



Molecular Investigation of the Impact of Thermal Processing Techniques on Tropomyosin Crustacean Allergens

Elif Tuğçe Aksun Tümerkan^{1,2,a,*}

¹Department of Food Processing-Food Technology, Ankara Yıldırım Beyazıt University, Vocational School of Health Services, Ankara, Türkiye

²AYBU Central Research Laboratory, Application and Research Center, Ankara Yıldırım Beyazıt University, Ankara 06010, Türkiye

*Corresponding author

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ABSTRACT

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While shellfish species are widely consumed due to their nutritional advantages, they are also among the top eight food items for food-borne allergies. Five distinct thermal processing techniques were applied to the crustacean to investigate the tropomyosin level variations caused by heat processing. Fresh shrimp and prawns were utilized as controls for the determination of allergen-encoding genes. Prior to molecular analysis, the proximate composition and acidity of raw and processed samples were also performed. The yield and purity of DNA were also determined. Melting curve and gel electrophoresis tests verified the existence of allergen-coding genes. Thermal processing procedures affected the proximate composition, particularly the total protein and fat concentrations, according to the findings. Following the heat treatment, the pH levels decreased, particularly in the grilled samples. There were also significant differences in the quantity and quality of the extracted DNA. Regardless of crustacean species, the tropomyosin-encoding gene was detected in both fried and grilled samples. These findings demonstrated that RT-PCR identification and validation of the crustacean allergy gene by gel electrophoresis might be a reliable approach for the thermally treated shrimp and prawn samples. This study shows that investigating the allergen coding gene might provide a viable way for detecting food-borne allergens in other thermally processed food items, which are becoming more concerned about food safety.

^a etaksun.tumerkan@aybu.edu.tr

<https://orcid.org/0000-0003-1993-0569>



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Introduction

Because of its health-promoting characteristics and ease of processing, seafood has become an important part of the human diet. With a better understanding of the necessity of these crucial animal-based protein sources, global seafood consumption has increased. Shellfish is a significant portion of the seafood sector, with yearly consumption reaching around 4.50 kg per capita globally (Kumar, Yadav et al., 2023). Shrimp and prawns are considered beneficial seafood items that account for approximately 15% of the total market size of the global traded fishery and aquaculture industries, accounting for nearly US\$ 43 billion, and this rate is expected to reach approximately 6 million tons per year by 2024 (Khan and Azam, 2021). Similar to other seafood, stir-frying, steaming, boiling, and baking are the most commonly applied processing techniques for both shrimp and prawns. Food-borne allergens can result from consumption, inhalation, or contact with other allergen-specific compounds found in food items (Ward 2015; Nayak; Li et

al., 2017). Due to the lack of complete treatment for food allergies, food-borne allergies have been declared the fourth most critical public health problem by the World Health Organization (Amponsah and Nayak, 2018). Seafood allergens, specifically parvalbumin isolated from fish species and tropomyosin isolated from shellfish, have been recognized as major allergens in the food industry and belong in the “big eight” list, which represents the eight food items responsible for approximately 90% of all food-borne allergies. (Nwaru, Hickstein et al., 2014). Shrimp and prawns are responsible for more than 80% of the total occurrences of crustacean allergy, and tropomyosin is accepted as the primary allergenic protein in these species (Ruethers et al., 2018). Due to the thermal resistance characteristic of tropomyosin, several studies have been performed on the determination of the variation in allergenicity driven by different heat processing approaches.

Xu et al. (2020) and Lv et al. (2021) highlighted that stir-frying, boiling, and canning processes lead to variation in the allergenicity of tropomyosin. It's well known that proximate composition, especially fat and protein levels of food items, causes variation in the allergenicity of food items; additionally to protein-based interaction in the allergen mechanism, lipid structures also widely impact the allergenicity of food products (Moreno, 2007). Acidity of food products is another factor playing an important role in the allergen capacity that can differ depending on the properties of the food, processing methods, and storage conditions.

Tiwari, (2004) highlighted the impact of pH on both the allergenicity of almonds and the achievements of allergen detection analyses. Detecting food-borne allergens with a reliable method is the first step in the allergen treatment, elimination, and correct labeling system (Fu et al., 2019). As an alternative to traditional protein-based analyses, DNA-based techniques are mostly preferred due to their high achievement rate caused by DNA being more resistant than protein to heat, pressure, or acidity (Jayasena et al., 2019). DNA-based techniques, especially real-time PCR, have been accepted as official methods by governmental laboratories for the detection of food-borne allergens in Japan and Germany in recent decades (Xu et al., 2020). The achievement of real-time PCR methods in the detection of allergens from various foods, from peanuts to lobsters, has been reported by several researchers; more recently, Aksun Tümerkan (2022) reported that the parvalbumin differences were detected by RT-PCR and confirmed by gel electrophoresis in commercial canned tuna.

