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The immune response of seafood allergen and their detection methods: A review

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Abstract
Seafood is highly nutritive and has an excellent source of proteins, vitamins, minerals, and essential polyunsaturated fatty acids such as Eicosatetraenoic acid (ETA) and Docosahexaenoic acid (DHA) from the n-3 series (omega-3). Due to great nutritive value and large health benefits, their consumption has been increasing continuously. Sometimes but significantly, in a small number of individuals consumption of seafood often leads to adverse reactions like a food allergy. It is a global public health issue in regards to food safety that decline the quality of life and may endanger the lives in severe cases. Seafood allergen can induce hypersensitive immune responses due to their ingestion, skin contact, and inhalation. Recent advancement in the food processing sector implies a great focus on the identification of food allergens by developing a rapid and user-friendly method to assess risk through proper labeling of foods and proper management. According to recent European Union regulations, food producers are advised to declare the presence of foods that are potentially allergic ingredients and highlight them by proper labeling. These efforts enable consumers' health to be guaranteed. There are various techniques for detection such as DNA-based, Aptamer based, and protein-based. These are more efficient and reliable detection methods for seafood allergen and will ensure the food safety and health of the consumer.

Keywords: Seafood allergy, food safety, hypersensitivity, aptamer- based, protein, DNA

1. Introduction

Seafood refers to various groups of edible aquatic animals including arthropods mainly crustaceans (such as prawns, shrimp, and crab), mollusks, and fishes like cods, salmon. Seafood is highly nutritive and has an excellent source of proteins, vitamins, minerals, and essential polyunsaturated fatty acids such as Eicosatetraenoic acid (ETA) and Docosahexaenoic acid (DHA) from the n-3 series (omega-3). Scientific background depicted that there is a wide range of health benefits associated with ingestion of omega-3 fatty acids like prevention of cancer, cardiovascular diseases, and improvement in glycaemic level. Due to great nutritive value and large health benefits, their consumption has been increasing continuously. Sometimes but significantly, in a small number of individuals consumption of seafood often leads to adverse reactions like a food allergy. Food allergy may be defined as an adverse effect due to immunogenic reactions on exposure to a particular food. The immune system protects our body against numerous invaders such as bacteria, viruses, fungi, and foreign substances. The defending human immune system sometimes produces harmful responses to the host. These harmful immune responses are termed hypersensitivity. Hypersensitivity is an immune state in which the immune system hyper react to a foreign body and their reaction itself is more dangerous than antigen. These immune responses recruit a series of effectors molecules that induce inflammatory reactions for the removal of antigen. It is a global public health issue in regards to food safety that decline the quality of life and may endanger the lives in severe cases. It was reported that there are 150 deaths annually due to food allergies. According to FDA (Food and Drug Administration), about 2% of adults and 3-8% of children less than 3 years are more affected due to food allergies. A survey from FAO (Food and Agriculture Organization) reported that peanuts, wheat, soybean, egg, milk, fishes, and shellfish like shrimp, crab are the most common source that leads to food allergy. Seafood is known to induce hypersensitivity reaction mediated by IgE antibody in allergic\sensitized individuals. These allergic reactions are generally immediate within 2 hours in most cases however; in the case of shellfish, late reactions up to 8 hrs have been also reported. Seafood allergen can induce hypersensitive immune responses due to their ingestion, skin contact, and inhalation. After ingestion, it can cause vomiting, abdominal pain, coughing, itching, and swelling in lips, mouth, and pharynx.

