Effect of simulated digestion on antigenicity of banana prawn (Fenneropenaeus merguiensis) after high pressure processing at different temperatures

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ABSTRACT

Changes in tropomyosin derived antigenicity of banana prawn (*Fenneropenaeus merguiensis*) due to high pressure processing (HPP) at 600 MPa for 5 or 10 min at various temperatures (40, 80, 120 °C) were investigated. HPP of prawn samples at 40 and 80 °C for 5 min increased tropomyosin derived antigenicity by almost double, whereas HPP at 120 °C for 10 min decreased antigenicity by 65%, detected using ELISA kit. A significant ($P \leq 0.05$) reduction of tropomyosin antigenicity after pepsin digestion was noticeable in prawns after HPP, but not in control prawn sample. However, further digestion of the control and HPP sample with pancreatin enzyme decreased antigenicity to ~0 mg mL^{-1}. The combination of HPP and high temperature (120 °C) in the current study can potentially reduce tropomyosin-derived antigenicity in whole prawn muscle, whereas SIF digestion with pancreatin enzyme may present a new prospective method to produce hypo-antigenic, enzymatically digested prawn products.

Key words: Antigenicity; tropomyosin; high pressure processing; simulated digestion; banana prawn

1. INTRODUCTION

Prawn is one of the widely consumed seafood products all over the world due to its delicacy and high nutritional properties (Ravichandran et al., 2009; Hoffmann, 2000). It also plays a substantial role in international seafood trade (Oosterveer 2006) having high economic value. However, it has been declared by World Health Organization (WHO) as one of the eight major sources of food allergens due to its high antigenicity (WHO, 2001). Prawn antigenicity causes mild to severe reactions including life-threatening anaphylaxis and usually persists throughout life (Albrecht et al., 2008). The muscle protein tropomyosin has been identified as the major allergen (Steensma, 2003), although arginine kinase (García et al., 2007), myosin light chain (Ayuso et al., 2008), sarcoplasmic calcium-binding protein (Shiomi et al., 2008), triosephosphate isomerase and troponin C (Bauermeister et al., 2011) have also been implicated and characterized as minor allergens in prawns. In spite of high allergic incidence, treatments are only available for accidental consumption of prawns and avoidance is the recommended therapy to prevent prawn allergies (Jones et al., 2014).

Growing demand for safe, fresh-tasting, additive-free and extended shelf-life of foods, have fostered the development of high pressure processing (HPP), a technology that is used to reduce microbial load but retain flavour, texture, colour and nutritional quality of many foods (Kaur et al., 2016; Barba et al., 2015; Briones et al., 2010). Studies have described several structural and biochemical changes of prawn proteins due to HPP (Joseph et al., 2017; Bindu et al., 2013; Büyükcan et al., 2009). Moreover, HPP has become one of the best commercial alternatives to traditional heat processing methods for the preservation of prawns, e.g. black tiger prawn (Kaur et al., 2013). In addition, Dang et al. (2018) stated that HPP could become a potential processing method to remove shells of shrimp as well as to prepare ready to eat shrimp. However, impact of HPP on antigenicity of prawn remains unclear.

As a novel technology HPP can change the nutritional and sensory quality suitable for human consumption (Dang et al., 2018; Barba et al. 2015), therefore its impact on antigenicity of prawn need to be studied clearly. Moreover, the changes through gastrointestinal digestion of HPP treated prawns has not been studied in-depth. Therefore, the aim of this study was to assess the effect of HPP at 600 MPa applied at different temperatures for 5 and 10 min, respectively, on tropomyosin derived antigenicity of banana prawn protein and its fate during gastrointestinal digestion.

2. MATERIALS AND METHODS

2.1. Treatment and extraction of proteins from prawn

Extraction of proteins from prawn is an important step for isolation and analysis of antigenic components. Fresh banana prawns (*Fenneropenaeus merguiensis*) were collected from a local supermarket in Australia. The prawns were washed for 2-3 min in Milli-Q water to remove external contaminants, after which the external shells were removed and deveined using the tip of a sharp blade.

HPP of whole prawn muscle was performed using a Stansted ISO-LAB FPG11501 High Pressure 3.6 L unit (Stansted Fluid Power Ltd., Stansted, Essex, UK) described elsewhere (Knoerzer et al., 2010). The pressure vessel has a permitted initial temperature range from -20 to 110 °C for pressures up to 800 MPa. The maximum temperature within the vessel during pressure holding is 130 °C. A deionised water/propylene-glycol mixture (40% glycol) was used as the pressure-transmitting medium.