Due to the fact that food-borne allergens are considered one of the riskiest fraudulent actions in terms of public health and food security concerns, a better understanding of allergen variations in crustaceans could be useful for both industry and academia. Thus, the aims of this study were to determine the impact of both different thermal processes and proximal differences on the crustacean's allergen. Within these aims, any potential variations in the presence of crustacean allergens depending on thermal processes such as boiling, grilling, frying, baking, and steaming are investigated based on the allergen-encoding gene by RT-PCR and confirmed by gel electrophoresis.

Materials and Methods

Deep-water pink shrimp (*Parapenaeus longirostris*, L) and green tiger prawn (*Penaeus semisulcatus*) were supplied by a local retailer in Izmir, Türkiye, in early 2022. The caught individuals from prawns and shrimp were kept in ice-covered polystyrene boxes and transported to the laboratory directly within the cold chain. Fresh shrimp and prawns were washed in distilled water until they were free of external contaminants. Shells, heads, legs, tails, and veins were removed by a sharp blade prior to further cooking treatments. The total abdominal muscle amount of shrimp and prawns was divided into six groups; one of the sub-groups was stored in raw form and considered the control group.

Thermal treatments

Baking: Edible shrimp and prawn samples are placed on the aluminum foil-covered metal tray and then

transferred to the preheated electric oven. The samples were baked at 200°C for 4 minutes (Lasekan and Nayak, 2016).

Frying: 500 g of the shrimp and prawn meats were fried in boiled sunflower oil (1:2, g/mL) for 10 min. in the non-stick deep fryer. The shrimp and prawn samples were stir-fried for 10 min until they became crisp-tender (AlFaris et al., 2021).

Grilling: Shrimp and prawn samples are placed in a gas-operated, aluminum foil-covered oven. The prawn and shrimp samples were grilled individually at 180 °C for 10 min. (Abd-Elghany et al., 2020).

Boiling: 500 g of shrimp and prawn meat and 300 mL of boiling water were poured into a pot for 10 min. in a stainless-steel pan. Then, they were placed on filter paper to drain excess water excess (Abd-Elghany et al., 2020).

Steaming: Edible shrimp and prawn samples are placed on the steamer basket, which is found at the top of the steamer pot. When the water started boiling, the temperature was measured by the probe that was located in the water, reaching up to 100 °C, and then samples were steamed for 10 min. (Khan and Azam, 2021).

After thermal processing, all the moisture and oil were removed by special filter papers. The processed samples were homogenized, stored in sterile falcon tubes, and stored at -80°C until the time of further experiments.

Proximate Composition and pH Measurements

The proximate composition of the raw and thermally processed crustacean samples was analyzed by the following techniques: The crude protein level was calculated according to the Kjeldahl method (AOAC, 1998a), and the conversion factor was accepted as 6.25 for the determination of the crude protein level calculation. The moisture and ash content of raw and processed crustacean samples were analyzed following the AOAC method by the gravimetric method (AOAC, 2000). The total lipid content of the sample was analyzed according to the method of Bligh and Dyer (1959) based on chloroform-methanol solution extraction. The pH of the raw and thermally processed samples was measured using a calibrated 315i/SET pH meter (Weilheim, Germany), as described by Mohan (Mohan et al., 2014).

DNA extraction

DNA was extracted from the raw and cooked shrimp and prawn samples using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol with minor modifications such as extending the Proteinase K digestion at 56 °C. Briefly, a 20 mg grounded sample was lysed with lysis buffer and Proteinase K (Thermo Scientific™) until no tissue was visible. The DNA pellet was dissolved and applied to the silica column. Then the spin column was washed with AW1 and AW2 solutions. Finally, DNA was eluted with pre-heated elution buffer. The purified DNA was stored at -20 °C until further experiments.

Quality and yield assessment of Extracted DNA

The DNA yield and quality parameters, such as purity of DNA and the presence of any contaminants in the isolated DNA, were determined by the absorption rate at 230, 260, and 280 nm by a NanoDrop 1000

spectrophotometer (NanoDrop™ 2000/2000c, Thermo Scientific, Pittsburgh, PA, USA). All the analyses were performed in triplicate for each group.