If allergen enters through skin contacts then symptoms are different like urticaria, rashes, pruritus, angioedema, etc. sometimes causing life-threatening anaphylaxis. Seafood is mainly derived from two major groups like fish and shellfish. Allergens derived from fish are Parvalbumin, enolase, aldolase, collagen, glyceraldehydes dehydrogenase, fish hormone vitellogenin, etc. and from shellfish Arginine kinase, myosin light chain, a sarcoplasmic calcium-binding protein, troponin C and trios phosphate isomerase, etc. Among various food allergen parvalbumin in fish and tropomyosin in shellfish are most dominant and represent two large classes of animal-derived allergen according to allergen databases AllFam (Radauer *et al*., 2008) [41]. Some allergen was also reported from Mollusk such as Hal m1found in abalone (Lopata *et al*., 1997) [33] but they have not been studied in detail and but not fully identified. There is no reliable cure for food allergens, unfortunately. The effective way to prevent these adverse reactions is total avoidance of seafood by allergic person, identification, proper labeling, and sensitization. Thus, it became necessary to improve consumers' protection through a proper labeling system to prevent life-threatening risks for allergic reactions (Costa *et al*., 2014 [8]; Rencova *et al*., 2013) [42]. Recent advancement in the food processing sector implies a great focus on the identification of food allergens by developing a rapid and user-friendly method to assess risk through proper labeling of foods and proper management. Recently, According to recent European Union regulations, food producers are advised to declare the presence of foods that are potentially allergic such as crustaceans, mollusks, fish, celery, peanuts, milk, eggs, soybeans, lupine sulfite, etc. and highlight them by proper labeling. These efforts enable consumers' health to be guaranteed. There are various methods for detection such as ELISA, DNA based, Aptamer based, and protein-based.

2. Prevalence of Seafood allergy

The frequency of seafood allergy varies among regions and also between children to adults. Shellfish allergy is more common worldwide than fish allergy. In descending frequency, shrimp, crab, oyster, and mussels were the common source of shellfish allergen. In the United States, the frequency of seafood allergy was higher in adults than children. Among fish tuna, salmon, and catfish were common that contain the most frequently diagnosed allergens in the United States. In the Spanish population, sole and hake were common fish associated with allergies. In Germany, about 0.4-0.5% population were allergic to mackerel, herring fish, approximately 0.1-1.2% were allergic to shellfish like mussels and crabs. In Asian countries, the prevalence of seafood allergy was more than in western countries due to the large intake of seafood products. In Taiwan, shellfish allergies are common and affect about 7% of adults and children. Similarly, in Singapore and the Philippines, shellfish allergy was more prevalent about 5% than fish allergy among children than adults.

3. Fish Allergen

There is some protein family such as aldolase, enolase, and parvalbumin that has been classified as a fish allergen. But parvalbumin is most dominant among various allergens in fish and is currently reported in 95% of food allergies occurs due to fish consumption. After contact with allergenic food, symptoms arise within 30 minutes (Kuehn *et al*., 2014 [28]; Lee & Taylor, 2011 [29]; Weber *et al*., 2010) [51].

a. **Parvalbumin:** These are small proteins with low molecular weight, generally insoluble in water, acidic in nature, resistant to high temperature, denaturation, and proteolytic cleavage. There are two classes of parvalbumin- alpha isoform that is non-allergic and beta isoform that is highly allergic in nature. These are abundant in the white muscle tissue of many fish and perform the role in the relaxation of muscles by binding to calcium ions. In parvalbumin, the binding of calcium is responsible for binding to IgE epitopes. This type of protein is present at a high proportion in the fish that are bottom dwellers like cod, flounder, or whiff. Codfish allergy is mostly intolerable by many patients. The main allergen is Gad c1 which is isolated by *Gadus callarias,* used as a reference molecule for the study of parvalbumin. Homologous allergen have been isolated from other fish like *Gadus morhua*, *Cyprinus carpio*, *Atlantic salmon*, *Salmo salar*, *Trachurus japonicas*, *Thunnus obesus* and *Merluccius merluccius*.

b. **Enolase and aldolase:** These allergens are most commonly present in cod, tuna, and salmon fish. These two enzymes play a very important role in carbohydrate metabolism like glycolysis for the breakdown of glucose and the production of energy. The biochemical characterization of enolase is dimeric but aldolase is oligomeric in nature. Both are heat labile, temperature above 90 °C for 5 minutes destroy their structure. But during food processing, new linear epitopic regions are created which could increase allergenicity.

