Allergen and Selenium Content in *Lutjanus Campechanus* and *Peneaus Vannaimei*

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Abstract: - Seafood is rich in primary protein source. However, seafood allergen causes health problem which affects the quality of life and may even threaten lives. Seafood comprises of fish and shellfish, whereas major allergen of fish is parvalbumin and for shellfish is tropomyosin. Prawn and crab are the major causes of anaphylaxis in both children and adults. *Lutjanus campechanus* and *Peneaus vannaimei* was analyzed by RT-PCR method to detect allergen content. Selenium is the one of trace elements which is necessary for proper functioning of human body and it contains both nutritional and toxicology interest. Selenium content in *Lutjanus campechanus* and *Peneaus vannaimei* were determined by AOAC method (Association of official Analytical Chemists). *Peneaus vannaimei* (126.32 mg/kg) contains more selenium content level compare to *Lutjanus campechanus* (85.32 mg/kg). The nutritional bioavailability of selenium in seafood depends on the fish and shellfish species and/or place where they are produced.

Key Words: — ***Lutjanus campechanus, Peneaus vannamei, Allergen, Selenium*.**

I. INTRODUCTION

Seafood is an increasingly important primary protein source to feed the world's ever increasing population. Seafood is an organism regarded as food from sea and fresh water by capture or culture, with fin fish and shellfish as two major subgroups (Linglin Fu et al., 2019). Major seafood allergen for fin fish is parvalbumin and for shellfish is tropomyosin, representing the two largest classes of animal-derived allergens according to the allergen database AllFam (Fu et al., 2017; Radaeur et al., 2008). So far, immunotherapy is hardly available to tackle seafood allergies (Zuidmeer-Jongejan et al., 2012).

Fish allergens have been identified in various parts of the fish, including fish muscle (Arif, 2009), skin, bones (Hamada et al., 2001; Kobayashi et al., 2016b), roe (Perez Gordo et al., 2008; Shimizu et al., 2009), milt (seminal fluid) (Liu et al., 2016) and blood (Nakamura et al., 2009). The major fish allergen is the small muscle protein parvalbumin (10–13 kDa, pI 4.1–5.2) with a conserved protein structure.

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The most commonly characterized allergenic fish parvalbumins from cod, salmon and carp were demonstrated to be responsible for 70 -100% of allergic reactions to fish and fish products (Sharp et al., 2015; Matricardi et al., 2016).

Shellfish contain two subgroups; they are molluscan (Abalones, Limpets, Squid, Clams, Oysters) and crustacean species (prawn, shrimp, crabs, lobsters). Tropomyosin is major allergen in shellfish species. Three allergenic proteins: tropomyosin, arginine kinase and triosephosphate isomerase, have also been identified in other shellfish groups (Thimo Ruethers et al., 2018). Tropomyosin is considered the major invertebrate pan-allergen found in all edible crustacean species. Clinical manifestations of shellfish allergy are very similar to fish allergy, resulting, not only from the ingestion of the offending food, but also from manipulating or inhaling the cooking vapours during food processing. Commonly, symptoms begin within minutes and may include oral allergy syndrome and cutaneous (urticaria, angioedema), gastrointestinal (vomiting, abdominal pains) and/or respiratory symptoms. Although less frequent, severe and systemic responses such as anaphylactic shocks may also occur upon shellfish consumption (Carrapatoso, 2004; Lopata et al., 2010; Yu et al., 2011).

Selenium is an essential trace element for humans, as obvious from its biochemical role as part of the active site in selenoproteins, such as glutathione peroxidase (Maria Plessi et al., 2001).The influence of dietary selenium upon the activity

of glutathione peroxidase is evident, and minimum selenium requirements have been fixed: the selenium requirement of adults is calculated to be 70 and 55µg/day for males and females, respectively (RDA, 1989). However, selenium plays an ambivalent role in relation to its concentration, high amounts possibly having toxic effects (Maria Plessi et al., 2001).