Assay design and Real-time PCR

The shrimp tropomyosin gene sequence performed according to the procedure described by Kim et al., (2019), libraries were prepared using as the target sequence a hypervariable 71 bp region by the Clustal Omega program (Table 1). As an internal control, the primer pair was utilized to amplify the 18S rRNA gene region. Real-time PCR was performed in a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). SYBR Green reaction mix and thermal cycles were performed according to the manufacturer's method. The melting curve was acquired by quantifying fluorescence during heating from 65 to 95 °C at a rate of 5 °C per second in the Applied Biosystems system.

Gel Electrophoresis Analysis

Strop-qPCR positivity and the correction of PCR product size were authenticated by gel electrophoresis. 2% agarose gel in Tris-Sodium Acetate-EDTA (TAE) buffer, including 1× SYBR Safe DNA Gel Stain at 100 V. Following the electrophoresis, the gel was visualized in the gel documentation system.

Statistical analysis

The data was analyzed using SPSS 22.0 software (Chicago, IL, USA) by ANOVA and Tukey post-hoc tests at a P value of 0.05 (P<0.05) to identify significant variance among the groups. All analyses were performed in triplicate.

Results and Discussion

Proximate composition and pH Differences among Raw and Processed Samples

The proximal composition of crustaceans is changed not only by the properties of the raw material but also by the type of processing methods, which differ by processing time, temperature, and whether there is any external oil or brining solution (Mielcarek et al., 2020). The differences in proximal compositions of raw and thermally processed crustaceans are given in Table 2. The proximal analyses results revealed that the protein level was altered among the raw and processed crustacean samples. A common reduction in protein level can be explained by protein denaturation based on high temperatures during thermal processes (Puthanangadi Dasan et al., 2021). Similarly, moisture and ash levels also decreased during thermal processing in both shrimp and prawn samples, which can

be explained by physically removing moisture during the heat process. As seen in Table 2, for the fried samples, the total lipid rate decreased in other processing methods compared to the raw form of the crustacean sample. Statistically significant differences were found in the lipid content between raw and fried shrimp and prawn. This increase can be related to the absorption of external oil during the frying process (Arwani et al., 2022).

Statically important variations were found in the protein, lipid, and ash levels between raw (unprocessed) shrimp and prawn. While the protein content was determined to be 22.1% in raw shrimp, this rate was found to be 24.7% in raw prawn. There were significant differences detected in the thermally processed shrimp and prawn samples, which can be driven by processing temperature and processing time. The highest and lowest crude protein rates were determined in baked (21.4%) and fried samples (5.8%) among the shrimp groups. In the prawn samples, the highest and lowest crude protein rates were found in the steamed (22.6%) and fried samples (6.4%). Relatively higher lipid levels were found in both fried shrimp (16.9%) and fried prawn (12.6%) among the thermally processed samples. The lowest lipid content was found in the grilled shrimp (2.2%) and grilled prawn samples (1.1%). The frying process also significantly decreased (P<0.05) the moisture content in both shrimp and prawn samples, which can be related to an increased lipid level and applied heat transfer. Among the thermally processed samples, relatively higher moisture levels were detected in the boiled shrimp (71.4%) and boiled prawn (70.4%). This higher moisture can be related to water retention in the edible part of crustaceans during the boiling process. These results clearly show that the usage of external oil or process temperature directly impacts the proximal composition of crustacean species. The proximal composition has importance for not only nutritional benefits to human health and, consequently, public health, but also for the determination of any hazardous components in the food matrix (Rao et al., 2020). The proximal differences in the raw and processed crustacean samples were detected. Significant differences were found in the raw and prawn samples in terms of protein and lipid levels. Similar differences were also reported in the prawn (*Microtrichium rosenbergii*) and shrimp (*Penaeus monodon*) (Islam et al., 2017). The main differences were observed in the protein and lipid content following the frying process. The protein level decreased and the lipid level increased in both fried shrimp and prawn samples. These differences are in accordance with Arwani et al., (2022) who stated that protein reduction and lipid levels increased following the frying process in the shrimp.

Table 1. Primer sets assessed in this study.