4. Shellfish allergen

There are four allergens reported in shrimp that have been discovered to be the major contributor to allergenicity including tropomyosin, arginine kinase, a sarcoplasmic calcium-binding protein, and myosin light chain.

a. **Tropomyosin:** Shellfish allergens that trigger observable symptoms and are present in muscle and non-muscle cells with actin and myosins. Motoyama *et al*., 2007 [38] concluded that tropomyosin can be classified into three fast classes, slow switch, and slow tonic based on the muscle fiber. It was estimated that about 75% of people are allergic to tropomyosin. After food processing, their allergenicity becomes reduced but after cooling they can regain their property. Pen a1 from *Penaeus azetcus*, Pen I 1 from *Penaeus indicus*, Pen m1 from *Penaeus monodon*, and Lit v1 *Litopenaeus vannamei* are common allergen reported in shrimp.

b. **Arginine kinase:** This enzyme is monomeric phosphagen ATP phosphotransferase of 40kD molecular weight that is generally found in invertebrates like shrimp, water-soluble present in myosinogen that is involved in cell metabolism of invertebrates. These enzymes are unstable at a temperature between 40-80°C. At high temperatures, they became partially unfolded and revealed hidden epitopes that are responsible for increasing hypersensitivity. Pen m 2 and Lit v2 are arginine kinase reported in black tiger and white pacific shrimp that play an essential role in cross-reactivity and in raising hypersensitivity. Arginine kinase can be characterized by molecular genetics and mass spectrometry techniques.

c. **Sarcoplasmic calcium-binding protein:** It is a calcium-binding protein found in white leg shrimp. It is also known as Lit v4 and has 22 kD molecular weight, 194 amino acids, and 4.7 isoelectric points. It was also

reported in American lobster *(Homarus americanus)* and snow crab *(Chionoecetes opilio)* (Shiomi *et al*., 2008) [45]. To characterize SCP few methods such as ELISA, Western blotting, Edman, Liquid chromatography-tandem mass spectrometry (LC-MS), Mass spectrometry, or Molecular genetics (Garcia-Orozco *et al*., 2007) [18] can be used.

d. **Myosin light chain:** *Lit* v3 was recognized as shrimp allergen. Ayuso *et al*., 2008 [5] concluded that MLC is a muscle protein with 20 kD and 4.2 isoelectric points that is similar to Tropomyosin, thus provoking difficulties to recognize which protein is responsible for allergic reaction in patient's IgE antibodies by following standard laboratory methods.

5. Seafood Allergens and epitopes

Allergen is the chemical substance that causes allergy by inducing an immune response. As discussed earlier, the parvalbumin in fish and tropomyosin in shrimp are major seafood allergens. Epitopes are the specific chemical group that can determine the allergenicity of allergens. There are two categories of epitopes conformational and linear. Linear epitopes contain conserved amino acid sequences and conformational have the discontinuous amino acid sequence.

6. Mechanism of seafood allergy

It is an immediate or anaphylactic Reaction. This reaction takes 15-20 minutes for initiation from the time of exposure to the seafood allergen. This allergic reaction is mediated by IgE (Adkinson & Hamilton, 2015) [2]. The basic cellular component is basophile or mast cell. The mechanism of this reaction involves the production of IgE in response to allergens. IgE antibody has a high affinity for their receptor present on basophiles and mast cells.

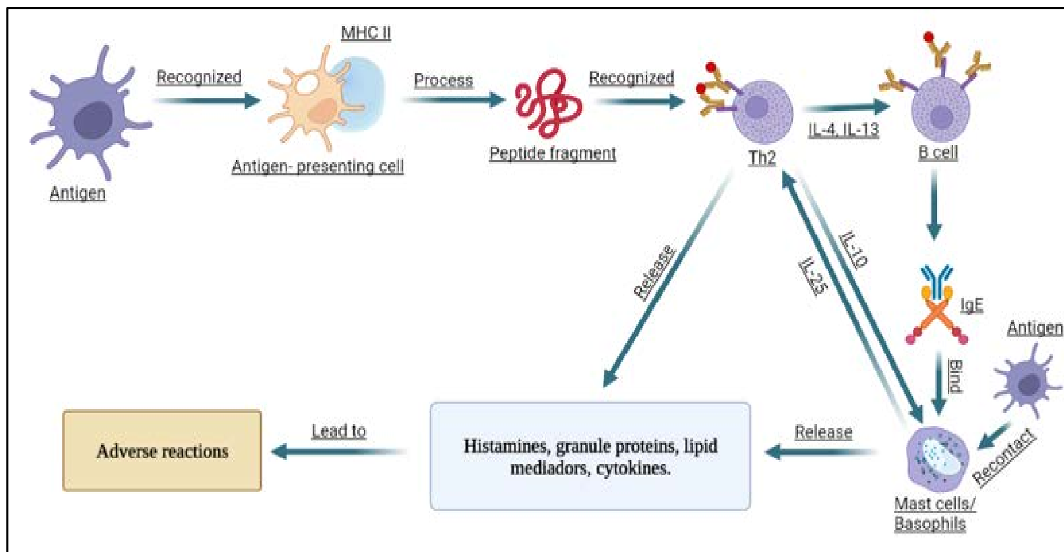

Fig 1: Show mechanism of food allergy (Xu *et al*., 2021) [53]

