sections.

3.2. Arginine kinase

Arginine kinase has been identified in several crustacean species (Table 3). Yu et al. (2003) identified AK, Pen m 2, in black tiger shrimp (*Penaeus monodon*). It is a monomeric phosphagen ATP phosphor transferase, which is generally found in invertebrates (Yao et al. 2005). This enzyme is a 40-kDa water-soluble protein present in myosinogen and is the key to energy metabolism. It has been considered as an important allergen of crab, shrimp (Yu et al. 2003) and crayfish (Chen et al. 2013a), and is likely to be involved in cross-reactions between different species and to elicit hypersensitivity responses (Srinroch et al. 2015). As with TM, AK is an invertebrate panallergen, as it has been described, not only in crustaceans and mollusks, but also in moths (Binder et al. 2001), mites (Hales et al. 2007), silkworms (Liu et al. 2009), spiders (Bobolea et al. 2011) and cockroaches (Sookrung et al. 2006). AK was reported as an allergen in different crustacean species, being identified in Chinese shrimp or fleshy prawn (*Fenneropenaeus chinensis*) (Yao et al. 2005), Pacific white shrimp (*Litopenaeus vannamei*) (García-Orozco et al. 2007), black tiger prawn (*P. monodon*) and other shrimp species using proteomics (Khanaruksombat et al. 2014; Yu et al. 2003). AK is a thermo-labile allergen, though IgE-binding has been attributed to AK of thermally treated shrimps, which might be due to the presence of intact IgE epitopes on aggregated AK (Kamath et al. 2014; Shen et al. 2012). Thermal processing (between 40 and 80°C) partially unfolds and reveals novel hidden epitopes that may be the cause of IgE-reactivity. Processing above 80°C seems to decrease or even eliminate the IgE-binding capacity, probably as the result of the complete unfolding of AK (Chen et al. 2013a; Giuffrida et al. 2014).

3.3. Sarcoplasmic calcium-binding protein

The SCP contributes to the control of Ca^{2+} concentration in the cytosol and participates in several cellular functions. They are acidic and possess a structural EF-hand domain, with an MW of 20–22 kDa. Shrimp SCP is a dimmer of polypeptide chains ($\alpha\alpha$, $\alpha\beta$, and $\beta\beta$) with three calcium-binding sites. SCP is believed to promote muscular relaxation by translocating Ca^{2+} from myofibrils to the sarcoplasmic reticulum and is considered equivalent to the vertebrate parvalbumins, which plays a role in the maintenance of intracellular calcium (Gao, Gillen, and Wheatly 2006; Khanaruksombat et al. 2014). The SCP has a significant amino acid sequence homology (80–98% in crustaceans) among taxonomically similar species, while the homology between mollusks and crustaceans SCP is only 15–21% (Mita et al. 2013). The SCP identified as allergens in crustaceans are presented in Table 4.

SCP from Pacific white shrimp is known as Lit v 4 and has an MW of 22 kDa, an isoelectric point of 4.7 and 194 amino acids. The sensitization to SCP seems to participate in cross-reactions among crustaceans only and is the main allergenic culprit in pediatric population (Ayuso et al. 2009). Shiomi et al. (2008) found SCP in various species of crustaceans (*Penaeus japonicas, Homarus americanus, Pandalus eous, Paralithodes camtschaticus* and *Chionoecetes opilio*) during the process of purification of AK. SCP is thermo-stable and resistant to acidic and alkaline treatments (stable at a pH range of 1–11). However, after 1 h of peptic digestion, it is completely degraded. SCP is a polymorphic allergen with three isoforms (SCP-I, SCP-II, and SCP-III), all of which show IgE-binding capacity (Chen et al. 2013b).

Table 2. List of TM found in various crustacean species (ALLERGEN 2017; Encyclopedia of Life, 2017; ALLERGOME 2017).

TM	Common names	Scientific names	Allergens
Shrimp	Atlantic white shrimp	*Litopenaeus setiferus*	Lit se 1
	Greasy back shrimp	*Metapenaeus ensis*	Met e 1
	Pacific white shrimp	*Litopenaeus vannamei*	Lit v 1
	Sand shrimp	*Crangon crangon*	Cra c 1
	Skeleton shrimp	*Caprella equilibra*	Cap e 1
	Akiami paste shrimp	*Acetes japonicas*	Ace ja 1
	Shiba shrimp	*Metapenaeus joyneri*	Met j 1
	Broad velvet shrimp	*Metapenaeopsis lata*	Met la 1
	Japanese Mantis Shrimp	*Oratosquilla oratoria*	Ora o 1
	Northern shrimp	*Pandalus borealis*	Pan b 1
	Alaskan pink shrimp	*Pandalus eous*	Pan e 1
	Northern brown shrimp	*Penaeus aztecus*	Pen a
	Neptune rose shrimp	*Parapenaeus fissurus*	Par f 1
	Indian white shrimp	*Penaeus indicus*	Pen i 1
	China Red Shrimp	*Solenocera melantho*	Sol me 1
	Japanese Mantis Shrimp	*Squilla oratoria*	Squ o 1
	Southern rough shrimp	*Trachysalambria curvirostris*	Tra c 1
	Sakura shrimp	*Sergia lucens*	Ser lu 1
Crab	Giant mud crab	*Scylla serrata*	Scy s 1
	Orange mud crab	*Scylla olivacea*	Scy o 1
	Green mud crab	*Scylla paramamosain*	Scy pa 1
	Spanner crab	*Ranina ranina*	Ran ra 1
	Edible crab	*Cancer pagurus*	Can p 1
	Hair crab	*Erimacrus isenbeckii*	Eri i 1
	Crucifix crab	*Charybdis feriatus*	Cha f 1
	Chinese freshwater edible crab	*Eriocheir sinensis*	Eri s 1
	Snow crab	*Chionoecetes opilio*	Chi o 1
	Horseshoe crab	*Limulus polyphemus*	Lim p 1
	Japanese Freshwater Crab	*Geothelphusa dehaani*	Geo de 1
	Ocean Sand Crab	*Ovalipes australiensis*	Ova au 1
	Red king crab	*Paralithodes camtschaticus*	Par c 1
	Swimming crab	*Portunus trituberculatus*	Por tr 1
	Blue swimming crab	*Portunus pelagicus*	Por p 1
	Three spot swimming crab	*Portunus sanguinolentus*	Por s 1
Lobster	Green lobster	*Panulirus stimpsoni*	Pan s 1
	Scalloped spiny lobster	*Panulirus homarus*	Pan h 1
	Northern lobster	*Homarus americanus*	Hom a 1
	Cape rock lobster	*Jasus lalandii*	Jas la 1
	Cape spiny lobster	*Jasus edwardsii*	Jas ed 1
	European lobster	*Homarus gammarus*	Hom g 1
	Japanese lobster	*Metanephrops japonicus*	Met ja 1
	Flathead locust lobster	*Thenus orientalis*	The or 1
Prawn	Banana prawn	*Fenneropenaeus merguiensis*	Fen me 1
	Kuruma prawn	*Marsupenaeus japonicus*	Mar j 1
	Fleshy prawn	*Fenneropenaeus chinensis*	Fen c 1
	Red rice prawn	*Metapenaeopsis barbata*	Met ba 1
	Western king prawn	*Melicertus latisulcatus*	Mel l 1
	Giant freshwater prawn	*Macrobrachium rosenbergii*	Mac r 1
	Fleshy prawn	*Penaeus orientalis*	Pen o 1
	Tiger prawn	*Penaeus monodon*	Pen m 1
	Green tiger prawn	*Penaeus semisulcatus*	Pen se 1

3.4. Myosin light chain

Myosins are a superfamily of motor proteins that hydrolyze adenosine triphosphate to move actin filaments. Myosin has two heavy and four light chains (each with 20 kDa). The MLC plays a fundamental role in muscle contraction since its phosphorylation affects the actin-myosin complex during the contraction process (Ayuso et al. 2008). This allergen was firstly identified in Pacific white shrimp (*L. vannamei*) and, later on, identified in black tiger prawn (*P. monodon*) (Ayuso et al. 2008; Rahman et al. 2010). To date, some MLC have been identified as allergens in various species of crustaceans (Table 5). Similarly to TM, the MLC are thermally stable and tend to retain IgE-reactivity upon processing at 100°C for 5 min (Ayuso et al. 2008). Currently, data on the immunological cross-reactivity of MLC among crustaceans or other shellfish species are still lacking.

Table 3. List of AK found in various crustacean species (ALLERGEN 2017; Encyclopedia of Life, 2017; ALLERGOME 2017).