Primer name	Sequence (5' 3') Access no.	Target gene region	Amplicon Base Pair (bp)	Access no.	Reference
StropF	TGTTGGTTGAGCACCTCCTA	Lit v 1 (Shrimp tropomyosin)	71	EU410072	(Kim et al., 2019)
StropR	GCTTCATCGCCTGCATCTTC				
M18S-F	CAGGTCTGTGATGCCCTTAG	18S rRNA	160	-	
M18S-R	GCTTCATCGCCTGCATCTTC				

Table 2. Proximate composition in the raw and processed crustacean samples

Sample	Crude Protein	Total lipid	Moisture	Ash	pH
SR	22.1±0.04 ^c	4.4±0.08 ^c	72.2±1.10 ^d	0.81±0.03 ^c	8.84 ^e
SBA	21.4±0.12 ^c	3.2±0.16 ^d	69.4±1.08 ^c	0.69±0.07 ^a	6.49 ^b
SS	20.9±0.08 ^b	3.0±0.08 ^d	68.2±0.84 ^c	0.67±0.17 ^a	6.72 ^b
SBO	19.6±0.14 ^b	2.6±0.13 ^c	71.4±0.36 ^d	0.75±0.13 ^b	7.05 ^c
SF	5.8±0.20 ^a	16.9±0.07 ^g	59.8±1.20 ^a	0.78±0.09 ^c	7.29 ^c
SG	20.4±0.06 ^b	2.2±0.19 ^c	68.5±1.07 ^c	0.85±0.07 ^d	6.14 ^b
PR	24.7±0.14 ^d	2.4±0.12 ^c	71.5±0.07 ^d	0.89±0.21 ^e	8.50 ^d
PBA	21.4±0.16 ^b	2.2±0.08 ^c	63.9±0.27 ^b	0.81±0.07 ^c	6.58 ^b
PS	22.6±0.22 ^c	1.8±0.04 ^b	65.7±0.16 ^b	0.71±0.03 ^b	6.29 ^a
PBO	20.3±0.07 ^b	1.4±0.08 ^b	70.4±0.16 ^d	0.84±0.08 ^d	7.16 ^c
PF	6.4±0.12 ^a	12.6±0.18 ^f	58.6±0.22 ^a	0.81±0.11 ^c	7.24 ^c
PG	21.7±0.18 ^b	1.1±0.05 ^a	64.8±1.28 ^b	0.76±0.12 ^b	6.05 ^a

SR: Raw shrimp, SBO: boiled shrimp, SF:fried shrimp,SBA:baked shrimp,SS steamed shrimp,SG:grilled shrimp,PR:raw prawn, PBO: boiled prawn, PF: fried prawn, PBA:baked prawn,PS:steamed prawn,PG:grilled prawn.Data are expressed as mean value ± standard deviation of triplicates. Values followed by different letters indicate significant differences (P<0.05) Values in a same column followed by different numbers indicate significant differences of the parameter with respect to groups

Table 3. DNA yield and Quality of raw and processed forms of crustacean samples

Sample	DNA Yield (µg/µL)	Purity (A260/A280)	Chemical Contamination (A260/A230)
SR	1551.20±0.13 ^c	2.02±0.02 ^c	2.30±0.01 ^c
SBO	556.70±0.02 ^c	2.10±0.04 ^c	2.30±0.02 ^c
SF	1189.10±0.06 ^c	2.06±0.02 ^c	2.30±0.05 ^c
SBA	842.70±0.08 ^d	2.10±0.03 ^c	2.11±0.04 ^c
SS	562.40±0.04 ^c	2.00±0.01 ^c	2.28±0.02 ^c
SG	1458.10±0.05 ^c	2.10±0.02 ^c	2.30±0.05 ^c
PR	52.50±0.04 ^b	1.26±0.03 ^b	-0.59±0.02 ^b
PBO	4.60±0.03 ^a	1.10±0.04 ^b	-0.70±0.02 ^b
PF	12.50±0.04 ^a	1.46±0.02 ^b	20.86±0.04 ^d
PBA	5.40±0.04 ^a	2.00±0.01 ^c	4.03±0.03 ^c
PS	1.00±0.04 ^a	0.62±0.02 ^a	-1.98±0.02 ^a
PG	4.00±0.04 ^a	1.15±0.03 ^b	-0.59±0.07 ^b
PR	52.50±0.05 ^b	2.02±0.04 ^c	2.30±0.03 ^c

SR: Raw shrimp, SBO: boiled shrimp, SF:fried shrimp,SBA:baked shrimp,SS steamed shrimp,SG:grilled shrimp,PR:raw prawn, PBO: boiled prawn, PF: fried prawn, PBA:baked prawn,PS:steamed prawn,PG:grilled prawn.Data are expressed as mean value ± standard deviation of triplicates. Values followed by different letters indicate significant differences (P<0.05) Values in a same column followed by different numbers indicate significant differences of the parameter with respect to groups