Selenium plays a significant role in many physiological processes in a direct or indirect way (R. Juhaszne Toth et al., 2018). The most important role of selenium is its antioxidant effect. This is expressed by the interaction with various enzymes (Awashti et al., 1975). It plays a key role in the function of the glutathione peroxidase enzyme, which responds to hydrogen peroxide and other harmful lipids and phospholipid hydroxides to prevent harmful free radicals, inhibit DNA damage and the development of metabolic active carcinogens. The amount is determined by the amount of selenium and reduced glutathione in our body (Meister and Anderson, 1983). Selenium is incorporated into the enzyme as selenocysteine, where the sulphur is located. In the body's antioxidant defence system, the biochemical property provides the importance of selenium to reduce the amount of sulphur more easily (R. Juhaszne Toth et al., 2018).

The normal human body (60-70 kg) contains 10 to 15 mg of selenium. Almost every cell of our body contains it, but most of the selenium accumulates in the kidneys, liver, spleen, pancreas, and testes. According to Codex Alimentarius Hungaricus (152/2009 (XI. 12) FVM), the recommended daily intake (RDA) of adults is 55 µg/day. According to the Institute of Medicine, Food and Nutrition Board (2000), the maximum limit of Se is 400 µg/day over which negative selenium effects are expected (Arthur, 1991). The first symptoms of selenium poisoning are metallic mouth taste, garlic smell breath, in chronic cases, hair loss, the loss of nails, skin rashes, discolouration of the teeth, and ultimately neurological disorders. Acute selenium toxicity only rarely causes death, and the lethal dose of selenium is 5-10 mg/kg (Olson, 1986).

II. Materials And Methods

A. RT-PCR Method for Detection of Allergen

Sample Collection, Storage and DNA Extraction:

Lutjanus campechanus and *Peneaus vannaimei* were collected from seafood market. They were stored in freezer and used for further studies.

CTAB buffer was prepared, use within 3 months, stored in the refrigerator at 4°C. CTAB solution was preheated at 65 °C before use to dissolve the precipitated particles.

DNA extraction was performed using a kit for automated isolation. The example given is for the MaxwellR 16 FFS Nucleic Acid Extraction Kit X9431. For the DNA extraction from the sample, continue as described:(a) 50 mg of sample (prawn and fish) material were weighed into a 1.5 mL tube.(b) 600 µL CTAB buffer was added, 30 µL of proteinase K (20 mg/ mL), and 2 µL of RNase A solution.(c) Vortex vigorously and incubated at least 60 min at 60 °C in a thermo shaker (1400 rpm).(d) Centrifuged for 10 min at ≥14,000 × *g*. The DNA obtained from the samples must be quantified with a spectrophotometer using the absorbance at 260 and 280 nm, and the concentration must be normalized to use in the downstream steps.

Design Primer and Probe:

A specific primers/probe set was designed by using Primer Express software (Applied Biosystems): a) forward primer for fish allergen (5′-GGC TCA TTA AAT CAG TTA TG-3′), reverse primer for fish allergen (5′-CCG AGT TAT CTA GAG TCA-3′) and fish allergen probe (5′-6-FAM-CCG TAC TTG GAT AAC TGT GGC AAT TC-BHQ1-3′) (Beatriz Herrero et al., 2013) and b) forward primer for crustacean allergen(5′-TAA AGT CTG GCC TGC CCA -3′), reverse primer for crustacean allergen (5′-GCT TTA TAG GGT CTT ATC GT-3′) and crustacean allergen probe(5′-6-FAM-TGC TAC CTT IGC ACG GTC A-3′) (Francisco J. Santaclara et al., 2017).

The PCR reactions were carried out in a total volume of 20 lL containing 100 ng of DNA template, 10 lL of TaqMan Fast Advanced Master Mix (Applied Bio systems), primers and probe and molecular biology grade water (Eppendorf) to adjust to the final volume.

The optimal concentrations of primers and probe were evaluated by preparing dilution series. To determine the minimum primer concentrations giving the maximum ΔRn (the maximum fluorescence emission intensity and the lowest threshold cycle, Ct), forward and reverse primer concentrations of 50, 300, 500, and 900 nmol/L were used. All were evaluated at a constant probe concentration of 50nmol/L. Subsequently, the probe concentration was optimised with concentrations of 50–250 nmol/LM (Murray & Curran, 2005).