Samples were high pressure (600 MPa) treated separately for 5 and 10 min at 40, 80, or 120°C, respectively. During high pressure treatment, the vessel was conditioned to an initial temperature, which then attained the target temperature after compression heating (Knoerzer et al., 2010). Conditioning times for samples were short (< 2 min) but varied slightly depending on the applied temperature. The compression and decompression rates were set to 600 or 1200 MPa min^{-1}, respectively. The temperature of the compression fluid and sample were monitored using type T thermocouple attached to the sample carrier (Knoerzer et al., 2010). All treatments were replicated on different days. The prawn muscle without any processing used as control.

The extraction of proteins from control and HPP samples were executed as described by Faisal et al. (2019). In brief, prawn muscle was homogenized using a laboratory blender (Waring 8011ES blender, NJ, USA) in phosphate buffered saline solution (pH 7.4) at 1:3 ratio for 5 min. The protein slurry was agitated for 3 h at 4 °C, trailed by centrifugation (Beckman Coulter Avanti J-26S XPI, Palo Alto, CA, USA) at 4 °C and speed of 29,400× g for 15 min. The supernatant of blend (control or HPP samples)

was deliberately isolated utilizing micropipette and stored in sealed containers with appropriate labelling at -80 °C until further analysis.

2.2. Determination of protein content

Determination of total protein content of each concentrate was performed by Kjeldahl method. Foss 2020 Digester Unit DS20 and Foss 2012 Distilling Unit (Hillerod, Sweden), were used for sample digestion and distillation respectively.

2.3. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The complete protein profile of extracted control and HPP samples was established by using reducing SDS-PAGE as described by Faisal et al. (2019). In brief, 2 mg mL^{-1} extract protein content from prawn sample was diluted 1:1 with 2x Laemmli buffer (containing 5% 2-mercaptoethanol) and heated at 95 °C for 3 min. Precisely 12 µg of protein was added onto each well in a 4–20% Mini-Protean TGX unstained precast gel (Bio-Rad Laboratories, Sydney, NSW, Australia). Electrophoretic separation of protein was accomplished by Bio-Rad prescribed process and Precision Plus Protein Unstained Standard was utilized as a molecular weight marker to highlight the molecular weight of separated protein bands. The protein profile on the gel was visualized through activation by Chemi-Doc imager (Chemidoc MP, Bio-Rad Laboratories).

2.4. Immunoblot analysis

Immunoblotting was performed as described by Faisal et al. (2019). Briefly, protein bands of SDS-PAGE were transferred into Polyvinylidene fluoride (PVDF) membrane utilizing the Trans-Blot Turbo Transfer System (Bio-Rad) as per manufacturer's guideline (Bio-Rad Laboratories). The membrane was blocked using 5% w/v skimmed milk in PBST followed by incubation with Anti-Tropomyosin antibody (MAC 141, Abcam Australia Pty Ltd, Melbourne, VIC, Australia) at 1:3,000 dilution with 2.5% w/v skimmed milk in PBST for overnight at 4 °C under steady shaking at 150 horizontal strokes per min (Ratek, Orbital mixer, Melbourne, VIC, Australia). Following washing 5 times with PBST, the membrane was further incubated with Goat Anti-Rat Immunoglobulin-G H&L, HRP preadsorbed (Abcam Australia Pty Ltd) diluted 1:3,000 in PBST for 2 h at 4 °C under constant shaking. Finally, chemiluminescence (Thermo Pierce ECL Western Blotting Substrate) was added to membrane to visualise the IgG

binding using Chemi-Doc imager (Chemidoc MP, Bio-Rad Laboratories, Sydney, NSW, Australia).

2.5. Enzyme-Linked Immunosorbent Assay (ELISA) kit

Sandwich ELISA (RIDASCREEN®FAST Crustacean, R-Biopharm, Darmstadt, Hessen, Germany) was used to measure the tropomyosin derived antigenicity in extracted protein samples. Each sample was replicated individually following manufacturer instruction. In brief, exactly 1 mL of protein extract was diluted with 19 mL of extraction buffer followed by centrifugation at 2,500× g (Eppendorf centrifuge 5810 R, Hamburg, Germany) for 10 min. Exactly 100 µL of sample supernatant was added into each well of antibody pre-coated microwell and incubated at room temperature for 10 min. The microwell was washed three times with 250 µL washed buffer to remove unbound proteins. An aliquot of 100 µL conjugate solution was added to each well, after gentle shaking, the plate was incubated at room temperature for 10 min. After subsequent washing, 100 µL of chromogen was added and incubated in the dark at room temperature for 10 min. Finally, 100 µL of stop solution was added to each well and absorbance was measured within 10 min using ELISA plate reader (iMark microplate absorbance reader, Bio-Rad, Tokyo, Japan) at 450 nm.