The pH differences in the raw and thermally processed shrimp and prawn samples are presented in Table 2. The higher pH values were determined in the raw form in both shrimp and prawn samples (8.84 and 8.5, respectively). The pH values of processed samples decreased among all thermally processed groups, regardless of the crustacean species. The highest pH values were determined in the fried shrimp (7.29) and fried prawns (7.24) among the thermally processed groups. This relatively higher pH value can be correlated with the lowest moisture level and denatured protein rate (Rabie et al., 2016). The sharp decreases were observed in the grilled sample, with 6.14 and 6.05 for grilled shrimp and grilled prawn, respectively. Relatively higher pH value and lowest protein level fried sample in both shrimp and prawn revealed that the pH impact on the protein level of the food sample Immersion of vapor, heat penetration, and interaction of other compounds cause variations in the protein structures and, consequently, the allergenicity of food (Chi and Cho, 2016). Venugopal and Gopakumar (2017) highlighted that boiling and steaming processes also affect the proximate composition of crustaceans. The pH level also changed depending on the thermal processing methods. The pH level decreased following all processing methods in both shrimp and prawn. This reduction is in accordance with Bello (2013), who reported that the pH level decreased from 8.50 to 4.50 depending on heat treatment in the prawn.

The yield and quality of extracted DNA

While the DNA yield has no effect on the PCR amplification, it is one of the key factors impacted by the thermal processing of food samples. The DNA yield was determined with the sample weight, DNA concentration, and the final volume for each group. The DNA yield of raw forms was determined to be significantly higher than that of thermally processed shrimp and prawn samples. Surprisingly, higher DNA yields were observed in all shrimp groups, regardless of whether they were raw or thermally processed, than in prawn groups; these significant differences revealed that even the same pre-treatments or extraction methods were applied to samples. The yield of extracted DNA can vary from species to species, which could be related to the thermal integrity of the raw material. In the processed shrimp and prawn sample groups, DNA yields differed depending on thermal treatment. These significant differences can be explained by the processing time and temperature that limit the DNA extraction process. The highest DNA yield was determined from the steamed samples (1458.10 µg/µL) and the prawn samples (12.90 µg/µL) for shrimp and prawn samples (Table 3). The relatively lower DNA yield determined from the grilled sample in both shrimp (502.40 µg/µL) and prawn samples (1.02 µg/µL) These significant variances can be explained by DNA degradation.

The purity of DNA from raw and thermally processed shrimp and prawn samples is shown in Table 3. The purity of shrimp samples was found to be relatively lower in the prawn samples that were processed with the same procedure, even in the raw form of the crustacean samples. The purity of DNA from raw and thermally treated prawn groups was found to be statistically different, and the highest and lowest purity values were found in the baked and steamed prawn samples, respectively. These differences can be explained by the different levels of thermally degraded DNA (Quintrel et al., 2021). Another key factor in the achievements of DNA-based methods in the food industry is the A260/A230 ratio, which indicates the presence of contaminants such as salts, oils, or carbohydrates (Babić et al., 2018). Among all the raw and thermally processed shrimp and prawn groups, the highest contaminants rate was found in the fried shrimp (22.30) and fried prawn (20.86), which can be related to the use of external oil during the frying process (Table 3). The optimal limits (2.0–2.2) were not detected in any of the prawn samples. These significant variances revealed that the same processing techniques can result in differences in DNA quality even in cryptic species. Thermal processing heavily affects the quality and integrity of isolated DNA from food items, especially seafood, which is known to be highly perishable due to quality degradation (Tsai et al., 2023). The quality loss of extracted DNA can directly result from the heat treatment, and the presence of any additives during processing can also increase the intensity of DNA fragmentation, which may cause a lack of amplification (Pascoal et al., 2008). The lowest DNA yield was found in the grilled samples, regardless of the crustacean species. These results agree with Tümerkan (2021), who reported that the lowest DNA yield was found in the thermally processed fish sample. The purity of DNA from raw and processed groups was found to be statistically different. These differences are consistent with the result of Musto (2011), who proved that the variances in the DNA quality and integrity of animal-based protein sources are impacted by various cooking methods. The highest contamination rates were found in the fried samples, which could be attributed to the damaging effects of oil accelerated by heat treatment on the raw samples during the frying process. This result is in agreement with previous findings that the frying process impacted the detection limit of food items in DNA-based methods (Eischeid, 2019). These findings were generally in agreement with other research interpreting the differences in the allergenicity of crustacean and mollusk species driven by thermal processing approaches.