The reactions were performed in triplicate on DNA samples in MicroAmp Optical 96-well reaction plates (Applied Biosystems) with MicroAmp optical caps (Applied

Biosystems) using the ViiA 7 Real-Time PCR System (Applied Biosystems). Amplification was carried out with the following conditions: 95 °C for 20 s and 40 cycles each of 95 °C for 1 s and 60°C for 20 s (Francisco J. Santaclara et al., 2017).

Program and Run Applied Biosystems Viia 7 System:

The Applied Biosystems ViiA 7 System and SDS (Sequence Detection System) software according to the manufacturer's recommendations was operated. The reaction volume was set upto 20 µL, and the amplification program (unless other parameters are established in the internal methodological validation).FAM/LNA as detector/quencher was used. Briefly, launch the SDS software and open a new plate template window to denote well locations on the 96-well plate for the controls, and testing samples. The template window was saved with the recorded data as a SDS run file. The "Connect" button was selected, and then loaded the 96-well plate into the instrument. The "Start" button was selected to begin the actual run. Run the PCR reaction to determine Ct (cycle threshold) values for each sample (Francisco J. Santaclara et al., 2017).

Data Collection and Analysis:

After the run is completed, selected the baseline, and place the threshold line at the exponential phase of amplification. Removed the plate from the instrument and saved the results of the run. When the analysis button is selected in the SDS software, the results were analyzed automatically if the standards and testing sample information were recorded as noted in the previous section. The Ct values obtained thoroughly reviewed and interpretation of the results must be made in relation to data obtained in the internal validation performed in each laboratory for a given detection limit, allowing to determine whether a sample contains or does not contain crustaceans in its composition (Francisco J. Santaclara et al.,2017).

B. AOAC Method for Detection of Selenium Content in Lutjanus Campechanus and Peneaus Vannaimei

Principle:

AOAC method measures element- emitted light by optical spectrometry. Digested materials are nebulized into radio-frequency inductively coupled plasma. Spectra of elements are dispersed by grating spectrometer, and intensities are measured by photomutiplier tubes. Correction for background and interferences are required (George W Latimer, 2019). .

Preparation of Standards:

Prepared 100ml of an aqueous solution that contains 2ml 50% HNO3 and 10ml 50% HCl, and (per litre) 200mg of selenium. Use to test that interference correction factors yield data within ±3 standard deviations of calibration means (George W Latimer, 2019).

Preparation of Test Solutions:

1-2 g of weighed test portion of well-mixed material and to nearest 0.01g and transfer to 250ml beaker. On dry weight basis, dry another portion of material to constant weight to determine wet/dry weight ratio, but do not digest and analyze during drying, or extractability of analytes could be altered.10ml 50% HNO3 was added to undried test portion and mix. Beaker was covered with watch glasses; heated to 95°C and let solution reflux 10-15 min. Let digest cool, 5ml concentrated HNO3 was added, watch glass was replaced and the solution was refluxed another 30 min at 95°C (George W Latimer, 2019).

Evaporated solution to 5 ml without letting any section of beaker bottom go dry. The solution was cooled, 2ml water was added and 3ml 30% H2O2, covered with watch glass, and heated slowly to initiate peroxide reaction. Continuously heating beaker until effervescence subsides. The solution was cooled, and 7 ml 30% H2O2 was added in 1ml portions with warming. The solution was cooled, 5ml concentrated HCl was added, and 10ml of water, covered with watch glass. And the solution reflux additional 15 min without boiling. The solution was cooled, diluted to 100ml with water and it was mixed. Any particulate matter was removed in digest by filtration, centrifugation or settling. If any analyte level exceeds linear range, dilute digest further with calibration blank solution (George W Latimer, 2019).