Diagnosis of type I hypersensitivity includes intradermal test or measurement of total IgE level in the blood (Yalew, 2020) [54]. Total IgE is measured by an Enzyme-linked immunosorbent assay. There are two stages of this reaction, IgE production, and symptom development. Firstly seafood allergens are processed into small peptides fragments then presented by antigen presenting cells on their surface through MHC class II. Later, CD4 Th helper T cells recognize them by T-cell receptors and secrete IL-4 and IL-13 that differentiate B-cells into plasma cells. Finally, plasma cells release IgE antibodies which have receptors on mast cells or basophiles cells. Again exposure to the same seafood allergen resulted in degranulation of mast cells. The chemical released during degranulation are histamines, tryptase, leukotrienes, cytokines, etc. are responsible for the symptoms that arise due to allergic reactions. Seafood allergy may be simple as urticaria and may cause life-threatening anaphylactic reactions (Prester, 2016) [40]. Depending on the location of the attack seafood allergy is categorized into four classes: dermatological allergy, gastrointestinal allergy, respiratory allergy, and systemic allergy, and the specific details are included in Table1. Based on the mechanism of seafood allergy their detection methods for allergens are subdivided into three categories of DNA-based, protein-based, and aptamer-based methods and their recent applications are as follows.

Table 1: The Adverse reactions occur due to seafood allergen

Allergy	Symptoms	Disease
Dermatological allergy	Edema, itching and erythema	Urticaria
	Loose muscle tissue, red skin, and dizziness]	Angioedema
	Swelling, papules, blisters	Erythema
	Eczematous contact dermatitis	Diffuse flushing, papules, blisters, erosion, exudates, scab
Respiratory allergy	Continuous wheezing, chest tightness, difficult breathing	Asthma
	Sneezing, stuffy nose, itchy nose	Rhinitis
Gastrointestinal allergy	Nausea, vomiting, abdominal pain, diarrhea, poor appetite, Intermittent abdominal pain, and malabsorption	Physical discomfort
	Abdominal pain, bloating, diarrhea, constipation	Gastroenteritis
	Itchy or tingling tongue, swollen or numb lips	Oral allergy syndrome

7. DNA Based methods

Application of DNA fragments of target *al*lergen is done by polymerization chain reaction (PCR). This is the fast, simple, sensitive, and specific technology in the detection of allergen (Mafra *et al*., 2008) [35]. DNA-based methods include PCR, Multiplex PCR, Real-time PCR and digital PCR.