Tropomyosin Gene Detection as a Marker for Crustacean Allergens in Raw and Processed sample

In this research, crustacean allergen-coding sequences were used as targets for reliable molecular methods for the detection of shrimp and prawn allergens in both raw and thermally processed samples. For that purpose, five different cooking methods were applied to both shrimp and prawn samples. Due to the less than 100-bp gene region, it commonly reduces the DNA degradation in highly processed food (Lit V3) gene regions used for tropomyosin detection (Rao et al., 2020). To confirm, experimentally, an internal primer (M18S) 18S rRNA was used for testing the specificity of the primers. Fig. 1 presents the assay of

PCR fluorescence plots, including melting curves, and the amplification for both tropomyosin (Fig. 1.A, C) and internal control (Fig. 1.B, D), respectively. These results clearly demonstrated that, allergen-coding gene region can be used alternatively for determination of the presence of the food-borne allergen in the different matrices.

Any tropomyosin-encoding gene was not detected in the raw form for shrimp and prawn; sharp peaks with identical melting points were observed by melting curve analysis in the fried and grilled shrimp and prawn samples. As seen in Figure 1.A, relatively higher T_m values were found in the grilled and fried shrimp and prawn samples (G9, G10, G11, and G12).

Fried and grilled samples were determined to be allergen-positive groups, which means the different processing techniques impact the allergenicity of crustacean samples. As seen in Fig. 1.C, clear distinctions between amplicons based on tropomyosin-encoding genes were determined. The results of the melting curve and amplicons clearly demonstrate that allergen-encoding genes were detected by the RT-PCR technique. Owing to the achievements of RT-PCR in the allergen coding gene, a wide range of research has been conducted to determine foodborne allergens in different products without any protein-based method confirmation. For example, Sanchiz et al., (2021) and Torricelli et al., (2020) reported the allergen encoding gene in the various food samples, such as peanut, sesame, and pistachio, was changed by different processing methods. More recently, Aksun Tümerkan (2022) highlighted the achievements of fish allergen encoding gene detection by RT-PCR. Due to gel electrophoresis, confirmation plays an important role in the achievements of RT-PCR analyses to prevent any misinterpretation resulted by SYBER-green dye. Following the RT-PCR, the allergen-encoding gene was also analyzed on the gel electrophoresis to confirm. As seen in Figure 2, clear bands were detected in the fried and grilled shrimp and prawn samples, which obviously confirmed the specificity of the RT-PCR and agarose electrophoresis techniques. There was no band observed in the other raw and thermally processed samples.

The results of gel electrophoresis are in accordance with Li et al., (2021) who reported the achievements of allergens in both dairy products and shrimp balls using the same techniques. Interestingly, (Khan et al., 2019), highlighted that the thermally degraded tropomyosin can be recovered by cooling at the ambient temperature ($\sim 25^\circ\text{C}$). This important characteristic of tropomyosin has increased attention for food safety due to the fact that shrimp and prawns can be consumed at room temperature. Structural changes of allergen-response proteins occur influenced by the properties of the food matrix, applied processing methods, and storage conditions until they reach consumers. The alteration of allergens driven by thermal treatment in food products is commonly caused by the peptide bonds hydrolysis, aggregation of both disulfide and non-covalent bonds and denaturation of allergen respond mechanisms (Laly et al., 2019). The tropomyosin coding gene were found in the fried and grilled crustacean samples. While, Shriver et al., (2011) claimed that boiling of shrimp did not change the tropomyosin, other researchers reported that major heat-resistance allergen was found in the boiled, giant river prawn and shrimp samples (Lasekan and Nayak, 2016).