AK	Common names	Scientific names	Allergens
Shrimp	Northern shrimp	*Pandalus borealis*	Pan b 2
	Greasyback shrimp	*Metapenaeus ensis*	Met e 2
	Shiba shrimp	*Metapenaeus joyneri*	Met j 2
	Sand shrimp	*Crangon crangon*	Cra c 2
	Pacific white shrimp	*Litopenaeus vannamei*	Lit v 2
Crab	Giant mud crab	*Scylla serrata*	Scy s 2
	Orange mud crab	*Scylla olivacea*	Scy o 2
	Green mud crab	*Scylla paramamosain*	Scy pa 2
	Crucifix crab	*Charybdis feriatus*	Cha f 2
	Chinese freshwater edible crab	*Eriocheir sinensis*	Eri s 2
	Snow crab	*Chionoecetes opilio*	Chi o 2
	Blue crab	*Callinectes sapidus*	Cal s 2
	Green crab	*Carcinus maenas*	Car ma 2
	Swimming crab	*Portunus trituberculatus*	Por tr 2
	Blue swimming crab	*Portunus pelagicus*	Por p 2
	Dungeness crab	*Cancer magister*	Can mg 2
	Marbled Rock Crab	*Pachygrapsus marmoratus*	Pac ma 2
	Horseshoe crab	*Limulus Polyphemus*	Lim p 2
Lobster	European lobster	*Homarus gammarus*	Hom g 2
	Red-banded lobster	*Metanephrops thomsoni*	Met t 2
Prawn	Northern lobster	*Homarus americanus*	Hom a 2
	Tiger prawn	*Penaeus monodon*	Pen m 2
	Giant freshwater prawn	*Macrobrachium rosenbergii*	Mac r 2
	Kuruma prawn	*Marsupenaeus japonicas*	Mar j 2
	Banana prawn	*Fenneropenaeus merguiensis*	Fen me 2
	Fleshy prawn	*Fenneropenaeus chinensis*	Fen c 2

Table 4. List of SCP found in various crustacean species (ALLERGEN 2017; Encyclopedia of Life, 2017; ALLERGOME 2017).

SCP	Common names	Scientific names	Allergens
Shrimp	Northern brown shrimp	*Penaeus aztecus*	Pen a 4
	Kolobri shrimp	*Solenocera agassizii*	Sol ag 4
	Indian white shrimp	*Penaeus indicus*	Pen i 4
	Sand shrimp	*Crangon crangon*	Cra c 4
	Pacific white shrimp	*Litopenaeus vannamei*	Lit v 4
	Northern shrimp	*Pandalus borealis*	Pan b 4
	Japanese Mantis Shrimp	*Oratosquilla oratoria*	Ora o 4
	Deep-water rose shrimp	*Parapenaeus longirostris*	Par lo 4
	Alaskan pink shrimp	*Pandalus eous*	Pan e 4
	Greasyback shrimp	*Metapenaeus ensis*	Met e 4
	Southern pink shrimp	*Farfantepenaeus notialis*	Far no 4
	Pink shrimp	*Farfantepenaeus brevirostris*	Far be 4
Crab	Green mud crab	*Scylla paramamosain*	Scy pa 4
	Blue swimming crab	*Portunus pelagicus*	Por p 4
	Chinese freshwater crab	*Eriocheir sinensis*	Eri s 4
	Crucifix crab	*Charybdis feriatus*	Cha f 4
	Snow crab	*Chionoecetes opilio*	Chi o 4
Lobster	Northern lobster	*Homarus americanus*	Hom a 4
Prawn	Tiger prawn	*Penaeus monodon*	Pen m 4
	Green tiger prawn	*Penaeus semisulcatus*	Pen se 4
	Giant freshwater prawn	*Macrobrachium rosenbergii*	Mac r 4
	Kuruma prawn	*Marsupenaeus japonicas*	Mar j 4
	Banana prawn	*Fenneropenaeus merguiensis*	Fen me 4
	Western king prawn	*Melicertus latisulcatus*	Mel l 4

Table 5. List of MLC and troponin C found in various crustacean species (ALLERGEN 2017; ALLERGOME 2017).

Classification	Common names	Scientific names	Allergens
MLC	Northern shrimp	*Pandalus borealis*	Pan b 3
	Pacific white shrimp	*Litopenaeus vannamei*	Lit v 3
	Sand shrimp	*Crangon crangon*	Cra c 5
	Northern lobster	*Homarus americanus*	Hom a 3
	Tiger prawn	*Penaeus monodon*	Pen m 3
Troponin C	Sand shrimp	*Crangon crangon*	Cra c 6
	Northern shrimp	*Pandalus borealis*	Pan b 6
	Snow crab	*Chionoecetes opilio*	Chi o 6
	Northern lobster	*Homarus americanus*	Hom a 6
	Tiger prawn	*Penaeus monodon*	Pen m 6

3.5. Troponin C

Bauermeister et al. (2011) described troponin C as an allergen for the first time in *Crangon crangon* (North Sea shrimp), termed as Cra c 6, which was recognized in 29% of the patients' sera. Later on, Rahman et al. (2013) found this allergen in *Pandalus borealis*, being IgE-reactive in 33% of the allergic patients. Troponin is composed of three subunits: (i) troponin C (binds to Ca^{2+}), (ii) troponin T (binds to TM), and (iii) troponin I, which binds to actin and hinders actin-myosin interaction (Pedrosa et al. 2015). Similarly, to SCP and MLC, troponin C is an EF-hand calcium binding protein. Its MW is approximately 20 kDa and its heat stability is not well understood. Troponin C allergens observed in various species of crustaceans are listed in Table 5. The IgE-binding of troponin C is 15% lower as compared to TM, SCP or AK (Lopata, Kleine-Tebbe, and Kamath 2016). Recently, Pascal et al. (2015) observed 17.2% sensitization to troponin C in 58 shrimp-allergic patients. Kalyanasundaram and Santiago (2015) used recombinant *P. monodon* (Indian black tiger shrimp) troponin C to effectively improve the diagnosis and treatment of seafood allergy. The recombinant allergen was evaluated with sera collected from allergic patients, revealing immunoreactivity in 8 patients out of 35, thus confirming the allergenic potential of troponin C.

3.6. Hemocyanin

Hemocyanin is another heat stable crustacean allergen, which is isolated from hemolymph. It shows cross-reactivity with snail and house dust mite (Faber et al. 2017; Khanaruksombat et al. 2014; Lu et al. 2015; Piboonpocanun et al. 2011; Srinroch et al. 2015). Hemocyanin is an oxygen-transport protein that accounts for 75–95% of the total protein content. It forms hexamers or multi-hexamers with 75-kDa subunits in its natural state and the number of subunits is species-specific (Hodgson and Spicer 2001). Piboonpocanun et al. (2011) obtained peptide bands (72 and 75 kDa) via SDS-PAGE from giant fresh water shrimp (*Macrobrachium rosenbergii*), being characterized as hemocyanin that showed 62.5% to 100% of sequence homology with different crustaceans. They further demonstrated that hemocyanin sensitization could cause selective allergy to *M. rosenbergii* in patients tolerating a black tiger shrimp (*P. monodon*) allergen. These selective allergies might possibly be due to the lower amino acid sequence homology (18.8–27.3%) between these shrimp species that belong to the Caridae and Penaeidae families, respectively. The IgE-binding was detected in both raw and boiled hemolymph extracts, exhibiting the thermal stability of this allergen.

3.7. Other allergens

Several other potential allergens have been identified in crustaceans. Paramyosin has a size of 100 kDa, being recognized as a thermo-labile allergen in various shellfish species. It is an invertebrate-specific protein that forms the core of filaments and shows cross-reactivity with TM (Suzuki et al. 2011). Triosephosphate isomerase (Cra c 8) is a minor allergen in crustaceans since it was reactive with 23% of patients' sera. Its approximate size is 28 kDa and is probably heat sensitive (Bauermeister et al. 2011; Kamath et al. 2014). However, its immunological cross-reactivity among various invertebrate species is not fully understood. Few other allergens identified in crustaceans are myosin heavy chain (Khanaruksombat et al. 2014; Rahman et al. 2013), α-actin (Rahman et al. 2011), smooth endoplasmic reticulum Ca^{2+} ATPase (SERCA) (Rahman et al. 2011) and glyceraldehyde-3-phosphate dehydrogenase (GADPH) (Kamath et al. 2014; Khanaruksombat et al. 2014). However, further research on their molecular characterization and allergenic potential is needed.

4. Effect of processing technologies on crustacean allergenicity

The immunoreactivity of most proteins depends on the retention of their native structural epitopes and on their recognition by IgG or IgE (Verhoeckx et al. 2015). Thermal and non-thermal processing technologies may alter physicochemical properties of foods in a manner that may cause denaturation, aggregation, crosslinking, oxidation, glycation and glycosylation, thereby masking or unmasking of allergenic epitopes. Consequently, processing technologies might potentially alter the sensitizing capacity of the offending foods. The protein region that causes an allergic reaction may be a simple stretch of a few amino acids along the primary structure or it may be a unique three-dimensional motif of the protein structure, referred to as linear or conformational epitopes, respectively. Clearly, these processing-related structural and chemical changes will have the potential to influence the allergenicity of proteins as reflected by their tendency to bind to specific IgE. Several attempts have been made to understand how different methods of processing affect the immunoreactivity of crustacean allergens (Ahmed et al. 2018; Liu et al. 2017a; Lv et al. 2017a; Lv et al. 2017b; Nayak et al. 2017; Vanga, Singh, and Raghavan 2017; Vanga and Raghavan 2016; Verhoeckx et al. 2015; Zhang, Deng, and Zhao 2017). The influence of processing technologies on the immunoreactivity of crustacean allergens, as well as their advantages and limitations are summarized in Table 6.

4.1. Thermal treatment

Food products are thermally processed in order to minimize the microbiological hazards and improve their quality and shelf life stability. Thermal treatments (i.e. cooking, boiling, roasting, baking, frying) of food proteins induce modifications including

Table 6. Summary of different processing technologies for mitigating crustacean allergenicity.