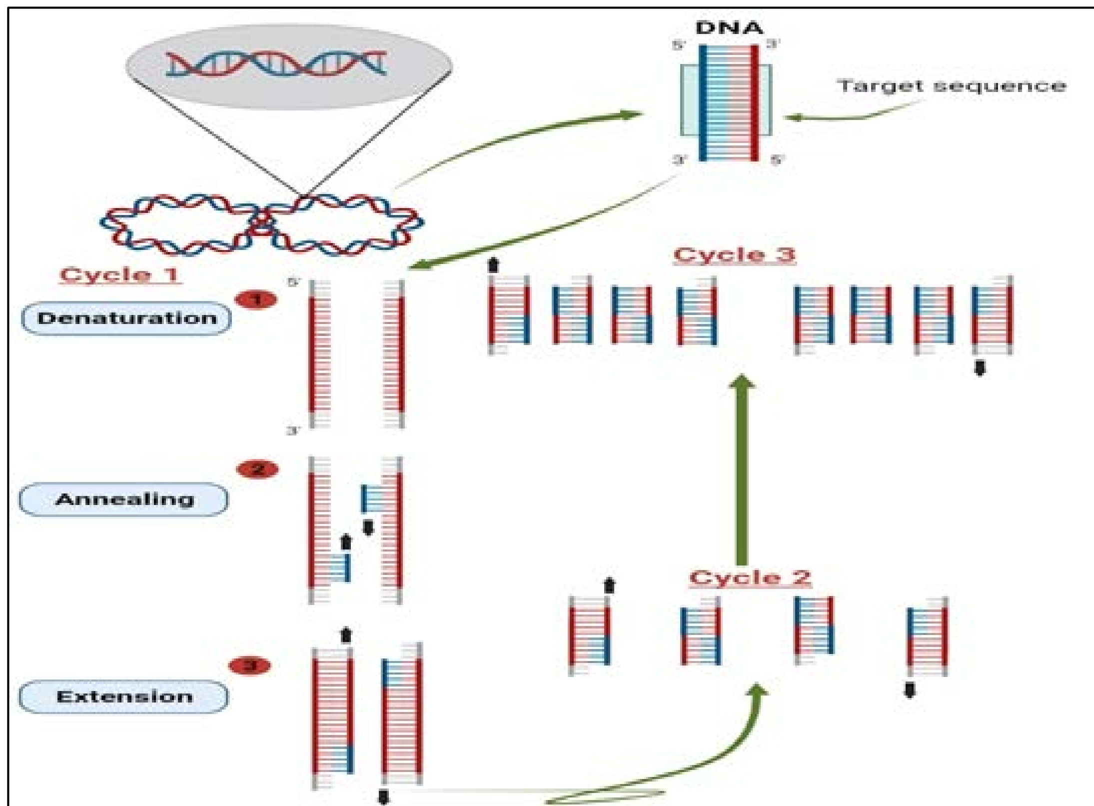

Fig 2: Show mechanism of Polymerase Chain Reaction (Linacero et al., 2020)

a. **PCR:** Consists of three basic steps:
1. **Denaturation:** In this step, double-stranded DNA molecules get converted into a single strand at a high temperature (94 °C).
2. **Renaturation:** In this step drops to 55°C temperature and primer bind to single-strand DNA.
a. **Primer extension:** In this step, the temperature rises to 72°C and a new double-strand DNA molecule is synthesized by using free nucleotides with the help of the Taq polymerase enzyme. By using this process repeatedly, the amount of DN molecule could be increased exponentially. This traditional PCR technique helps in the quantitative analysis of allergens.
b. **Real-time PCR:** Some shortcomings of the traditional PCR technique have led to the development of real-time PCR. It is a technology that can measure the total amount of DNA amplification product after each cycle by using some fluorescent probe. During real-time PCR, CT value is determined that is the number of cycles after amplification. Thus, Plotting a CT value on Y-axis and the logarithm of the initial copy number of a template on the Y-axis gives a linear curve and provides a basis for quantification analysis (Adams, 2006 [1]; Heid *et al*., 1996) [19]. The real-time PCR method consists of three steps: design and synthesis of probes and primer, design of PCR amplification system, validation of method (Fernandes *et al*., 2018a [16]; Herrero *et al*., 2012) [21]. The first step of Real-time PCR is to select a suitable target gene according to the requirement of test methods and test objects. In the second step, PCR amplification is optimized for faster and easy detection. For example, microfluidic chips have been developed to reduce the reaction time of thermal cycling. The last step is to validate the method that includes verification for its practicability and reliability with severe food processing methods. The unique thing about this method is the accuracy of detection results. (Eischeid, 2016) [13] developed a real-time PCR method and reported that his method could detect lobster without cross-reaction with shrimp and crab. In this method, a fragment of the mitochondrial 16S rRNA gene as the target gene was taken and proved to be specific for crustacean species.
c. **Multiplex PCR:** Multiplex PCR, also known as multiple primers PCR, allows for simultaneous amplification of two or more pairs of primers in the same PCR. For the detection of allergens, multiplex PCR is used to detect multiple allergens. Suh *et al*., 2020 [46] firstly performed the simultaneous analysis of allergen from mollusk species. They got the combination of a multiplex PCR by using capillary electrophoresis and expressing DNA concentration. Unterberger *et al*., 2014 [49] also performed simultaneous detection of allergens in cephalopods, fish, and shellfish components by using a multiplex (LPA) ligation-dependent Probe Amplification system. Unlike traditional multiplex PCR systems, LPA involves a single primer pair to detect different targets in a single assay simultaneously.
d. **Digital PCR:** In this method, the samples are diluted and dispersed into reaction chambers by ensuring that each chamber should contain one or no target sequence. After PCR amplification, each reaction chamber can be defined as either positive that contains PCR products or negative

that does not contain PCR products). The total amount of positive reaction chambers and negative reaction chambers were analyzed by counting using Poisson distribution, the nucleic acid amount can be determined (Baker, 2012 [6]; Morisset *et al.*, 2013) [37]. As Compared with other PCR technology mentioned above, digital PCR does not require the establishment of a standard curve that's why it is considered to be an absolute method for quantitative analysis. Information about DNA- based detection methods used for seafood allergen is summarized in table 2 below.