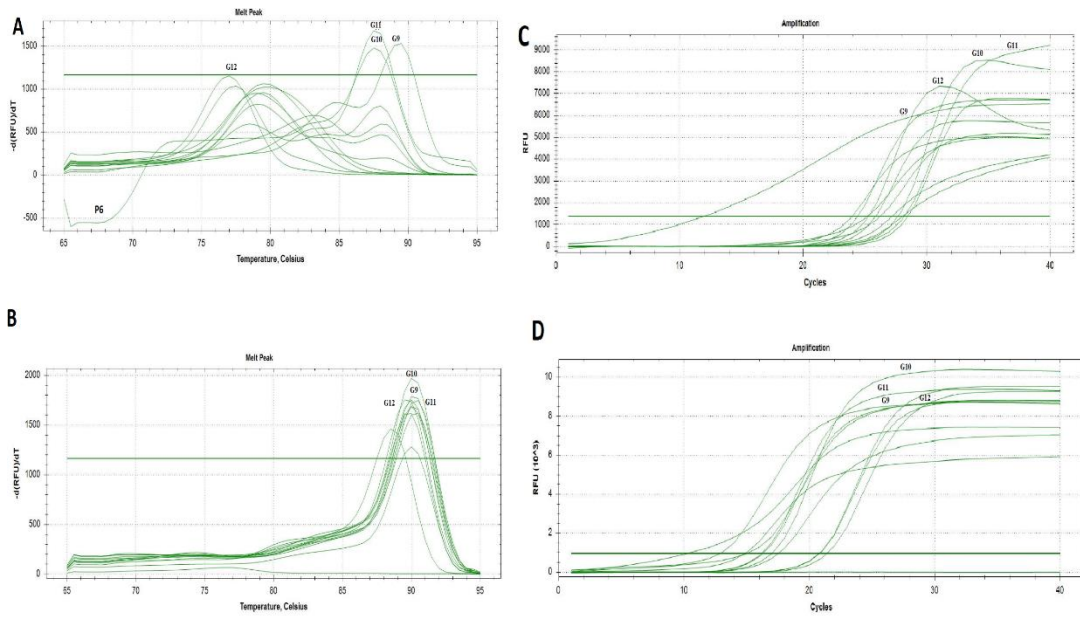


Figure 1. Melting curves (A, B) with Sybr-Green dye targeting the Litv3 and M18s of crustacean sample, Real-time PCR amplification (C, D)
 (G1: Raw shrimp; G2: raw prawn; G3: Baked shrimp; G4: Baked prawn; G5: Steamed shrimp; G6: Steamed prawn; G7: Boiled shrimp; G8: Boiled prawn; G9: Fried shrimp; G10: fried prawn; G11: Grilled shrimp; G12: Grilled prawn)