2.6. Simulated gastrointestinal digestion

Simulated gastrointestinal digestion of untreated and treated prawn samples was performed using the INFOGEST protocol as described by Minekus et al. (2014) with slight modifications. Briefly, 5 g of prawn sample was blended with 5 mL of simulated salivary fluid (SSF) electrolyte stock solution using a laboratory blender (Waring 8011ES blender, East Windsor, NJ, USA) for 2 min. The protein slurry was further mixed with 7.5 mL of simulated gastric fluid (SGF) electrolyte stock solution, 1.25 mL pepsin stock solution [10,000 U mL^{-1} (Pepsin, Sigma, MO, USA) in SGF electrolyte stock solution] and 5 µL of $CaCl_2$ (0.3 M). The pH of the mixture was adjusted to 3.0, the volume made up to 20 mL with Milli-Q water, and digested at 37 °C in a rotary shaker (Thermo Scientific MaxQ Shaker, Marietta, OH, USA) at 100 horizontal strokes per min for 2.5 h. Afterward, 20 mL of gastric-chyme was mixed with 11 mL of simulated intestinal fluid (SIF) electrolyte stock solution, 5.0 mL of a pancreatin stock solution [100 mg mL^{-1} (Pancreatin 1X, USB, OH, USA) in SIF electrolyte stock solution], 2 mL of bile stock solution [50 mg mL^{-1} (Bile, Sigma, MO, USA) in SIF

electrolyte stock solution] and 40 µL of $CaCl_2$ (0.3 M). The pH of mixture was adjusted to 7.0, and the volume made up to 40 mL with Milli-Q water, and digested at 37 °C in a rotary shaker (Thermo Scientific MaxQ Shaker) at 100 horizontal strokes per min for 2.5 h. After complete digestion Na_2CO_3 (0.2 M) was added to solution to inactivate the enzymes and then stored immediately at -80 °C.

2.7. Statistical analysis

Statistical analysis of results was performed using a one way ANOVA by the Statistical Analysis System (v. 9.2). The experimental design was replicated three times. The means were compared using Tukey's Studentised Range (HDS) test. The antigenicity of HPP and enzyme digested samples was considered to be significant at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Protein profile of control and HPP prawn extracts by SDS-PAGE

The SDS-PAGE protein profile of banana prawn shows several protein bands having various molecular weights (Figure 1A). In the control extract, 14 protein bands were observed of which molecular weights 20, 34, 37, 40, 75, 90 and over 250 kDa were more visible in intensity, whereas 25, 30, 45, 55, 105, 175 and 213 kDa were less potent (lane 2). Protein bands with similar molecular weights in untreated prawn protein extracts have also been reported in banana prawn and other prawn species in previous studies (Faisal et al., 2019; Wu et al., 2015; Kamath et al., 2013).

On the other hand, protein extracts of HPP treated prawn samples for 5 min (lanes 3 to 5) showed comparatively less number of protein bands compared to control (Figure 1A). In lane 3, out of 9 visible protein bands, 20, 40 and 75 kDa bands were prominent, whereas in lanes 4 and 5, only 3 less visible protein bands were present respectively. Moreover protein extracts from the 10 min HPP treatment of prawn samples (lanes 6 to 8) showed similar protein profiles, but with lower intensity compared to the 5 min HPP treatments. In a previous study, Faisal et al. (2019), reported less number of protein bands (7 bands) in banana prawns treated at 100 °C (atmospheric pressure) and autoclaved pressure (121 °C at 0.2 MPa), respectively, for 15 minutes compared to the control. The probable reason of less protein bands in HPP samples was due to the combined effect of heat and high pressure resulting in disintegration of proteins

into smaller molecular weight protein fragments (peptides) (less than 10 kDa). In addition, at high temperature (120 °C) proteins likely start to re-aggregate in the presence of high pressure (600 MPa) resulting in the formation of some higher molecular weight aggregates, which appeared on top of lanes 5 and 8 (Figure 1A), whereas smaller proteins ultimately pass through the gel into the buffer (Shriver and Yang, 2011). HPP can alter the tertiary structure as well as induce denaturation of proteins by affecting the ionic, hydrogen and hydrophobic bands (Wang et al., 2013), whereas, heat can result in changes of the secondary and tertiary structures of protein through changes in inter and intra molecular bonds (Chatterjee et al., 2006).