Determination:

An instrument response were stable (usually 30 min after plasma is started), profile and calibrate instrument according to manufacturer instructions. Flush system for ≥1 min with calibration blank solution before introducing standards or test solutions. Calibration accuracy was verified (±10%) by analysis of certified reference solutions as quality control standards before any test solutions are analyzed. Instrument check standards and calibration blank was analyzed before each 10 test solutions to confirm that calibration is still acceptable. Interelement and background corrections (±3 standard deviations of mean) were verified by analysis of interference check standard at beginning and end of analysis session and at least twice in each 8h period. Recommended quality control includes at least reagent blank for each batch of test solutions, and one duplicate digest each 20 test solutions. For new or unusual matrices, absence of significant interference is

indicated by 5-fold dilution that is within 10% of expected value (spikelevel should be 2-4 times unspiked concentration). When matrix interference is indicated, diution or the method of standard additions can usually compensate for the effect (George W Latimer, 2019).

III. Results And Discussion

A. Allergen Content in Lutjanus Campechanus and Peneaus Vannaimei

A RT-PCR probe system targeting the 18S rRNA and 16S rRNA gene was proposed for the detection and quantification of fish and crustacean species. RT-PCR method was used for detection of allergen in Lutjanus campechanus and Peneaus vannaimei samples. Both Lutjanus campechanus and Peneaus vannaimei does not contain tropomyosin and parvalbumin allergen content, so they are eatable seafood which will not cause allergic reaction to humans. RT-PCR method provides advantages with respect to accuracy, sensitivity, specificity, dynamic range, high-throughput capacity, and lower risk of contamination, and reduces the time for analysis.

B. Selenium Content in Lutjanus Campechanus and Peneaus Vannaimei

Selenium is important trace elements which contain both nutritional and toxicological effect. Selenium content level in Lutjanus campechanus and Peneaus vannaimei are determined by AOAC method. Selenium in Peneaus vannaimei contains 126.32 mg/kg whereas Lutjanus campechanus contain 85.32 mg/kg. Peneaus vannaimei contain higher selenium content when compare to Lutjanus campechanus.

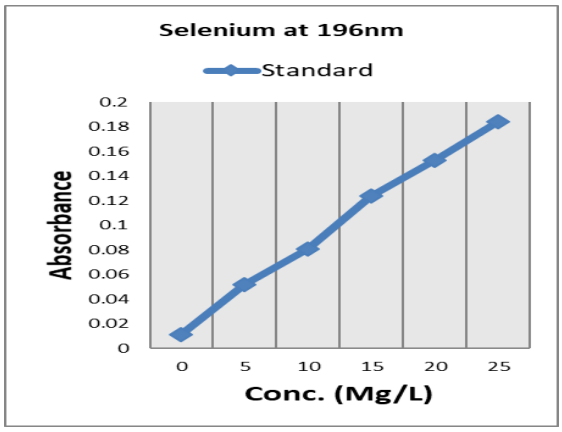

Fig.1. Absorbance versus concentration (mg/l)

Fig.1 graph was plotted between absorbance vs concentration (mg/l) were selenium calibrated standard concentration are represents the x-axis and their calibrated absorbance value represent the y-axis. Using calibrated standard unknown sample concentration was determined.

IV. Conclusion

Food allergies are recognized as a food safety issue. Fish are one of the main causes of food hypersensitive reactions. A major allergen in fish is parvalbumin, whereas tropomyosin is major allergen in crustacean and molluscan shellfish. Fish and shellfish allergen causes IgE antibody mediated adverse reactions. A real-time PCR probe system targeting the 18S rRNA and 16S rRNA gene was proposed for the detection and quantification of fish and crustacean. Lutjanus campechanus and Peneaus vannaimei does not contain allergen, so they are eatable seafoods which will not cause any allergy to humans. A real-time PCR method provides advantages with respect to accuracy, sensitivity, specificity, dynamic range, high-throughput capacity, and lower risk of contamination, and reduces the time for analysis. RT-PCR method is a useful tool to address questions regarding food quality and security.

Selenium plays an important role in the proper functioning of the entire human body. Selenium in Peneaus vannaimei contains 126.32 mg/kg whereas Lutjanus campechanus contain 85.32 mg/kg. The nutritional bioavailability of selenium in seafood depends on the fish and shellfish species and/or place where they are produced. Seafood materials appear to contain nutritionally effective low-molecular-mass organo-selenium compounds that have not yet been chemically identified. High consumption of selenium intake in humans causes toxicity humans.

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