Processing method	Mechanism	Effect on allergenicity	Advantages	Limitations	References
Heat treatments	• Unfolding and aggregation of allergenic proteins. • Modification of protein by hydrolysis of peptide bonds and Maillard adducts in sugar-rich foods upon heating. • Changes in the secondary and tertiary structure of allergens due to heat treatment i.e., high pressure steaming and boiling.	Increase/ decrease or without any obvious effect.	• The process is safe and easy. • No chemicals are added. • Reduce allergenicity of heat labile allergens to a great extent. • Can be used for both purified crustacean allergens and protein extracts.	• The reduction of allergenicity is very limited. • Heat stable protein such as TM retains allergenicity even after heat treatment. • The process can sometime generate neo-allergens by the creation of new epitopes or exposure of the existing inaccessible epitopes.	Abramovitch et al. (2017); Kamath et al. (2013, 2014); Lasekan and Nayak (2016); Liu et al. (2010); Mejrhit et al. (2017); Shriver et al. (2011); Sockalingam, Misnan, and Yadzir (2017); Yu et al. (2011)
Maillard reaction	• Denaturation and unfolding of proteins by formation of Maillard reaction products due to covalent bonding between amino group and carbonyl group upon heating. • Formation of insoluble aggregates to form networks.	Decrease	• The process is safe and has potential for mitigating food allergenicity. • Can be used for purified crustacean allergens as well as protein extracts.	• Difficulty in controlling the reaction, long-time reaction, and potential formation of some undesired products. • The process cannot entirely abolish crustacean allergenicity.	Gupta et al. (2016); Teodorowicz, van Neerven, and Savelkoul (2017); Yuan et al. (2017);
Enzymatic crosslinking	• Proteins can become crosslinked with other proteins or polysaccharides in the presence of enzymes. • Crosslinking lead to the modification of the conformational structure of allergens.	Decrease	• The process is simple and is carried out under mild conditions. • Used for development of low allergenic crustacean products. • No toxic chemicals are introduced during the process and the enzymes used are food grade.	• Efficiency is not very high, so cannot entirely abolish the allergenicity of crustacean allergens.	Ahmed et al. (2018); Fei et al. (2016); Liu et al. (2017b); Yuan et al. (2017)
Enzymatic hydrolysis	• Proteases partially or severely hydrolyze the allergenic protein into small peptide fragments and/ or amino acids that reduce the number of IgG/IgE epitopes. • Hydrolysis lead to the disruption of conformational and linear epitopes	Decrease	• High efficacy in reducing IgE-binding capacity. • Actin and myosin heavy chain degraded quickly during protease digestion. • The process is simple and safe, since no chemicals are used. • Enzymes used are food grade.	• May produce bitter peptides, which can affect the taste of the product. • TM showed resistance against pepsin and trypsin/chymotrypsin digestions, while actin were resistant only to trypsin/ chymotrypsin digestion.	Huang et al. (2010); Liu et al. (2011); Shimakura et al. (2005)

(*Continued on next page*)

Table 6. (*Continued*)

Processing method	Mechanism	Effect on allergenicity	Advantages	Limitations	References
Acid treatment	• Low pH induce protein denaturation by altering the conformational structure.	Decrease	• Acid condition show high efficiency in reducing the allergenicity of crustaceans.	• Acidic condition might not be effective in reducing the allergenic potential of proteins, if most epitopes are linear.	Lasekan et al. (2017);
				• Decrease the solubility of allergenic protein in extraction solution, thereby influencing their detection and accurate quantification.	Lin et al. (2015);
					Perez-Macalalag, Sumpaico, and Agbayani (2007)
HPP	• HPP causes reversible or irreversible structural modifications in proteins, leading to their denaturation and aggregation.	Decrease or no effect	• No chemicals are added.	• High-pressure equipment is needed.	Hu and Xie (2013);
			• Shown potential in mitigating the allergenicity of crustaceans. • Assists in allergen extraction and enzymatic hydrolysis.	• The efficiency to reduce crustacean allergenicity is not very high.	Yohannes et al. (2008)
High-intensity ultrasound	• High-intensity ultrasound modify food proteins by inducing mechanical, physical and chemical/ biochemical changes through cavitation phenomenon. • Induce conformational changes in native proteins, cleaving peptide bonds near the collapsed cavities and increasing the polymerization/ depolymerization	Decrease	• No chemicals are used and the process is carried out without any thermal treatment.	• The technology needs to be rigorously tested and proved to be safe, while being commercially viable.	Li et al. (2006);
			• Substantially reduces the allergenicity with potential application to prepare hypoallergenic crustacean products	• Still, the optimization and application of this technique for effective reduction of allergenicity is not fully understood.	Li et al. (2011)
			• Product degradation is reduced.	• The process is complex and equipment is expensive.	
Irradiation	• Exposing protein to irradiation lead to fragmentation, aggregation, crosslinking, oxidation and amino acid modification.	Decrease	• No chemicals are used.	• Expensive equipment is needed and the operation of the process requires skillful personnel.	Byun et al. (2000);
			• It is regarded as cold pasteurization technique.	• Treatment with low doses of radiation increases the IgE-binding capacity of shrimp extracts and TM	Li. et al. (2007);
			• Shows enough potential for mitigating crustacean allergenicity. • Products are safe for consumption without any toxicological, nutritional and microbiological hazards	• Most consumers are reluctant to consume irradiated food products.	Liu et al. (2017)

PUV light	• The process involves discharge of high voltage electric pulses into the food product, which produces photochemical and photothermic effects. • These effects lead to aggregation, unfolding and fragmentation of allergenic proteins by oxidative reactions, as well as, creation and cleavage of hydrophobic and hydrogen bonds.	Decrease	• Fast process (4–5 min). • Potential use for mitigating allergenicity in different forms of allergenic protein. • No chemicals are used.	• Expensive equipment is required. • Prolonged UV-light radiation may cause unsaturated fatty acid peroxidation and generate harmful free radicals. • Reduce solubility of allergens.	Shriver et al. (2011); Yang et al. (2012)

hydrolysis of peptide bonds, aggregation by disulfide and non-covalent bonds, denaturation and reactions with other food components, such as carbohydrates and lipids. In consequence of all these reactions, the allergenic proteins will be substantially influenced, either decreasing (loss of conformational epitopes) or increasing (generation of new epitopes or exposure of existing ones) the allergenicity (Jiménez-Saiz et al. 2015; Mejrhit et al. 2017; Rahaman, Vasiljevic, and Ramchandran 2015). Figure 3 shows the heat-induced conformational changes and their impact on the allergenic potential of food allergens.

Heat treatment appears to exert a low impact on crustacean allergens because most of them are thermostable. It is worth noticing that all the studies came to an understanding that TM retains a great allergenicity even after heat treatment (Lasekan and Nayak 2016; Liu et al. 2010; Lopata, O'hehir, and Lehrer 2010). Motoyama et al. (2007) observed 90% homology in the TM sequences of six crustacean species (pink shrimp, kuruma prawn, black tiger prawn, snow crab, horsehair crab and king crab) and confirmed their allergenicity by immunoblotting. Despite the high consumption of crustaceans as grilled or boiled, the allergic individuals still suffer from adverse immunological reactions. This is mainly attributed to the high immunoreactivity of TM, even after high-temperature treatment and after being digested by trypsin or chymotrypsin (Yu et al. 2011). Nonetheless, the analysis by circular dichroism revealed that the α-helical structure of TM (Pen j 1) collapsed upon heating at 80°C. Additionally, there was no evidence of insoluble aggregate formation after heating and the protein regained its original conformation when cooled to 25°C. A small change in the total IgG production was observed between mice sensitized with untreated and heated Pen j 1, thus maintaining its antigenicity (Usui et al. 2013).

Lasekan and Nayak (2016) observed that TM bands were more intense in different heat-treated shrimp extracts, while the pattern of proteins appeared to be reduced in case of high-pressure steaming, as obtained by SDS-PAGE analysis. The IgE-binding potential of shrimp TM extracts after being heat processed was comparable to that of untreated sample, while the extracts of high pressure steaming exhibited reduced IgE-reactivity. The increase of TM band intensities might be due to the unfolding of these proteins upon heat treatment. Several studies have confirmed the stability of TM during heating. Shriver et al. (2011) stated that boiling of shrimp extract for 4 min did not influence the band intensity of TM. Kamath et al. (2013) reported complex protein bands between 18–20 kDa and 35–40 kDa, assessing the patterns of 11 raw and boiled (10 min) crustaceans. The heated crustacean extracts evidenced stronger monoclonal antibody binding in the range of 30–39 kDa when compared to raw extracts. Additionally, the heated extracts presented immunoreactivity to multiple bands of similar MW (most likely TM) (Kamath et al. 2013). Likewise, Sockalingam, Misnan, and Yadzir (2017) observed prominent heat stable bands in the range of 32 to 38 kDa in all the extracts of cooked (boiled, steamed and fried) giant river prawn, assuming to be TM. Other relevant bands (17 to 20 kDa) were noticed in all treated extracts, while protein bands between 24 to 27 kDa were observed only in boiled and steamed prawn extracts. With these MW, the identified allergens most likely correspond to SCP, MLC and troponin C, respectively. The heat-sensitive proteins with different MW were not detected in thermally processed prawn extracts (Sockalingam, Misnan, and Yadzir 2017). The reduction in band intensity, in case of high pressure steamed samples, is also in accordance with other studies. Similarly, Yu et al. (2011) observed reduced band intensity in crab (*Scylla paramamosain*) TM samples heated at high pressure (0.14 MPa at 121°C for 20 min), although with a minor decrease in their allergenicity. This might be due to the denaturation of TM under high pressure steaming, while other heat processing methods did not significantly alter the IgE-binding epitopes of crustacean TM. It is worth noticing that high pressure steaming significantly mitigate the degree of antigen-antibody binding required to cause an allergic response,