3.2. Protein profile of simulated digested prawn extracts by SDS-PAGE

Figure 1B shows SDS-PAGE protein profiles of the control (lane 2) and HPP prawn extracts subjected to SGF digestion (lanes 3 to 8). Single protein bands of approximately 39 kDa were observed in lanes 2 to 8. The 39 kDa protein band was not from the sample but from the added enzyme (pepsin) as indicated in lane 9. Gámez et al. (2015) also reported the 39 kDa protein band on SDS-PAGE as pepsin enzyme used for SGF digestion. Moreover, protein bands above 39 kDa were completely absent (lanes 2 to 8) indicating breakdown of proteins due to pepsin enzyme activity during SGF digestion. Barrett et al. (2004) reported that pepsin enzymes hydrolyze peptide bonds of tyrosine and phenylalanine residues. Furthermore, the intensity of aggregated proteins at the top of lanes 5 and 8 (Figure 1B) was also reduced compared to undigested proteins (Figure 1A) due to enzymatic digestion. In addition, the smearing of protein bands below 39 kDa to less than 10 kDa (lanes 2 to 8) resolved on SDS-PAGE can be attributed to the breakdown of proteins into peptides as well as intramolecular crosslinking, preventing linearization of protein bands with any specific molecular weight (Shriver and Yang 2011).

Figure 1C depicts changes in protein profiles after SIF digestion of control (lane 2) and HPP prawn samples treated with pancreatin enzymes, respectively (lanes 3 to 8), compared to undigested (Figure 1A) and SGF digested protein profiles (Figure 1B). On SDS-PAGE, one prominent protein band at the 50 kDa mark and 4 less intense bands in between 20 to 30 kDa can be observed in lanes 2 to 8 (Figure 1C). These protein bands were not from the sample but from the added enzyme (pancreatin) and bile salt as indicated in lane 9. The disappearance of other protein bands (lanes 2 to

8) in Figure 1C, were likely caused by pancreatin enzymes cleaving proteins at multiple sites including glutamic acid, lysine and arginine (Mikita and Padlan, 2007; Beck, 1973) resulting in complete hydrolysis of proteins into peptides. Smaller protein fragments from digestion, with molecular weight less than 10 kDa, passed through the gel during electrophoretic mobility (Taheri-Kafrani et al., 2009). Jin et al. (2015) treated squid at 600 MPa at 20 °C for 20 min and reported similar degradation of protein bands after SGF and SIF digestion.

3.3. Detection of antigenic tropomyosin in control and HPP prawn extracts

Detection of antigenic tropomyosin in control and HPP treated extracts was performed by immunoblotting. The binding of monoclonal antibody with prawn proteins on PVDF membrane at 37 kDa for control and HPP prawn extracts is shown in Figure 2A. Faisal et al. (2019) reported similar results for IgG binding (37 kDa as tropomyosin) in untreated banana prawn. The IgG binding for HPP prawns treated at 40 °C for 5 or 10 min, respectively (lanes 3 & 6), showed double band intensity compared to that of the control. On the other hand, HPP prawn samples treated at 120 °C for 5 or 10 min, respectively, showed less band intensity (64 and 48%, respectively) due to fewer IgG binding sites compared to that of the control (lanes 5 & 8), whereas HPP samples treated at 80 °C (5 and 10 min) resulted in similar IgG binding to the control. In a previous study, banana prawn samples were treated at 100 °C atmospheric pressure and 121 °C at 0.2 MPa for 15 min, respectively, IgG binding was reported to be 4 and 2.5 times higher respectively, compared to the control (Faisal et al., 2019). This indicates that combining HPP at 600 MPa with temperature ranging from 40 to 120°C has a positive impact on reducing antigenicity.