Table 2: Information about DNA- based detection methods used for seafood allergen

Method	Species	Allergen	Limit of detection	References
Digital PCR	Fish	18S rRNA	0.18 pg	Daga *et al.*, 2018 [9]
Real-time PCR	Shrimp and Crab	For shrimp:12S/16S For crab: cytochrome oxidase I/cytochrome b	0.1-1 ppm	Eischeid *et al.*, 2013 [14]
Real-time PCR	Lobster	12S rRNA	0.1-1 ppm	Eischeid, 2016 [13]
Real-time PCR	Shrimp	16S rRNA	0.1 pg	Fernandes *et al.*, 2018b [17]
PCR	Atlantic and Pacific herrings	pvalb 2 gene	10 pg/mL	Rencova *et al.*, 2013 [42]
Real-time PCR-	Crustacean	16S rRNA	0.04-2.5 pg	Zagon *et al.*, 2017 [55]
Real-time PCR-	Crustacean	16S rRNA	1.25 pg	Herrero *et al.*, 2012 [21]
Fast real-time PCR	Crustacean	16S rRNA	NR*	Santaclara & Espi~neira, 2017 [43]
Ultrafast real-time PCR	Shrimp	tropomyosin genes	3.2 pg	Kim *et al.*, 2019 [26]
Fast real-time PCR	Fish	18S rRNA	0.05 ng	Herrero *et al.*, 2014 [20]
PCR	Salmonid fish	Cytochrome b	0.02 fg/mL	Ishizaki *et al.*, 2012 [23]
Real-time PCR	Cod and Pollock	Mitochondrial cytochrome oxidase I	1-10 ppm	Eischeid, 2019 [12]
Multiplex PCR	Oyster, mussel	tropomyosin genes/ 18S rRNA	16 pg	Suh *et al.*, 2020 [46]
Multiplex PCR	Snow crab	COI/ITS genes	0.005 ng/lL.	Kang, 2019 [25]

NR*: Not Recorded, PCR: Polymerase Chain Reaction

8. Protein-based detection method

Various protein-based detection methods use the specificity of antibodies to allergens like ELISA, immunochromatography, and immunosensors. The first two methods are traditional and in use for a long time but immunosensors is a new technique that requires less time to detect antigens. Summary of detection of seafood allergens by using protein-based methods is provided in the table 3 below.

a. **ELISA:** It was first described by Engvall and Perlmann in 1971 and now it is widely used in different fields like medicine, biology, and chemistry for detection purposes. The most common advantage of ELISA is its high sensitivity to antigen, simple design, and large-scale detection method (Lequin, 2005) [30]. There is also a various kit of ELISA commercially available in the market. There are different modes of ELISA reaction like it can be competitive, direct, indirect, and sandwich in nature. The first competitive mode is generally used for the detection of small-sized allergens and the sandwich method is employed for the detection of large size allergens like globulins. The direct and indirect method mainly differs from each other whether HRP- conjugated secondary antibody is used or not. For the detection of allergen, a sandwich-type reaction is generally used due to its high specificity and sensitivity to allergens. Sometimes preference is also given to indirect ELISA as it reduces the time per detection and there is no problem with antibody conjugation in this method (Pereira *et al.*, 2020) [39]. According to Kamath *et al.*, 2014 [24] the procedure of sandwich and indirect ELISA have the following steps:

- Coating: In this step coating of capture antibodies is done after that these antibodies are diluted in the buffer in a 96 well plate and then incubated at 4 °C overnight.
- Blocking: It is done to avoid the non-specific binding of antibodies by using a blocking solution.
- Addition of analytes: sample is added into the well and then incubated for 3hours.
- Addition of signal molecule: detection antibodies after diluting in the buffer are added into the well then streptavidinhorse radish peroxide conjugate is added to wells and incubated for 30 minutes. After this wells are washed and allowed to dry.
- Color reaction: a substrate is added to the wells to form coloration in the blank wells and hydrochloric acid (1N HCl) is also used to stop the reaction.
- Reading. Readings are taken by observing color change at 450nm by using a micro plate reader.