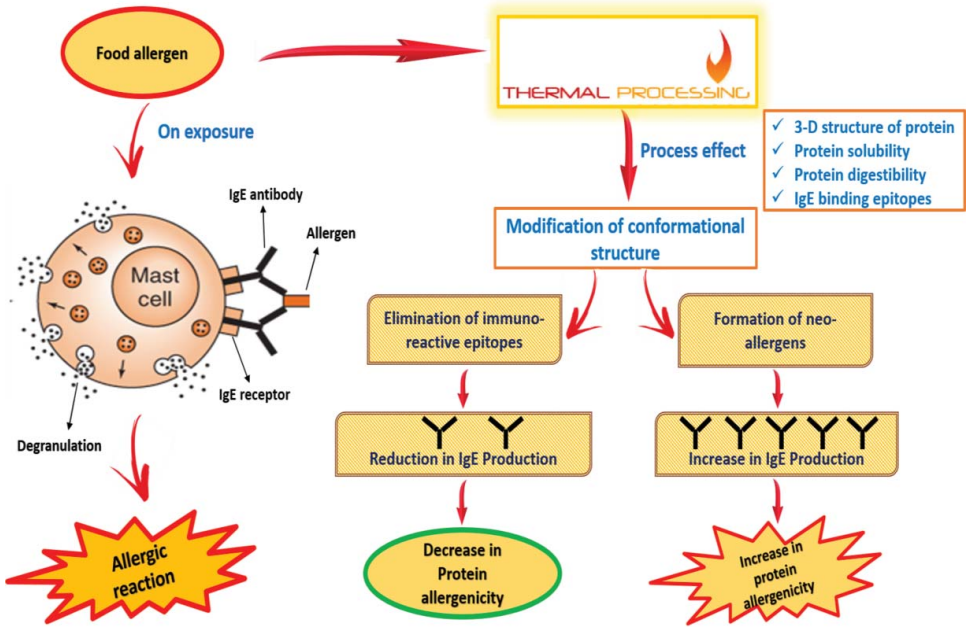

Figure 3. General heat induced conformational changes and their consequences on allergenicity of food protein.

though it does not completely diminish the immunoreactivity of all the allergens (Yu et al. 2011). Thus, the potency of thermal treatment for modulating the immunoreactivity of food allergens is mainly dependent on the processing types and conditions (temperature and time), as well as on the properties of allergenic proteins.

Recently, Abramovitch et al. (2017) investigated the effect of heat processing on the cellular immunoreactivity of crustacean extracts (banana prawn, black tiger prawn, blue swimmer crab and mud crab). Thermal processing did not modify the overall T-cell proliferative or cytokine reactivity of crustacean extracts, but it mitigated the induction of regulatory T-cells. In opposition, the IgE-reactivity of cooked extracts was significantly increased, probably due to TM (38–39 kDa) and its aggregates (78 and 102 kDa). Mejrhit et al. (2017) evaluated the IgE-sensitization to shrimp TM in Moroccan population from Fez region and then studied the influence of heat treatment on the allergenicity of TM. ELISA and dot-blot analyses indicated that TM showed a reduction in IgE-binding upon thermal treatment. The authors suggested that most epitopes recognized by Moroccans' IgE were conformational, being altered by heating and enzymatic digestion.

4.2. Maillard reaction

The Maillard reaction (glycation) is one of the well-known non-enzymatic interactions between proteins and sugars, which ensues in food following thermal processing or after long-term storage. In this process, Schiff bases are formed due to covalent bonding between the carbonyl groups of a reducing sugar and the free amine groups of amino acids (typically arginine and lysine) (Henle 2005; Jiménez-Saiz et al. 2015; Teodorowicz, van Neerven, and Savelkoul 2017). The mechanism of Maillard reaction consists of a series of chemical rearrangements (condensation, hydration and oxidation), resulting in the Maillard reaction products. The Schiff bases are followed by their Amadori rearrangements and subsequent glycoxidation (oxidative modification), causing the formation of advanced glycation end-products, such as Nε-(carboxymethyl-)lysine, methylglyoxal-H1, pyrraline and pentosidine (Gupta et al. 2016; Teodorowicz, van Neerven, and Savelkoul 2017).

The types and levels of Maillard reaction products formed are dependent on various factors, including the structural diversity of proteins and (poly)saccharides, the ratio of amine groups and reducing sugars, temperature and time, water activity and pH (Heilmann et al. 2014; Henle 2005). These by-products cause changes in the functional properties, bioavailability, digestibility, immunogenicity and, subsequently, in the allergenicity of food products. Amino acid and sugar interaction lead to the modification of the tertiary structure of the proteins, altering their conformational epitopes. Moreover, the formation of high MW and insoluble aggregates help masking conformational and linear epitopes, thereby mitigating the potential allergenicity of glycated proteins (Yuan et al. 2017; Apostolovic et al. 2016; Gupta et al. 2016; Iwan et al. 2011; Jiménez-Saiz et al. 2015; Teodorowicz, van Neerven, and Savelkoul 2017).

So far, some studies have been developed to address the effects of glycation on the allergenicity of TM from mollusks. Recently, Yuan et al. (2017) reported alteration in the secondary and tertiary structures of shrimp TM following glucosamine-catalyzed glycation, which subsequently reduced their IgG/IgE binding potentials. The change in allergenicity of Japanese scallops (*Patiopecten yessoensis)* following Maillard reactions was studied by Nakamura et al. (2005). In this study, an increase in IgE-binding ability was found for TM in the early stages of the reaction with glucose, ribose and maltose, but not with maltotriose. The researchers concluded that the loss of positive charges on the surface of the protein was not responsible for the increased allergenicity, but rather the structural changes resulting from glycation. Maillard reaction might also lead to the formation of neoantigens, as it generates sugar conjugated protein derivatives that, subsequently, enhance the allergenicity of protein. The neo-antigens may provoke the immunoreactivity in the same way as allergens (Davis, Smales, and James 2001). Conversely, IgE-binding ability was reduced as the reaction progressed and persisted, despite the peptic digestion in the case of squid TM (*Todarodes pacificus*) (Nakamura et al., 2006). Since the amino acid sequence homology between scallop and squid TM is high (69.7%), this finding might be explained due to differences in their epitope sites. The effects of the Maillard reaction appear to be dependent on the sample and the amount and type of reducing sugars, as well as many other variables, such as treatment temperature and time.

4.3. Enzymatic treatment

Enzymatic treatment has been considered an adequate option to reduce food allergenicity. Unlike other treatments, the hydrolysis of allergenic proteins using food grade proteases is regarded as a mild and safe method to destroy food allergens. Enzymatic methods are generally of two types: one is the crosslinking of food proteins to hide IgE-reactive epitopes and the second involves the proteolytic hydrolysis of food allergens by breaking down proteins into small fragments/peptides to decrease allergenicity (Yu 2016). The crosslinking can result from direct enzymatic catalysis of crosslink formation or from the indirect enzymatic production of a crosslinking agent, such as hydrogen peroxide, which oxidizes reactive structures with consequent crosslink formation. Food proteins contain several reactive groups for enzymatic crosslinking, such as lysine, tyrosine, glutamine and cysteine residues. The ultimate reactions are achieved depending on the enzyme used, the availability of the target reactive groups and the applied process conditions (Buchert et al. 2010; Yu 2016). Currently, food industry employs transglutaminases, peroxidases, laccases and tyrosinases for enzymatic crosslinking of dietary proteins. Crosslinking alters the protein molecular weight, the conformational structure, the charge moiety, the surface properties of the molecules and the biological properties of food allergens, including ligand binding, solubility, IgE-binding, antigenicity and susceptibility to proteolysis by digestive enzymes (Fei et al. 2016; Radosavljevic et al. 2014: Stanić-Vučinić and Veličković 2012; Yuan et al. 2017). Fei et al. (2016) investigated the influence of tyrosinase crosslinking on thermally polymerized crab (*Scylla paramamosain*) AK. The IgE-binding activity and digestive stability were analyzed by proteomics and by immunological methods, while sensitization and potency to cause oral