Tropomyosin, having α-helix coiled structure, is twisted tightly to the surface of actin filament through gestalt-binding interactions (Faisal et al., 2019; Holmes & Lehman 2008). HPP treatment of prawns at 600 MPa is likely to breakdown gestalt-binding interactions in tropomyosin and expose internal binding epitopes. As a result, HPP prawn samples treated at 40 °C showed significantly higher antigenicity compare to the control. Milk proteins treated at 600 MPa at 40 °C for 10 min have been shown to reveal antigenic epitopes resulting in higher antigenicity (Kleber et al., 2007). On the other hand, Ma et al., (2011) reported that beef muscle treated at 600 MPa for 20 min showed increasing solubility of myofibrillar proteins (actin, myosin, tropomyosin and

troponin) with increasing temperature. Similarly, our study showed that at higher temperatures (80 and 120 °C), the solubility of tropomyosin increased due to breakdown of the protein structure, resulting in decreased IgG binding. Furthermore, the treatment time also plays an important role in tropomyosin solubility. For example, IgG binding was lower in samples with high pressure treated for 10 min compare to those that were treated for 5 min under similar temperature conditions.

Jin et al. (2015) reported that HPP at 600 MPa and 20 °C for 20 min, converted 53% of the α-helix of squid tropomyosin into β-sheets and random coils, resulting in substantial changes of the secondary structure and decreased antigenicity. The structural changes likely masked or destroyed binding epitopes within the protein molecule causing less IgG binding. Long et al. (2015) investigated a range of high pressure (100 to 600 MPa) treatments at various temperatures (25 to 75 °C) on isolated tropomyosin extract from *Litopenaeus vannamei* shrimp and reported low antigenicity for 500 MPa at 55 °C for 10 min. The main reason for the contradiction of this result with the current study is probably due to the use of different treatment conditions. On the contrary, the current study subjected whole prawn muscles to high pressure at various temperatures and found that tropomyosin behaved differently from isolated tropomyosin extracts (Gámez et al., 2015) possibly due to presence of surrounding actin, myosin and troponin molecules within the muscle.

3.4. Detection of antigenic tropomyosin in simulated digested prawn extracts

An immunoblotting method was employed to detect the presence of antigenic tropomyosin in digested samples. IgG binding observed at 37 kDa (tropomyosin) on the PVDF membrane for the pepsin digested control sample is shown in Figure 2B (lane 2). The resistance of tropomyosin against pepsin digestion is due to the presence of its high lysine content (Li et al., 2012; Huang et al., 2010). A similar response of tropomyosin of various crustacean species to pepsin has also been reported in several studies (Gámez et al., 2015; Wu et al., 2015). The IgG binding for pepsin digested HPP samples (lane 3) showed increased band intensity, whereas lanes 4 and 5 exhibited decreased IgG binding compared to digested control sample, due to tropomyosin solubility at higher (80 and 120 °C) temperatures. Similar IgG binding trends were observed for lanes 6 and 7, whereas lane 8 (HPP at 120 °C for 10 min) did not show any band indicating non-IgG binding. In comparison to undigested prawn

extracts (Figure 2A), digested prawn extracts (Figure 2B) showed much lower IgG binding on the PVDF membrane. The combined effect of high pressure and temperature likely caused structural changes and partial denaturation of tropomyosin, thus accelerating pepsin digestion (Mikita and Padlan, 2007). Similar effects have been reported for autoclave treated (121 °C for 20 min) Scylla crab tropomyosin samples (Yu et al., 2011).

The pancreatin enzyme activity on control and HPP prawn samples is shown in Figure 2C. No IgG binding for tropomyosin was observed on the PVDF membrane. Jin et al. (2015) similarly reported the absence of tropomyosin after 60 min of SIF digestion for samples treated at 600 MPa for 20 min at 20 °C. Moreover Yu et al. (2011) also reported the absence of IgG binding for tropomyosin after 120 min of SIF digestion for autoclaved treated (121 °C at 0.14 MPa for 20 min) crab sample. The authors (Yu et al., 2011) further showed that tropomyosin and its fragments were still detectable by immunoblotting after 240 min of digestion for boiled (100 °C for 20 min) sample, indicating the impact of pressure on tropomyosin degradation during SIF digestion.