Werner *et al.*, 2007 [52] achieved a quantitative sandwich ELISA for the detection of tropomyosin in crustaceans in five food matrices. Faeste & Plassen, 2008 [15] successfully construct a

Sandwich ELISA for quantitative detection of parvalbumin in fish.

b. **Immunochromatography assay:** It is also known as the lateral flow test. This is the combination of two things immunology and chromatography for the detection of allergen. The advantage of this method is that it does not require any professional expertise; fast detection time, simple procedure, and can detect large-scale allergen on site. Major steps in Immunochromatography assay (ICA)are:

- Firstly labeled and capture antibodies are prepared against the target sample.
- Antibodies are immobilized on the conjugate pad, and the capture antibody is immobilized onto the strip to form the Control line\Test.
- Then assemble all the components on the backing card after dispensing of reagents at their proper pads.
- After that samples are added and buffer on the sample pad.
- Wait for the sample flow through the test and control line for about 5-10minutes.
- At last the result can be observed when the color reveals.

- Some scientists construct colloidal gold immunochromatography by using colloidal gold-antibody conjugate as a detection antibody, because of its good biocompatibility and easy preparation of colloidal gold (Ambrosi *et al*., 2007) [3]. In 2014, a colloidal gold immunochromatography assay was achieved to detect crustacean allergen in processed food and their limit of detection was 25 ng/mL and this whole process took less than 20 min (Koizumi *et al*., 2014) [27].

c. Immunosensors: These are indiscernible, highly sensitive, and novel methods for quick detection of allergen. Immunosensors consists of three main components: Biosignal recognition unit is an antibody, aptamer etc., Signal processing unit, and transducer which may be electrochemical, optical, thermal etc. At present, two main types of immunosensors are used for sea food allergen detection electrochemical immunosensors and optical immunosensors (according to transducer component). All of them are listed in table 3.

Table 3: Information about protein-based detection methods used for seafood allergen

Method	Allergen	Limit of detection	Working range	References
ELISA	Parvalbumin	0.02 mg/kg	0.49–31.3 lg/L	Faeste & Plassen, 2008 [15]
ELISA	Tropomyosin	1µg/g	0.38 to 49 ng/mL	Werner *et al*., 2007 [52]
ELISA	Tropomyosin	60 pg/mL	0.02–1.25 ng/mL	Kamath *et al*., 2014 [24]
ICA	Tropomyosin	12.4 ng/mL	0.01-20 lg/mL	Shi *et al*., 2011 [44]
ICA	crustacean protein	25 lg/L	NR	Koizumi *et al*., 2014 [27]
ICA	Tropomyosin	0.05 lg/mL	NR	Wang *et al*., 2019 [50]
ICA	Tropomyosin	0.5 ng/mL	NR	Zeng *et al*., 2019 [56]
ICA	Parvalbumin	40 ng/mL	50-1000 ng/mL	Du *et al*., 2014 [11]
EI	Tropomyosin	0.01pg/mL	0.01-100 pg/mL	Mohamad *et al*., 2020 [36]
OI	Tropomyosin	1µg/mL	3-50 mg/mL	Zhou *et al*., 2020 [59]
EI	Tropomyosin	46.9 pg/mL	0-218.7 ng/mL	Angulo-Ibanez *et al*., 2019 [4]
OI	Parvalbumin	3.55 mg/L	NR*	Lu *et al*., 2004 [34]

*NR: Not Recorded; ELISA: Enzyme Linked Immunosorbent Assay; ICA: Immuno Chromatography Assay; EI: Electrochemical Immunosensors; OI: Optical Immunosensors

9. Aptamer-based detection method

Aptamer are a small sequence of short peptides or oligonucleotides. This method is based on aptamer-based detection and it can overcome the shortcoming of the protein-based detection method. Generally, antibodies are expensive and have long synthesis cycles and aptamer synthesis is short, the main advantage of using aptamer for the detection of allergens. Summary of Aptamer-based detection of allergen detection is provided in Table 4. Similar to the detection method based on specific allergen antibody binding, the aptamer-based detection method is also based on specific binding of the aptamer and the allergen. (Dong *et al*., 2014 [10]; Chinnappan *et al*., 2020) [7]. Subsequently, Zhang *et al*., 2018 [57] achieved aptamer-based detection methods for tropomyosin by combining nanoparticles. There are few studies related to aptamer-based detection of seafood allergens and further research is needed.