tolerance was evaluated via *in vivo* assays and a cell model. The ELISA results showed that the inhibitory concentration of crosslinked AK changed from 1.13 μg/mL to 228.36 μg/mL, while the *in vitro* digestion assay showed that thermal polymerized AK is relatively resistant to gastrointestinal digestion compared to native AK. The mice model studies demonstrated low allergenicity and capability to induce oral tolerance, as observed by the sera levels of AK specific antibodies and T-cell cytokine production. Moreover, the exposure of RBL-2H3 cells to crosslinked AK resulted in lower mast cell degranulation and histamine release compared to untreated AK (Fei et al. 2016). Liu et al. (2017b) studied the effect of enzymatic crosslinking on the IgG/IgE-binding activity, digestibility and oral tolerance of crosslinked crab TM with horseradish peroxidase or tyrosinase. ELISA results demonstrated that horseradish peroxidase and tyrosinase mitigated its IgE-binding activity by 63.5% and 34.5%, respectively. Compared with native TM or crosslinked TM with tyrosinase, the crosslinked TM with horseradish peroxidase was more easily digested into small fragments, decreasing the degranulation of RBL-2H3 cells and increasing the endocytosis by dendritic cells. These findings indicate that enzymatic treatment has more potential to reduce the allergenicity by influencing the structure of the protein, modifying the original way of antigen presentation and T_H1/T_H2 immunobalance, as well as inducing the oral tolerance of the allergen TM. The enzymatic crosslinking of allergens creates high MW compounds that may alter their sensitization potential (Liu et al. 2017b). Yuan et al. (2017) evaluated the effect of transglutaminase-catalyzed glycosylation on the allergenic potency and conformational structure of shrimp (*M. ensis*) TM, demonstrating that its allergenicity decreased and it was well correlated with the structural changes. The authors suggested that transglutaminase-catalyzed glycosylation has the potential to serve as a mild approach for mitigating the allergenicity of shrimp products. Similarly, Ahmed et al. (2018) studied the influence of tyrosinase-catalyzed crosslinking on the IgE-binding potential and structure of shrimp (*M. ensis*) TM. Their results revealed the unfolding of the tertiary structure and loss of the secondary structure following protein crosslinking. Western blotting and indirect ELISA revealed that the antigenicity and potential allergenicity of crosslinked shrimp TM decreased, which were attributed to the alteration of molecular and immunological properties of the crustacean proteins.

Enzymatic hydrolysis via proteolytic enzymes is also an effective way to modify the immunoreactivity of allergenic proteins from various food sources, as these are very resistant to gastrointestinal digestion. This approach resulted in the degradation of linear epitopes by the partial or complete hydrolysis of allergens into small peptide fragments and/or amino acids. Hydrolysis leads to the disrupture of conformational and linear epitopes (Kasera et al. 2015; Liu et al. 2011; Yu 2016). Shimakura et al. (2005) showed that crustacean extracts (manufactured by boiling raw crustaceans) used in the food industry are allergenic since they have TM and/or its degraded peptide fragments with IgE-binding capability. However, after digestion with proteases, the extracts seemed to have lost almost completely their allergenicity. Therefore, the authors highlight the effectiveness of protease digestion to reduce the allergenicity of TM of heat-treated crustaceans. Huang et al. (2010) studied the digestive stability of TM and other proteins from mud crab in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) digestion systems. As observed by SDS-PAGE and Western blotting, actin and myosin heavy chain were degraded rapidly, while TM showed resistance to pepsin digestion. In the case of SIF system, the myosin heavy chain was easily degraded, while TM and actin were similarly resistant to digestion. The results evidenced that the allergenicity of TM was partially decreased, suggesting that proteolytic digestion is effective in reducing its immunoreactivity. In a similar work, Liu et al. (2011) demonstrated the proteolytic stability of crustacean proteins from Pacific white shrimp (*L. vannamei*) and grass prawn (*P. monodon*) using pepsin, trypsin and chymotrypsin. Both myosin heavy chain and actin were rapidly degraded within a short period, while TM showed resistance against pepsin digestion. In the SIF system, myosin heavy chain was easily decomposed, and actin and TM were resistant to trypsin/chymotrypsin digestion, being in good agreement with Huang et al. (2010). From the above discussion, it is concluded that the modifications of the allergenic proteins using both enzyme-catalyzed protein crosslinking and proteolytic hydrolysis offer mild and safe approaches to mitigate the allergenicity of crustacean allergens.

4.4. Acid treatment

The available data on the modulation of the allergenic activity of crustacean allergens after exposure to acidic conditions are scanty. Perez-Macalalag, Sumpaico, and Agbayani (2007) observed that the protein extracts obtained from boiled shrimp pre-soaked in vinegar (up to 8 h) revealed low immunoreactivity, as evidenced by the small wheal size during the skin prick test. However, the researchers did not elucidate the mechanism by which acid soaking led to the attenuation of the allergenic potential of shrimp TM. Lin et al. (2015) studied the influence of different pH (1.0–11) on the conformational structure and allergenicity of short-neck clam (*Ruditapes philippinarum*) TM. The low pH range (1–5) caused significant modulation of the secondary and tertiary structure of TM compared to the alkaline conditions (pH 8.0–11). The alteration in the secondary structure of TM was characterized by circular dichroism spectroscopy, which revealed an increasing trend in the positive molar residue ellipticity peak when the pH was shifted to 1.0, thereby indicating the rise in the number of α-helices. On the other hand, when the pH was shifted to 2.0, the negative molar residue ellipticity peak increased and the positive one reduced, which showed the number of β-sheets increased, while the number of α-helices decreased. An unfolded structure was observed between a pH of 2.0–5.0, followed by a considerable alteration in the secondary structure at pH 1.0. Likewise, a red shift in the absorbance peaks of UV spectra was observed when the pH was shifted from acidic to neutral, as conformational changes in protein can also be assessed by variations in UV absorption spectra. Correspondingly, the potential allergenicity of short-neck clam TM was reduced as observed by indirect ELISA and dot-blot assay, which correlated well with the structural changes. The authors highlighted that conformational changes in TM induced by pH shifting considerably affected the allergenicity of TM, mainly in the acidic pH range (Lin et al. 2015).

Recently, Lasekan et al. (2017) elucidated the allergenic potential of shrimp TM in acidic conditions. The effect of acid-induced denaturation on TM immunoreactivity was assessed using different marination processes with white vinegar adjusted to diverse pH (1.0, 2.5, 3.5 and 4.8). The soluble myofibrillar protein extract was substantially reduced among shrimps marinated in vinegar below pH 3.5 and a considerable amount of TM was retained in the insoluble pellets. In consequence, the IgE-binding potential of TM was reduced significantly in the soluble shrimp protein extract, compared to samples marinated at pH 4.8 and control. On the other hand, TM in the insoluble shrimp pellets showed greater IgE-binding capacity in all marinating conditions. Therefore, the acidic conditions seem to modulate the allergenicity of proteins via modification of the conformational epitopes. However, an acidic condition might not be operational in mitigating the allergenic potential of food protein in case of the presence of abundant linear epitopes (Lasekan et al. 2017).

5. Novel approaches for mitigating crustacean allergenicity

Novel processing technologies are adapted in response to the consumer choices towards minimally processed, eco-friendly, safe and quality food products. Although the thermal treatment can alter the allergenic potential of many foods, it can also adversely affect the nutritional and the organoleptic properties of foods. Therefore, novel approaches (high-pressure processing, high intensity ultrasound, gamma irradiation and pulsed ultraviolet light) have ascertained as being safe alternatives to the conventional thermal and chemical processes for attenuating the allergenicity of various foods, along with retaining their properties (Kramer, Wunderlich, and Muranyi 2016; Liu et al. 2017a; Wang et al. 2017; Zhang, Deng, and Zhao 2017).

5.1. High-pressure processing

HPP (also known as high hydrostatic pressure processing or ultrahigh pressure processing) is a novel processing technology that involves the use of pressures in the range of 100–800 MPa to inactivate pathogenic organisms and to extend the shelf life of foods. HPP at ambient or chilled temperature has a minor impact on product chemistry, thereby protecting a variety of bioactive compounds (Balasubramaniam, Martínez-Monteagudo, and Gupta 2015; Pottier, Villamonte, and de Lamballerie 2017). It causes reversible or irreversible structural modifications in proteins, leading to their denaturation, aggregation, or gelatinization. Compared with the conventional thermal processing, the original color, flavor, and nutrients of foods can be preserved, while leading to a limited reduction in allergenicity (Ahmed, Qazi, and Jamal 2016; Huang et al. 2014; Jin et al. 2015; Kaur, Rao, and Nema 2016; Zhang, Deng, and Zhao 2017). HPP is characterized by three processing parameters: temperature, pressure, and exposure time, thus allowing great flexibility in the design of the process (Heinz and Buckow 2010; Kaur, Rao, and Nema 2016). Generally, the pressure is transmitted instantaneously and uniformly throughout the food system, independently of the size and geometry of the food product, unlike heat processing (Balasubramaniam, Martínez-Monteagudo, and Gupta 2015).

In recent years, the impact of HPP on the allergenic potential of foods has been explored. The possible mechanisms involve protein denaturation, conformational alterations, allergen extraction, enzymatic hydrolysis facilitation, which subsequently modulate the allergenicity of food proteins. Furthermore, HPP has contributed for mitigating the allergenicity of some foods without eliminating the allergenic proteins themselves. Through the extraction or release of membrane-bound allergens into the environment, these molecules can be removed or destroyed by hydrolytic enzymes. In some cases, HPP can accelerate the activity of some enzymes, reducing the allergenic potential of certain foods (Hu and Xie 2013; Júnior, Tribst, and Cristianini 2017; Knorr, Heinz, and Buckow 2006). The application of HPP (600 MPa) to king prawns and black tiger prawns at a temperature of 30 °C for 10 min resulted in a significant loss of TM immunoreactivity (Yohannes et al. 2008). Hu and Xie (2013) applied HPP (450 MPa, 40°C, 55 min) combined with enzymatic hydrolysis to prepare hypoallergenic shrimp product. The processing promoted the diffusion of proteases into the internal structure of shrimp and attenuated its immunoreactivity due to the enzymatic hydrolysis. This technology has also been applied to process other allergenic foods, namely mollusks and fish (Jin et al. 2015; Liu and Xue 2010). In both cases, HPP seemed to induce structural changes in TM and to facilitate their digestibility, thus contributing to a reduction in their allergenicity. Based on the available literature, it can be considered that HPP has the potential to decrease the allergenicity of proteins by promoting modifications on their secondary structure and unfolding.