3.5. Quantification of antigenicity by ELISA

The quantification of antigenicity in control and HPP prawn samples is shown in Figure 3. HPP samples treated at 40 and 80 °C for 5 or 10 min, respectively, showed significant ($P < 0.05$) increase in antigenicity compared to the control. However, antigenicity of HPP samples treated at 120 °C for 10 min decreased significantly by 65%, similar to trends discussed in immunoblotting of HPP samples. On the other hand, the same control sample subjected to SGF digestion with pepsin enzyme showed no significant ($P > 0.05$) difference for tropomyosin antigenicity. Whereas HPP samples digested with pancreatin enzymes following pepsin digestion resulted in significant ($P < 0.05$) reduction in antigenicity similar to immunoblotting results. HPP samples treated at 120 °C for 10 min digested with pepsin enzyme showed a slight deviation compared to immunoblotting. The immunoblotting result for this sample showed complete disappearance of antigenicity, whereas the ELISA results indicated the presence of antigenicity at 1.21 mg mL^{-1}. The cause of this antigenicity is likely due to IgG binding epitopes still active in peptides resulting from enzymatic hydrolysis of proteins. Long et al. (2015) showed a declining trend of IgG binding for squid tropomyosin extract treated with 600 MPa for 10 min compared to 5 min treated

samples using inhibition ELISA. The authors further reported that antigenicity of squid tropomyosin extract treated at 600 MPa decreased with increasing temperature treatment from 25 up to 75 °C.

4. CONCLUSION

Prawn muscles treated with 600 MPa at 40 and 80 °C for 5 or 10 min showed significant ($P < 0.05$) increased antigenicity, whereas samples treated at 600 MPa and 120 °C for 10 min decreased antigenicity by 65% compare to control. Therefore, the combination of high pressure (600 MPa) and temperature (120 °C) can potentially reduce tropomyosin-derived antigenicity in whole prawn muscle. On the other hand, prawn muscles digested in presence of pepsin enzyme showed more than 50% reduction of tropomyosin antigenicity for HPP samples, yet no significant difference for the control sample. Moreover, further digestion with pancreatin enzymes decreased antigenicity of tropomyosin up to ~0 mg mL^{-1} for control and HPP prawn samples. This potential reduction indicates digestion with pancreatin enzymes can possibly open new opportunities to produce hypo-tropomyosin-antigenic, enzymatically digested prawn protein powders. This hypo-antigenic prawn protein powders can be used as prawn seasoning in different meals, as well as flavour and taste enhancer in various biscuits, cakes, and other snack products.

5. CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

6. ACKNOWLEDGEMENT

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List of Figures

Figure 1: SDS-PAGE of control and HPP prawn protein extracts (A), SGF (pepsin) digested protein extracts (B), and SIF (pancreatin) digested protein extracts (C): Lane 1 - standard; lane 2- control; lane 3, 4 and 5 - HPP (600 MPa) at 40, 80, and 120 °C for 5 min, respectively; lane 6, 7 and 8- HPP (600 MPa) at 40, 80, and 120 °C for 10 min, respectively, lane 9 - SGF (including pepsin (B) or pancreatin (C) enzyme) without having prawn protein extract.

Figure 2: Immunoblotting of control and HPP prawn protein extracts (A), SGF (pepsin) digested protein extracts (B), and SIF (pancreatin) digested protein extracts (C): Lane 1 - standard; lane 2- control; lane 3, 4 and 5 - HPP (600 MPa) at 40, 80, and 120 °C for 5 min, respectively; lane 6, 7 and 8- HPP (600 MPa) at 40, 80, and 120 °C for 10 min, respectively, lane 9 - SGF (including pepsin (B) or pancreatin (C) enzyme) without having prawn protein extract.

Figure 3: Quantification of antigenicity of prawn protein extracts before and after digestion (SGF and SIF) using ELISA method for control and HPP samples processed at 600 MPa and 40, 80 and 120 °C for 5 and 10 min, respectively. (■ Undigested prawn protein extract; ◪ SGF digested prawn protein extract, ◱ SIF digested prawn protein extract). Here the lower cases (a-m) represent significant differences ($P < 0.05$) among the treatments.