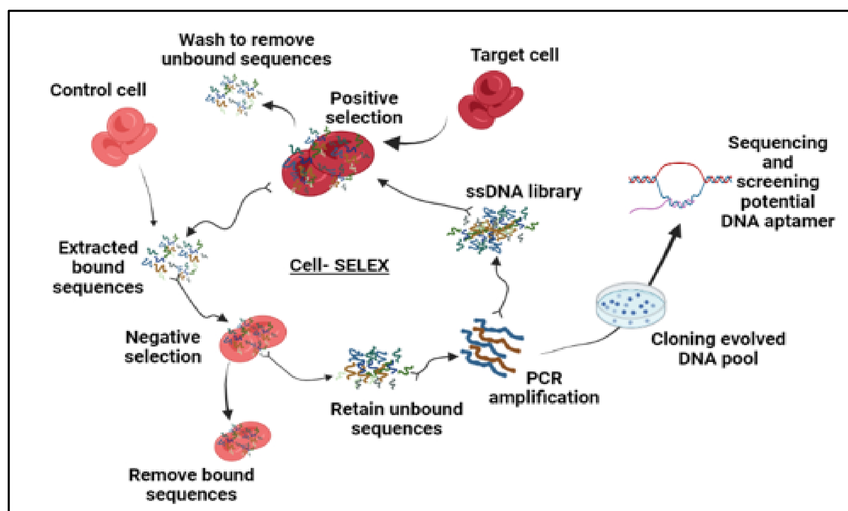

Fig 3: Show Aptamer-based detection of allergen (Liu *et al*., 2015) [32]

Table 4: Information about aptamer-based detection methods used for seafood allergens.

Allergen	Limit of detection	Working range	References
Tropomyosin	0.23 ng/mL	1-400 ng/mL	Tabrizi *et al*., 2017 [47]
Tropomyosin	2 nM	NR	Chinnappan *et al*., 2020 [7]
Tropomyosin	77 ng/mL	0.4-5 mg/mL	Zhang *et al*., 2018 [57]
Tropomyosin	4.2 nM	0.5-50 lg/mL	Zhang *et al*., 2017 [58]
Shrimp allergen	6.2 nM	25-1000 nM	Tah *et al*., 2018 [48]

*NR: Not recorded

10. Advantages and disadvantages of detection methods: DNA based detection methods have the following advantages

- **Strong stability:** DNA molecules are relatively more stable than proteins reducing the difficulty of allergen detection in processed foods.
- **Low cost of DNA molecules:** The probes and primers can be chemically synthesized at low cost than protein (Holzhauser, 2018) [22].
- **High selectivity:** It is easy to differentiate the same allergens DNA detection from shrimp and crab with the high homology of crustacean allergen.

Disadvantage: DNA-based detection methods cannot be used for identifying allergens with unknown nucleotide sequences.
The advantages of the protein-based detection methods are as follows:

- **High reliability:** Protein-based detection method directly uses allergens as the detection object thus reducing the possibility of false-positive results.
- **High sensitivity:** These methods are very sensitive can be achieved at a low detection limit.

Recently most studies on aptamer-based detection methods are coupled with biosensors that increase the cost of detection. Thus, the reliability of the aptamer-based detection method needs to be further verified and studied.

11. Conclusion

Seafood allergy is a major problem as seafood consumption is continuously increasing. Highly efficient, sensitive, and low-cost detection methods are needed to protect consumers. In this article, Seafood allergen detection methods are categorized into three classes: DNA- based, protein-based, and aptamer-based. There are several advantages and disadvantages of each method. This multiple detection methods is the main development trend in the future that can link the advantages of different methods and can improve the accuracy, specificity, cost reliability of these methods. Finally, the research on these novel technologies in these detection methods should be focused more and can be strengthened in future.

12. Disclosure statement

No potential conflict of interest was reported by the authors.

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