5.2. High-intensity ultrasound

High-intensity ultrasound is an emerging concept in food industries, frequently used for homogenization, filtration, tenderization, dehydration, allergenicity reduction and overall improvement of food products. However, the mitigation of food allergenicity via ultrasound treatment is still at a preliminary stage. High-intensity ultrasound uses high-energy mechanical waves (20–100 kHz), which induce cyclic generation and the collapse of cavities (sonication bubbles) in a food system, followed by the formation of localized regions surrounding these collapsed cavities. These ultrasonic waves induce conformational changes in native proteins, cleaving peptide bonds in the vicinity of collapsed cavities via sheer force, thereby influencing their immunoreactivity (Rodríguez et al. 2018; Nowacka et al. 2018; Corzo−Martínez, Villamiel, and Moreno 2017; Li et al. 2013; Shriver and Yang 2011). Cavitation increases with the rise in pressure and frequency, initiating various effects by creating free radicals and enhancing the polymerization/depolymerization of the reactions. This process is advantageous in a sense that it is carried out at ambient temperature without any thermal treatment, minimizing product degradation (Nowacka et al. 2014).

The modification of food proteins via the ultrasound technique is predominantly based on the cavitation phenomenon (Mawson et al. 2011). Acoustic waves are generated by ultrasound, which subsequently promote the formation of

sonication bubbles in foods and, ultimately, lead to recurrent compression and rarefaction. This phenomenon leads to the collapse of the bubbles until certain sizes and to the release of energy that increases the pressure and temperature in the medium, causing chemical and mechanical changes. Low frequencies (higher intensities) encourage acoustic cavitation due to the formation and growth of bubbles and that the rupture of these large bubbles release high energies. Furthermore, microstreaming induces extreme agitation, which could possibly disrupt hydrogen bonds and Van der Waals interactions in polypeptides. Consequently, the conformational structure of the native protein/allergen is altered (Corzo−Martínez, Villamiel, and Moreno 2017).

Ultrasound treatment can alter the secondary structure of proteins causing an increase in the formation of random coil α-helices, as reported for beta-lactoglobulin. Secondary structure measurements reveal that the exposure of the allergenic protein to ultrasound treatment for various time intervals lead to compositional alterations after refolding, although presenting a minor effect on the IgE−binding properties of beta-lactoglobulin (Stanić-Vučinić and Veličković 2012). In the case of crustaceans, Li et al. (2011) have studied the allergenic and textural properties of shrimps (*L. vannamei*) after treating them, as raw and boiled, with power ultrasound (800 W, 30 kHz) for 0, 2, 8, 10, and 30 minutes at 0°C and 50°C, respectively. The results revealed that ultrasound treatment substantially reduced the allergenicity of the boiled compared to the raw shrimps. The allergenic potential of boiled shrimps was reduced by approximately 50% or 40% by treating the samples for 10 min at 0°C or 50°C, respectively. In the case of raw shrimps, the allergenicity increased in the first 10 min with the treatment at 0°C and then decreased to an initial value, while at 50°C no changes in the allergenicity were observed. In another study, the researchers assessed the influence of high-intensity ultrasound on the IgE-binding potential of major shrimp TM (Pen a 1) and shrimp protein extracts (Li et al. 2006). Shrimp muscles were treated with power ultrasound (800 W, 30 Hz) at 0°C and 50°C, for 1.5 h respectively. The allergenicity of purified TM and shrimp protein extracts was substantially reduced, as analyzed by the competitive inhibition ELISA and enzyme allergosorbent test, using sera from shrimp-allergic patients. The IgE-reactivity of ultrasonic treated shrimp TM at 50°C was reduced by 81.3–88.5%, whereas a 68.9% allergenicity reduction was found in case of shrimp protein extracts, compared to the untreated samples. Moreover, lower MW crosslinks were also formed, indicating the denaturation of shrimp protein by means of ultrasound treatment (Li et al. 2006). These findings seem to indicate that crustacean allergenicity could be mitigated via ultrasound treatments with slight changes in textural properties.

5.3. Irradiation

Irradiation has been a fascinating technique for food preservation with minimal alteration in nutritive and sensorial characteristics of foods. There have been several investigations on the applicability of irradiation for minimizing food protein allergenicity. It is regarded as a cold pasteurization practice for preserving food and it is currently permitted for application to 40 food products in over 60 countries worldwide (Kume et al., 2009; Mostafavi, Mirmajlessi, and Fathollahi 2012). Exposing food to high-energy rays from irradiator bring about conformational changes, namely fragmentation, aggregation, crosslinking, oxidation and amino acid modification. All these processes are ascribed in modulating the immunoreactivity of allergenic foods (Harder, Arthur, and Harder 2017; Luo et al. 2013). A schematic diagram of a commercial gamma irradiator is shown in Figure 4.

Irradiation influences the immunoreactivity of several food proteins through the aforementioned processes. The amount of heat-stable allergenic shrimp protein in an irradiated solution was reduced by gamma irradiation, depending on the dose. The effects of gamma irradiation on the whole shrimp muscle lead to a decrease in the IgE-binding capacity of sera from allergic individuals to shrimp heat stable protein and to allergens in the sarcoplasmic and myofibrillar protein fractions, based on the amount of given doses (Byun et al. 2000; Byun et al. 2002). Moreover, above 7 kGy, the immunoreactivity of shrimp allergens was reduced in approximately 50% (Byun et al. 2002). Liu et al. (2017a) assessed the influence of electron beam irradiation on the immunoreactivity of shrimp TM and shrimp extracts at frozen stage. Initially, the specific IgE-binding capacity of shrimp extracts and TM increased up to 10% by irradiating at 3 kGy doses and then reduced by 20% with the increase of irradiation doses (10 kGy). Similarly, Li. et al. (2007) irradiated shrimp protein extract and the purified allergen (Pen a 1) with different dosages (1, 5, 10 and 15 kGy) and investigated their IgE-binding capacity. Immunochemical assays revealed a

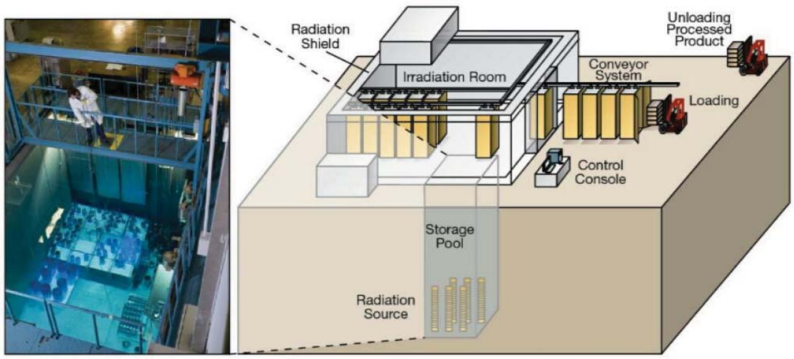

Figure 4. Schematic diagram of panoramic, wet storage commercial gamma irradiator. The sealed source is stored in water and raised into the air to irradiate a product that may be moved into the room on a conveyor system (Source: United States Nuclear Regulatory Commission 2016).

reduction in the reactivity of shrimp allergens, in a dose-dependent manner (Li. et al. 2007; Liu et al. 2017a).

A great proportion of the population is reluctant to consume irradiated food products since it is thought that "irradiated products are radioactive", being unfamiliar with the "Radura" symbol (https://en.wikipedia.org/wiki/Radura). The research on irradiated foods has shown that with an average dose of 10 kGy, the products are safe for human consumption without any toxicological, nutritional and microbiological hazards. Therefore, providing science-based information about the effectiveness of irradiation technology can lead to positive public perspectives (Junqueira-Gonçalves et al. 2011; Kebede et al. 2015; Konteles et al. 2009).

5.4. Pulsed ultraviolet light

Recently, PUV light has proclaimed itself as a good alternative or supplement to orthodox chemical or thermal intervention strategies for inactivating enzymes, retarding the growth of microorganisms, reducing allergenicity and other food processing purposes (Heinrich et al. 2016; Kramer, Wunderlich, and Muranyi 2016; Pellicer and Gómez-López 2017; Wang et al. 2017). The conventional preservation techniques likely impair the nutritional and perceived organoleptic traits of the processed products. The PUV light system captures and storse the electrical energy in a capacitor that eventually releases high-energy short pulses of ultraviolet, visible and infrared light (approximately 54%, 26% and 20%, respectively), which have great penetration capabilities. In fact, both irradiation and PUV light are different forms of radiation. Gamma irradiation is a form of ionizing radiation inducing changes in food properties in a dose-dependent manner, while PUV is non-ionizing radiation via instantaneous high-energy short pulses based on duration and rate of pulses (Harder, Arthur, and Harder 2017; Kramer, Wunderlich, and Muranyi 2016; Luo et al. 2013; Shriver and Yang 2011).