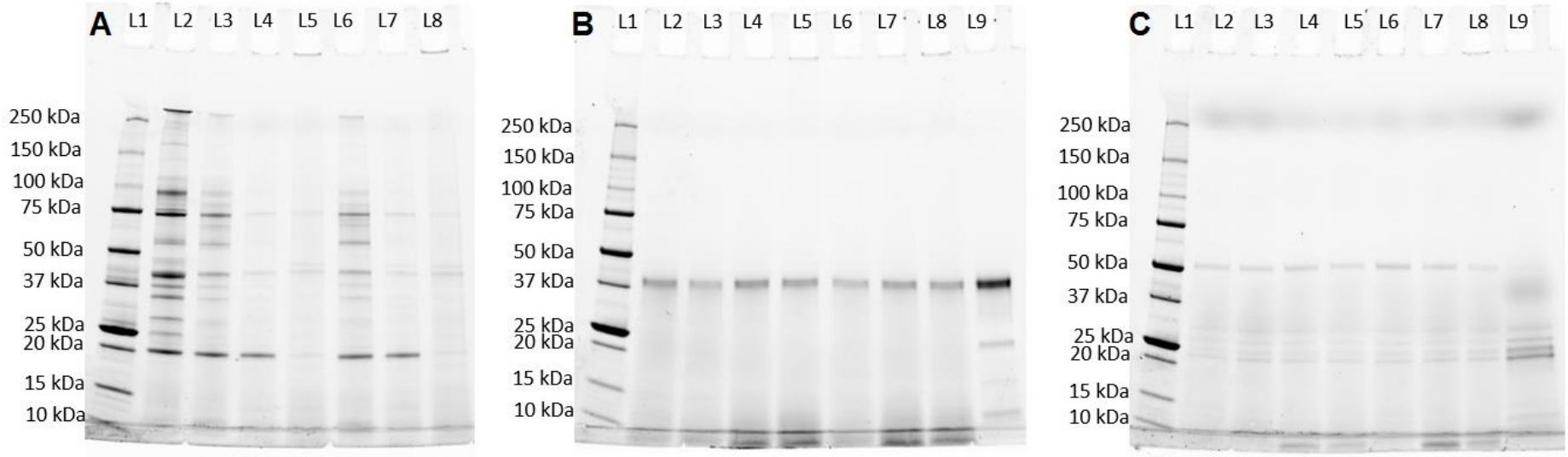
A
L1 L2 L3 L4 L5 L6 L7 L8
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
B
L1 L2 L3 L4 L5 L6 L7 L8 L9
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa
C
L1 L2 L3 L4 L5 L6 L7 L8 L9
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa

Figure 1.

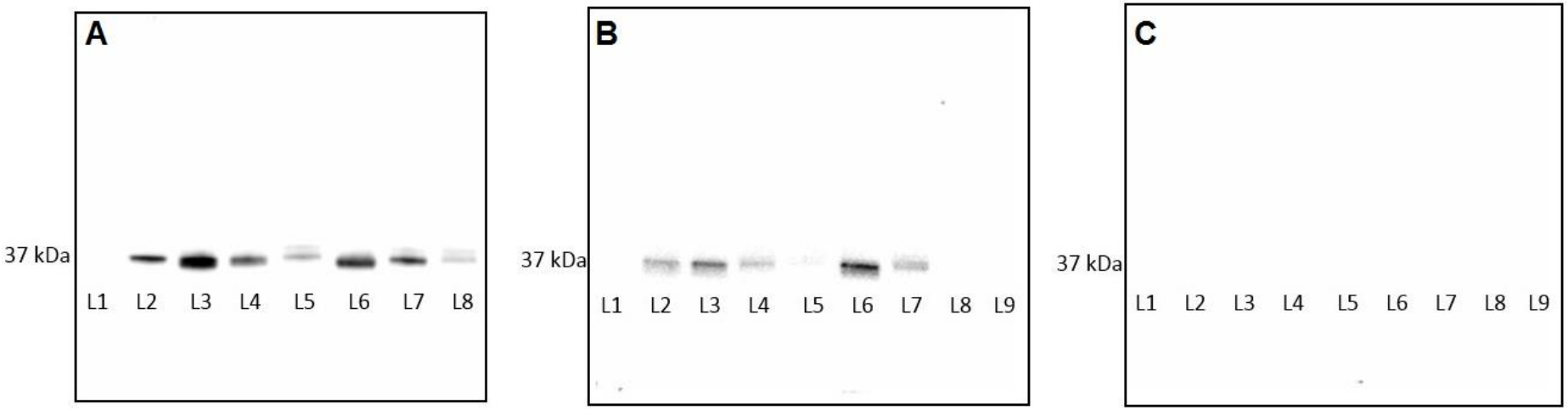
A
37 kDa
L1 L2 L3 L4 L5 L6 L7 L8
B
37 kDa
L1 L2 L3 L4 L5 L6 L7 L8 L9
C
37 kDa
L1 L2 L3 L4 L5 L6 L7 L8 L9