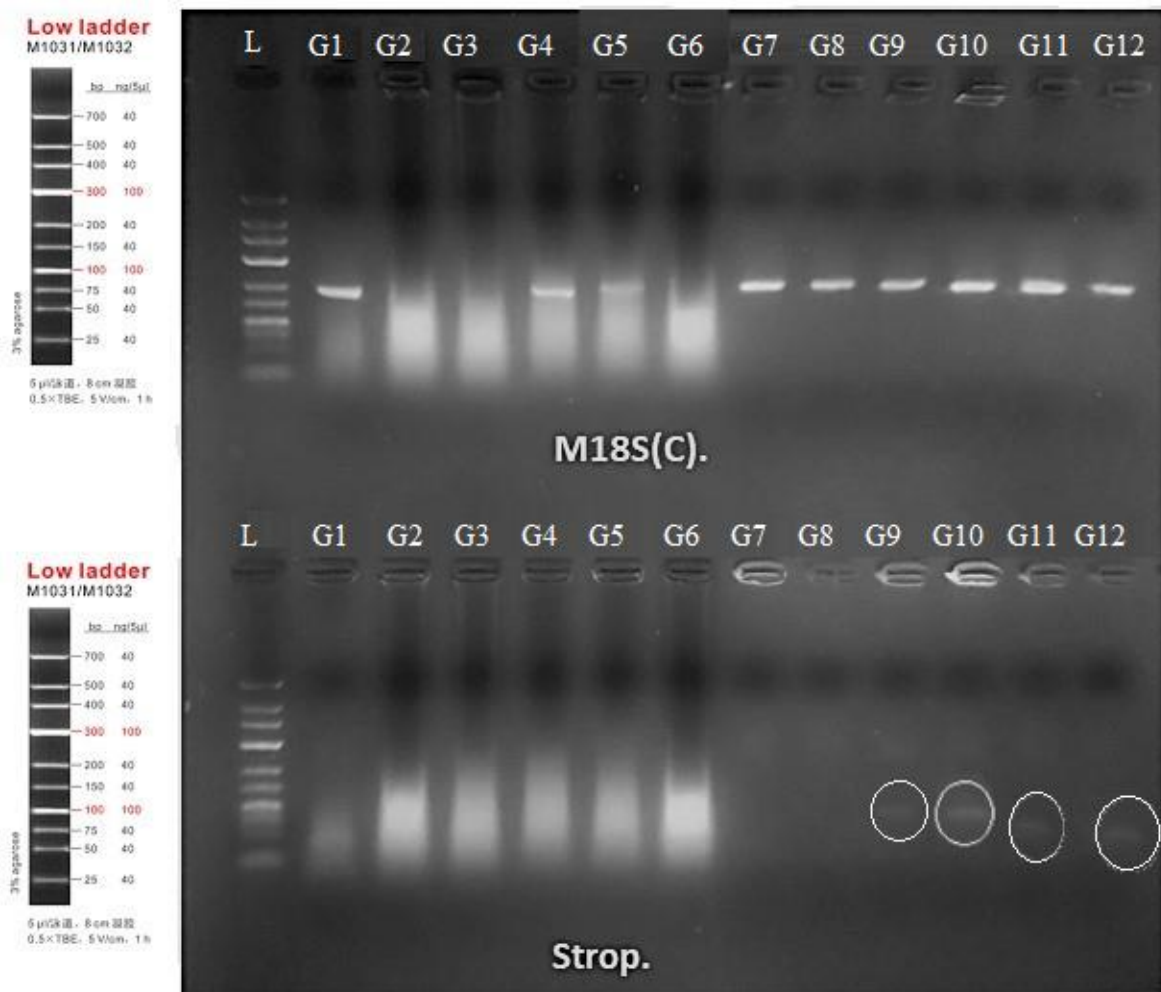


Figure 2. Gel electrophoresis of amplified tropomyosin and internal standard from raw and thermally processed samples
 (G1: Raw shrimp; G2: raw prawn; G3: Baked shrimp; G4: Baked prawn; G5: Steamed shrimp; G6: Steamed prawn; G7: Boiled shrimp; G8: Boiled prawn; G9: Fried shrimp; G10: fried prawn; G11: Grilled shrimp; G12: Grilled prawn)

Likewise, the higher stability of other main crustacean allergens, myosin light chain (Lit v 3) reported in the boiled sample (Ayuso et al., 2008).

More recently, Laly et al., (2019) reported that the identification of al-allergenic properties of tropomyosin during extended boiling was identified in flower tail shrimp. The results of this research also revealed the presence of tropomyosin encoding gene were found in fried and grilled crustacean sample. This result is in accordance with Lasekan(Lasekan and Nayak, 2016), who reported that grilling process increased tropomyosin level of shrimp (*Penaeus monodon*). These results are important for better understanding of how thermal process impact on the proximate composition and acidity of seafood that play important role in the DNA based allergen detection. The results could be valuable for the determination of the other food consumption originated allergens which are accepted as one of the important threats for the food safety and thereof public health. The alteration of allergens driven by thermal treatment in food products is commonly caused by peptide bond hydrolysis, aggregation of both disulfide and non-covalent bonds, and denaturation of allergen response mechanisms (Laly et al., 2019). The tropomyosin-coding gene was found in the fried and grilled crustacean samples. While Shriver et al., (2011) claimed that boiling shrimp did not change tropomyosin, other researchers reported that major heat-resistance allergens were found in the boiled giant river prawn and shrimp samples (Lasekan and Nayak, 2016). Likewise, the higher stability of other main crustacean allergens, myosin light chain (Lit v. 3), was reported in the boiled sample (Ayuso et al., 2008). More recently, Laly et al., (2019) reported that the identification of al-allergenic properties of tropomyosin during extended boiling was identified in flower tail shrimp. The results of this research also revealed the presence of tropomyosin-encoding genes in fried and grilled crustacean samples. This result is in accordance with Lasekan and Nayak, (2016), who reported that the grilling process increased the tropomyosin level of shrimp (*Penaeus monodon*). Taki et al., (2023) highlighted that the importance of molecular based methods on the detection of allergen in the different food matrices. Jabeen et al.,(2023) also pointed out that allergen detection in cereals by real-time PCR. The results of this research are important for a better understanding of how thermal processes impact the proximate composition and acidity of seafood, which play an important role in DNA-based allergen detection. The results could be valuable for determining the other food consumption-derived allergens, which are accepted as important threats to food safety and public health.

Conclusions

The findings of this research highlight that the tropomyosin encoding gene can be used for the crustacean allergens using different thermal processing methods, even in cryptic or close species. The proximate composition and pH values of raw and processed shrimp and prawn samples were also found to be significantly different. The allergen-coding gene was found in fried shrimp and prawn samples that could be related to the external oil effect. Grilled shrimp and prawn samples were other tropomyosin-positive groups, which could be related to pH variations.

This study is the first to achieve the simultaneous analysis of allergenic crustacean species driven by thermal process effects with molecular methods in different crustacean species in the same research. The findings revealed that different thermal processing impact both on the nutritional value and allergenicity which are crucial for public health. Due to the thermal processing methods applied by both consumers and producers, the outputs of this research could be beneficiary in domestic and industry level. The results could be beneficial for food processors, scientists, and decision-makers in public health. The scope of further research will be the stability of foodborne allergen detection and the allergen-detectable signal contained in packaging material.

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