Upon exposure of food products with highly intense light pulses, the molecules are excited and, after returning to the ground state, they liberate energy as heat or photons, being likely to induce photochemical, photophysical or photothermal reactions (Abida, Rayees, and Masoodi 2014; Heinrich et al. 2016). Similarly, prolonged UV light treatment resulted in the formation of insoluble complexes, unsaturated fatty acid peroxidation, starch depolymerization, protein unfolding, fragmentation, and carbohydrate and protein crosslinking. The changes in food protein structure induced by pulsed light areas following: aggregation (oxidative reactions such as formation of radical cessation reactions, SH-SS exchange and hydrophobic interaction), unfolding (alteration of protein binding potencies such as formation or cleavage of hydrophobic and hydrogen bonds) and fragmentation (oxidative reactions such as radical cessation reactions). These effects may contribute to the modification of the protein structure and the reduction of IgE-binding to allergens (Abida, Rayees, and Masoodi 2014; Kramer, Wunderlich, and Muranyi 2016; Manzocco 2015; Shriver et al. 2011; Yang et al. 2012).

Yang et al. (2012) studied the stability of crustacean allergen *in vitro* reactivity modified via PUV light. The changes in the allergenic potencies were evaluated after subjecting the PUV-treated shrimp extracts to SGF with pepsin and to SIF with trypsin and chymotrypsin enzymes. Western blot analysis revealed a reduction trend in the IgE-binding capacity of TM. The PUV-treated samples showed an obvious reduction in allergen reactivity under the conditions of SGF, SIF, and their combination (SGF + SIF) and did not regain their original reactive states (Figure 5). These findings suggest that PUV treatment could be a potential way to develop hypoallergenic shrimp products with reduced allergenic potencies under

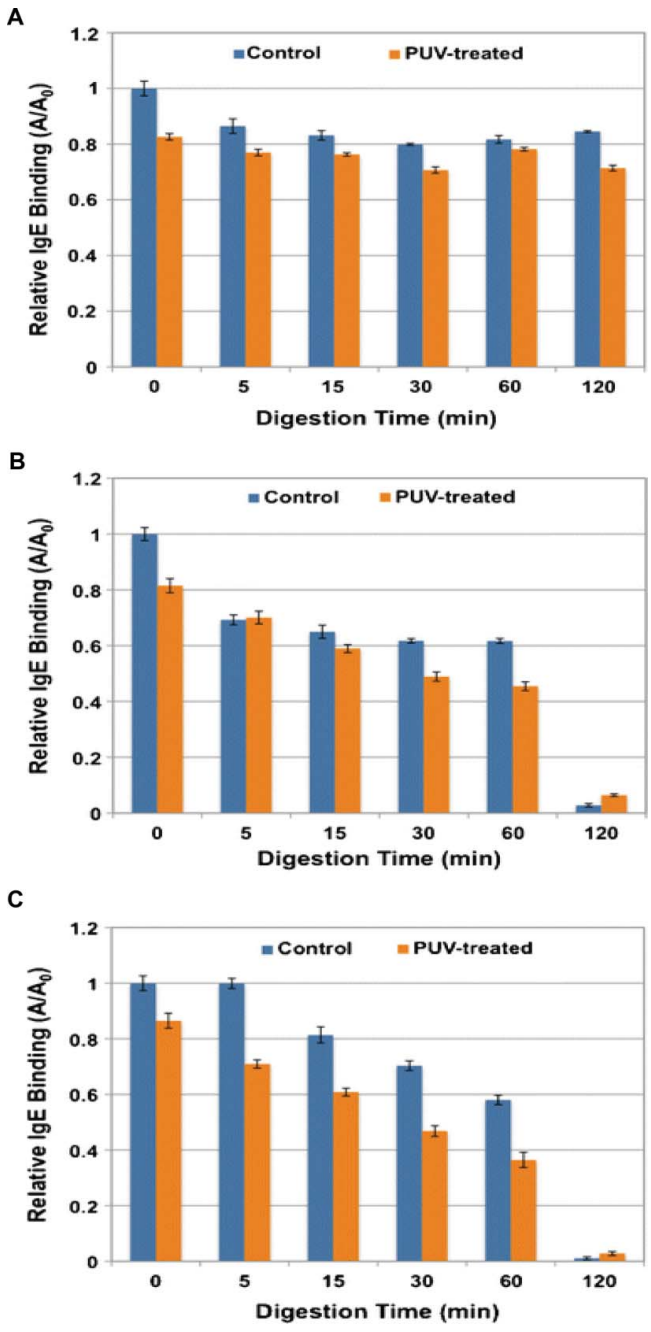

Figure 5. ELISA of non-PUV treated (indicated as control) and PUV-treated shrimp extracts that have been subjected to simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) digestion. Samples were digested for 0, 5, 15, 30, 60, and 120 min: A) pepsin in SGF; B) trypsin and α-chymotrypsin in SIF; C) pepsin in SGF, followed by a mixture of trypsin and α-chymotrypsin in SIF. Human IgE were used to determine shrimp allergen reactivity. Relative IgE-binding is expressed as A/A_o, where A is the absorbance of the sample divided by the absorbance of the untreated. Data are expressed as mean ± standard deviation (SD) (Reprinted from Yang et al. 2012 with permission from Springer-Humana Press Inc).

human gastric and intestinal digestive conditions. On the other hand, Shriver et al. (2011) found a reduction in the IgE-binding capacity of Atlantic white shrimp *(Litopenaeus setiferus*), following treatment with PUV light. Likewise, few other food products have been treated with PUV light in order to reduce their antigenic and allergenic potential.

Unlike thermal processing, very limited information has been reported on the impact of non-thermal treatments for reducing crustacean allergenicity. In most cases, these treatments are employed as a pretreatment method prior to other food processing operations. Although these technologies proved to be efficient in attenuating the crustacean allergy, optimization and realization of the system and *in vivo* studies need to be investigated prior to commercialization, in order to help the food industry and to protect the consumers.

6. Hurdle technology for mitigating crustacean allergenicity

In the modern era, the consumers demand minimally processed foods with reduced allergenicity and marginal changes in the organoleptic and nutritional attributes, which have prompted the hurdle concept. Hurdle technology, a combination of preservation/processing methods, has been successfully used globally, as an attempt to gently and effectively preserve foods (Pal et al. 2017; Singh and Shalini 2016). The various methods such as heating, refrigeration, irradiation, PUV light and enzymatic hydrolysis, involved in food processing, are considered hurdles when at least two methods are used in combination. The application of hurdle technology ensures the safety and stability of foods, as well as their sensorial and nutritive properties (Ahmed et al. 2017; Hu et al. 2016; Huang et al. 2014; Meinlschmidt et al. 2016, 2017; Pal et al. 2017). This technology has been applied in food of terrestrial and aquatic origins and has become a boon for efficient mitigation of food allergenicity.

The hurdle technologies reported in the literature for reducing crustacean allergenicity are summarized in Table 7. Li. et al. (2007) suggested that the combination of irradiation and heat induced a relevant effect on the integrity and structure of shrimp allergens, leading to a considerable decrease in the overall IgE-binding capacity. The authors hypothesized that a destruction of shrimp allergens was due to the radicals formed during irradiation and subsequent heat treatment. Additionally, the epitopes that were probably hidden were exposed by irradiation and further destroyed by heat. In case of combined ultrasound and boiling treatment, Yu et al. (2011) reported that the treated TM was degraded much more quickly than the untreated one in the SGF and SIF, thus contributing to decrease TM IgG/IgE-binding. Long et al. (2015) treated shrimp protein extracts with HPP and/or thermal treatment, analyzing the allergenicity of TM by competitive ELISA using pooled sera from patients with shrimp specific IgE. The combined treatments (55–75°C) completely blocked IgE inhibition, indicating that the allergenicity of TM might be eliminated. Moreover, a significant reduction in specific IgE titers was observed in mice (BALB/c mouse model of allergy) fed with TM treated with HPP and heat, accompanied by a decrease in histamine levels. In opposition to the aforementioned studies, the combination of heat treatment with PUV light seemed to have no effect on the immunoreactivity of shrimp TM. After boiling TM increased its reactivity, which was attenuated by the subsequent PUV light treatment (Shriver et al. 2011).

The combined technologies have also been applied to other foods, which considerably reduced their immunoreactivity (Meinlschmidt et al. 2017; Yang et al. 2017). Other processing combinations such as HPP with enzymatic hydrolysis proved to be effective and novel approaches for the development of low allergen food ingredients with enhanced functional and sensorial properties (Meinlschmidt et al. 2017). Undeniably, hurdle technology is prospected to have a crucial role in reducing the allergenicity of foods.

7. Strategies to manage crustacean allergens

7.1. Avoidance and labeling

Public awareness regarding food allergies is very important to reduce their incidence. Strict avoidance of the offending foods, along with the accessibility of epinephrine auto-injector is the best way for the allergic individuals to protect themselves from food-induced hypersensitivity. However, the eviction strategy is not always practicable, once the accidental exposure to allergenic proteins can sometimes occur, mainly because of

Table 7. Influence of hurdle technology on crustacean allergens.