Figure 2

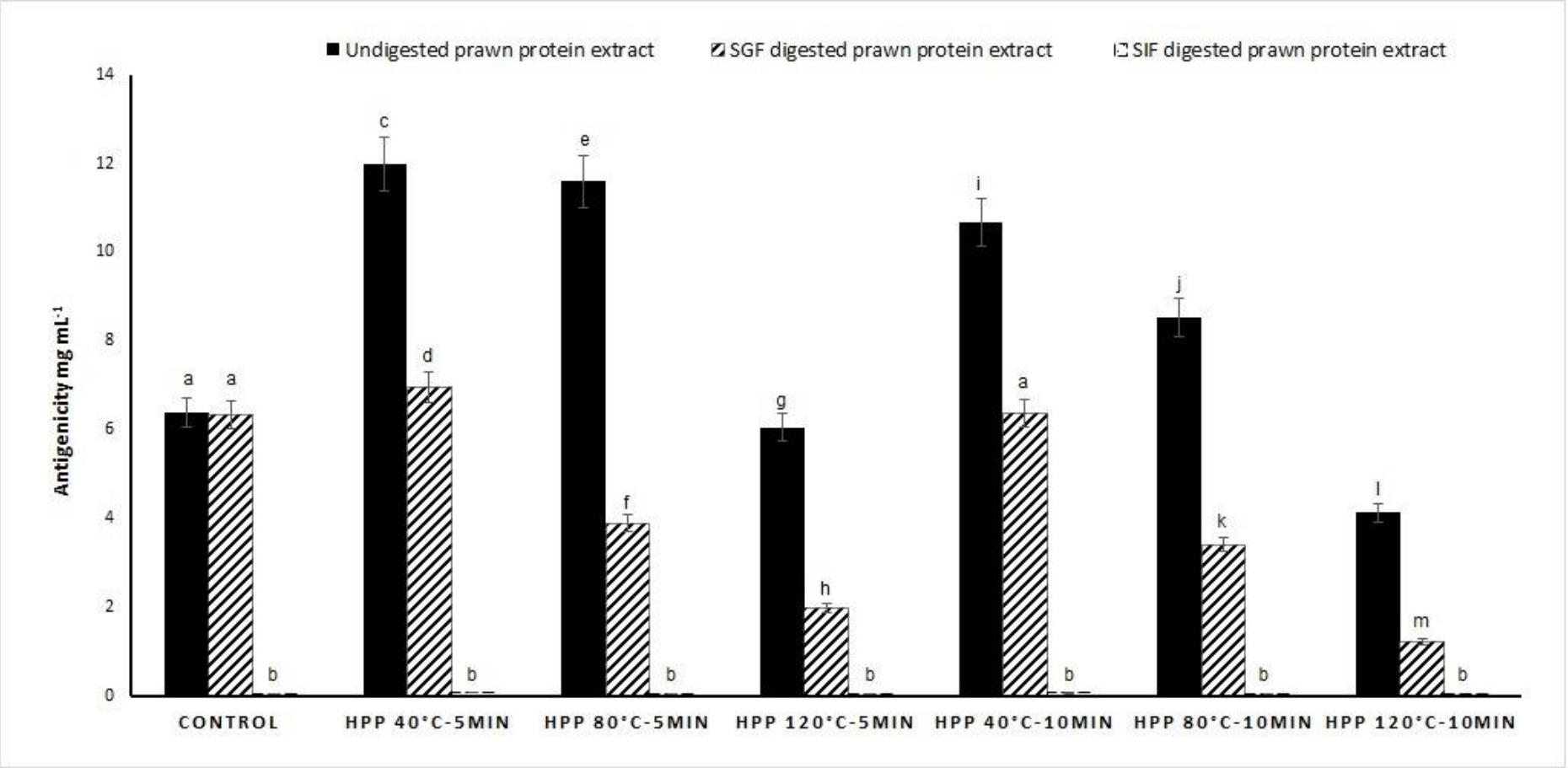
Undigested prawn protein extract
SGF digested prawn protein extract
SIF digested prawn protein extract
Antigenicity mg mL-1
14
12
10
8
6
4
2
0
a a b
c d b
e f b
g h b
i a b
j k b
l m b
CONTROL
HPP 40°C-5MIN
HPP 80°C-5MIN
HPP 120°C-5MIN
HPP 40°C-10MIN
HPP 80°C-10MIN
HPP 120°C-10MIN

Figure 3