Hurdles	Crustacean allergens	Impact on immunoreactivity	References
Gamma irradiation + Boiling	Shrimp (*Penaeus vannamei*) protein extracts and TM (Pen a 1)	Immunoreactivity decreased significantly.	Li. et al. (2007)
PUV light + Boiling	Atlantic white shrimp (*Litopenaeus setiferus*) TM	No change on the IgE-binding of TM. Boiling increased reactivity that was subsequently attenuated by PUV light treatment.	Shriver et al. (2011)
Ultrasound + Boiling	Crab (*Scylla paramamosain*) TM	IgG/IgE binding decreased. Enhance the digestion of TM in gastrointestinal digestion, and reduced the immunoreactivity.	Yu et al. (2011)
HPP + Heating	Shrimp (Litopenaeus vannamei) TM	Reduction in IgE binding potential. Significant reduction is specific IgE titers in BALB/c mouse model. Reduction in mRNA encoding interferon-γ, interleukin (IL)-4, and IL-10 in jejunum tissues.	Long et al. (2015)

mislabeling or cross-contamination during food processing (Choi, Ju, and Chang 2015; Costa et al., 2015; Muraro et al. 2014; Taylor et al. 2014). Therefore, food manufacturers should enhance the allergen labeling in food packaging. Countries within EU and USA, Canada, United Kingdom, Australia, People's Republic of China and Japan have set specific legislation concerning the mandatory labeling of allergenic foods to safeguard the health of consumers (Allen et al. 2014; Bucchini et al. 2016). Depending on each specific country legislation, the list of groups of allergenic foods might vary, although the foods included in the so-called big-8 list (cereals containing gluten, soybean, tree nuts, peanut, milk, fish, eggs and crustaceans) are normally covered. According to the EU legislation, food manufacturers must declare the existence of 14 groups of ingredients, documented as potentially allergenic, highlighting them from the rest of the list. These include the already mentioned "big-8" plus mollusks, sesame, mustard, lupine, celery and sulfites (Directive 2007/68/EC; Regulation (EU) No 1169/2011).

7.2. Detection of crustacean allergens

Despite the application of the legislation requesting the compulsory labeling of allergenic foods, the risk of allergic individuals suffer from an abnormal immune episode remains a reality, even when adopting a restrictive diet. Mislabeling or cross-contamination occurrences may happen during food processing, resulting in the inadvertent presence of hidden allergens. To avoid potential health risks, an effective allergen risk management is required. Therefore, the verification of labeling compliance of foods is crucial for the industrial management of allergenic foods, for the allergen control/monitoring by the regulatory authorities and for the allergic consumers. This relies on the availability of fast, reliable and highly sensitive analytical methodologies. Over the past years, several methods, either based on conventional or advanced technologies for proteins and/or DNA analysis, have been developed to detect food allergens (Costa et al. 2017). However, no unanimity has been reached with regard to the most suitable methodology for detecting food allergens.

Recently, methods targeting proteins and DNA have been proposed for the detection of crustacean allergens (mostly tropomyosins). The enzyme-linked immunosorbent assay (ELISA) is the most widely used immunochemical technique for protein analysis, being available as commercial kits for tropomyosin crustacean/tropomyosin detection (e.g. Crustacea ELISA kit (BioFront Technologies, Tallahassee, FL, USA) and Ridascreen Fast Crustacean (r-Biopharm AG, Darmstadt, Germany)). Additionally, some in-house ELISA using TM as the target protein for crustacean identification in foods have also been developed (Fuller, Goodwin, and Morris 2006; Seiki et al. 2007; Werner, Fæste, and Egaas 2007; Zhang et al. 2014). Immunoassays have major advantages associated with simplicity, speed, high specificity and sensitivity inherent to the antigen/antibody interaction. However, they are prone to cross-reactivity phenomena that can conduct to false positive results and they can be affected by food processing that cause structural changes in proteins, leading to false negative results (Costa et al. 2017; Prado et al. 2016).

Protein analysis based on liquid chromatography (LC) coupled to mass spectrometry (MS) platforms has greatly advanced in the past years, providing several applications for allergen detection (Prado et al. 2016). High accuracy, sensitivity, specificity and reproducibility are some of the advantages associated with this technology. Besides, it does not rely on the interaction of antibody/allergen (or marker protein), enabling the unequivocal identification of the target analytes (Monaci and Visconti 2009). Nonetheless, the high cost of the equipment/maintenance, together with the need for highly specialized personnel, are relevant drawbacks that constrain the application of this technology. In the case of crustacean allergens, some LC-MS methods have been developed for their detection in foods. Ortea, Cañas, and Gallardo (2011) exploited selected tandem mass spectrometry (MS/MS) ion monitoring (SMIM) scanning mode to detect and monitor diagnostic peptides, primarily focused on shrimp species identification, which can also be used for the detection of the crustacean allergenic protein arginine kinase in food products. Nagai, Minatani, and Goto (2015) developed a LC-MS/MS approach using multiple-reaction monitoring (MRM) to detect several marker peptides of TM and AK in crustaceans, obtaining results well correlated with those using the ELISA. Korte et al. (2016) developed a LC-MS/MS method with MRM-cubed mode for the detection of shrimp and lobster in model food matrices down to the level of 25 mg/kg (crustacean/food). The study of Chen, Pan, and Huang (2017) reported a LC tandem QTOF mass spectrometry (UPLC-QTOF-MS) method for crustacean TM and AK characterization using the 'bottom up' MS approach, which allowed identifying two specific heat-stable marker peptides suitable to screen potential addition of shrimp meat in foodstuffs.

Lately, methodologies based on DNA analysis have been referred as adequate alternatives to proteins for allergen analysis. The high stability of DNA molecules, compared to proteins, upon food processing conditions and their presence in most of the biological tissues have been pointed out as main advantages for the development of methods for allergen detection. Additionally, DNA-based methods targeting species-specific markers or sequences encoding for allergens are considered highly specific and sensitive approaches for allergen analysis in processed foods (Costa et al. 2017; Monaci and Visconti 2010), being real-time polymerase chain reaction (PCR) widely used. Despite the referred advantages, the fact that they are indirect methods of detecting allergens in foods is considered a drawback by some researchers. In the specific case of crustacean detection, multi-copy genes such as 16S rRNA (Cao et al. 2011; Pascoal et al. 2011; Herrero, Vieites, and Espiñeira 2012; Eischeid, Kim, and Kasko 2013; Mäde and Rohmberger 2017; Zagon et al. 2017; Wilwet et al. 2018), 12S rRNA (Eischeid, Kim, and Kasko 2013; Eischeid 2016; Eischeid and Stadig 2018) and cytochrome oxidase subunit I (COI) (Eischeid, Kim, and Kasko 2013; Fernandes et al. 2017) have been used as specific markers because they typically enable the development of PCR-based methods with high sensitivity. Several real-time PCR methods reported in the literature allow the detection of particular groups of crustacean species with high sensitivity (Cao et al. 2011; Herrero, Vieites, and Espiñeira 2012; Eischeid, Kim,

and Kasko 2013; Eischeid 2016; Mäde and Rohmberger 2017; Zagon et al. 2017; Eischeid and Stadig 2018). Despite the advantage of real-time PCR in providing quantitative results, very few studies describe quantitative methods (Eischeid, Kim, and Kasko 2013; Eischeid 2016; Eischeid and Stadig 2018). Eischeid, Kim, and Kasko (2013) developed two real-time PCR systems that allow estimating DNA from penaeid shrimps and crabs. Eischeid (2016) reported a real-time PCR quantitative method specific for lobsters based on model mixtures as calibrants, while Eischeid and Stadig (2018) proposed a similar approach for crabs, being both systems adequate for allergen quantification.

8. Final remarks

This review presents an update on the crustacean allergy, with focus on the impact of different processing strategies on crustacean allergens. So far, there are several crustacean allergenic proteins identified and fully characterized, belonging to different families of allergens. Among those, TM, AK, SCP, MLC, troponin C and hemocyanin have been described and included in the official list of allergens. Therefore, it is indispensable to comprehend the structural alterations of the allergenic proteins upon processing and to understand the effect of specific techniques, either conventional or novel, in the allergenicity of crustaceans. Distinct processing approaches affect the structure of crustacean allergens in different ways via denaturation, aggregation, masking or unmasking of the epitopes, oxidation, crosslinking, glycation, and glycosylation, which in turn influence their gastrointestinal digestion and allergenic potential. Inherent molecular characteristics of allergens, type of processing, intensity of treatment, environmental conditions (i.e. pH, time and temperature) and food matrix are determining factors in the allergenicity of food proteins. Most of the processing methods appear to be promising in mitigating the IgE-reactivity, although few show an enhanced immunoreactivity and the formation of neo-epitopes. Since the total elimination of the allergenic potential by processing is still unlikely, the selection of proper conditions could significantly reduce the allergenicity of foods.

The allergens are typically more resistant to proteolysis than other types of proteins, and although they may be partially hydrolyzed after the digestion process, the resultant fragments can still retain immunoreactivity. Consequently, it is also important to understand the impact of different processing methods on the allergenicity of foods upon the digestion process. From the available processing methods, the hurdle technology allows gathering the advantages of different treatments, being the most efficient in mitigating the allergenicity of foods. The development of appropriate processing technologies for mitigating the allergenic potential of foods or the removal of allergens to prepare hypoallergenic/non-allergenic foods are common goals of processing industry professionals to provide safe foods for allergic consumers. Moreover, the development of methodologies for the detection of crustacean allergens in processed food is very important to ensure the enforcement of labeling regulations and improve the life quality of crustacean-allergic individuals.

Conflict of interests

The authors declare that they have no conflict of interests.

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