



Altering the IgE binding capacity of king prawn (*Litopenaeus Vannamei*) tropomyosin through conformational changes induced by cold argon-plasma jet

Flora-Glad Chizoba Ekezie^{a,b,c}, Da-Wen Sun^{a,b,c,d,*}, Jun-Hu Cheng^{a,b,c,*}

^a School of Food Science and Engineering, South China University of Technology, Guangzhou 510641, China

^b Academy of Contemporary Food Engineering, South China University of Technology, Guangzhou Higher Education Mega Center, Guangzhou 510006, China

^c Engineering and Technological Research Centre of Guangdong Province on Intelligent Sensing and Process Control of Cold Chain Foods, Guangzhou Higher Education Mega Centre, Guangzhou 510006, China

^d Food Refrigeration and Computerized Food Technology (FRCFT), University College Dublin, National University of Ireland, Agriculture and Food Science Centre, Belfield, Dublin 4, Ireland

ARTICLE INFO

Keywords:

Cold plasma
Food allergy
Tropomyosin
Prawn immunoreactivity
IgE binding

ABSTRACT

Tropomyosin (TM), a myofibrillar protein, is a major allergen in most crustaceans including king prawn (*Litopenaeus vannamei*). In this study, the structural modifications and allergenic response of TM in fresh king prawn as induced by cold argon plasma jet (CAPJ) were examined. The result showed that the level of α -helix structures declined as treatment time progressed, while the level of β -sheets and random coils increased. The free sulfhydryl groups decreased as CAPJ treatment progressed due to the formation of disulphide bonds while surface hydrophobicity increased until equilibrium values. In addition, after 15 min of plasma exposure, the maximum reduction recorded for IgE and IgG binding capacities were 17.6% and 26.87% respectively as revealed by ELISA test. These findings correlated with the occurrence of protein unfolding and denaturation, evident by complementary structural analyses conducted. The study indicates that non-thermal plasma maybe a promising tool for developing hypoallergenic food products.

1. Introduction

In recent years, global production of seafood species and their derivative products has experienced massive growth, owing to their high nutritive value and increased preference across consumers of various groups worldwide (Chouljenko, Chotiko, Solval, Solval, & Sathivel, 2017; Dai, Zhang, Wei, Hider, & Zhou, 2016; Kaur, Kaushik, Rao, & Chauhan, 2013; Nirmal & Benjakul, 2012; Yuan, Pan, Li, Wang, & Chen, 2019; Wang et al., 2018; Wang et al., 2015; Cheng, Sun, Zhu, & Zhang, 2017). However, this surge in the consumption of seafood products have also been associated with more incidences of allergenic reactions among consumers (Lopata, Kleine-Tebbe, & Kamath, 2016). Although more than 170 foods reportedly cause allergic reactions, crustacean allergy induced predominantly by tropomyosin (TM) remains a leading cause of food allergies worldwide (Savage, Sicherer, & Wood, 2016; Ekezie, Cheng, & Sun, 2018b). Tropomyosin is a thermal stable and super-coiled dimeric protein having predominantly α -helix structures with a molecular weight (MW) of 36–41 kDa, containing 281 amino

acids. Other allergenic proteins are present in crustaceans but allergic reactions induced by tropomyosin have been reported in over 80% of susceptible patients. Most patients exhibit symptoms as simple as itching, hives, facial edema to pulmonary symptoms, gastrointestinal symptoms, and in rare severe cases, anaphylactic shock that can lead to death. Several techniques have been used to tackle prawn and crustacean allergies in general, but the most effective approach still remains complete avoidance of the food by the susceptible individual.

Nevertheless, researchers have sought substitute approaches like postharvest processing methods to mitigate the allergenicity of crustaceans before they reach consumers, so as to enable susceptible individuals benefit from their biological value. These processing methods have the ability to alter epitopes present in allergenic proteins via protein fragmentation or conformational changes. One of such approaches that can achieve this effect include heat treatment in various form but unfortunately, due to the heat stable nature of TM, simple heat treatment does not sufficiently reduce their allergenicity (Lasekan & Nayak, 2016). Thus as summarized in literature (Vanga, Singh, &

* Corresponding authors at: School of Food Science and Engineering, South China University of Technology, Guangzhou 510641, China.

E-mail address: dawen.sun@ucd.ie (D.-W. Sun).

URLs: <http://www.ucd.ie/refrig>, <http://www.ucd.ie/sun> (D.-W. Sun).

<https://doi.org/10.1016/j.foodchem.2019.125143>

Received 2 March 2019; Received in revised form 10 June 2019; Accepted 6 July 2019

Available online 08 July 2019

0308-8146/ © 2019 Elsevier Ltd. All rights reserved.

Raghavan, 2017), nonthermal techniques including gamma irradiation, power ultrasound, pulsed ultraviolet (PUV) light, high hydrostatic pressure processing (HPP) and/or the amalgamation of these technologies, which possess the ability to alter the structure of food proteins in diverse ways (*i.e.*, protein unfolding, cross linking, oxidation, glycation denaturation, association or aggregation) accompanied by subsequent effect on allergen reactivity, are being exploited. More recently, cold plasma (CP), *i.e.*, nonthermal plasma, has become increasingly popular as an alternative approach for microbial decontamination of food as well as for altering the functionality of food proteins via structural and conformational changes (Bußler, Steins, Ehlbeck, & Schlüter, 2015; Ekezie, Sun, & Cheng, 2017; Ji et al., 2017; Segat, Misra, Cullen, & Innocente, 2015).

With regards to CP, plasma oxidation presents ample advantages due to the presence of oxidizing species, radicals, ozone and hydrogen peroxide in the plasma glow and other physical effects (Ekezie, Cheng, & Sun, 2019; Pan, Cheng, Lv, & Sun, 2019; Han, Cheng, & Sun, 2019). CP functions at ambient temperatures, which aid applications even on heat sensitive materials, and the lack of toxic by-products, makes it environmentally friendly with tolerable exposure by operators and consumers. Recent attempts on using CP to reduce allergenic potential showed up to 89% reduction in allergen reactivity of soy protein isolates (Meinlschmidt et al., 2016). It was also reported to reduce immunoreactivity of wheat proteins (Nooji, 2011) and environmental aerosols containing Der p 1, Der f 1, Asp f 1, Alt a1, and Can f 1 (Wu et al., 2014). However, these studies employed a dielectric barrier discharge (DBD) plasma system, without demonstrating the possible and precise mechanism that may have led to attenuation of allergenicity. Besides, scarce information exists in literature regarding the impact of CP on food allergens. It was thus proposed in the current study that cold atmospheric plasma generated in argon with a jet system may have similar effect on IgE binding ability of tropomyosin extracted from *Litopenaeus vannamei*. Therefore, the objective of this study was to elucidate the structural changes in prawn TM and subsequent response on its antigenicity upon CAPJ treatment and to reveal the conceivable mechanism for modulation in TM allergenicity. In the case of plasma jet system employed in this work, the plasma gas is conveyed through a nozzle to the outlet with a limited diameter for whole sample treatment, which may hinder uniform interaction of the chemical species with the specific protein of interest. Thus, a model solution was utilized. The findings of the current study are very much beneficial for understanding the potential interaction of argon plasma with tropomyosin and the efficacy of the process which provide basic insights for future process design and optimization.

2. Materials and methods

2.1. Reagents

3, 3', 5, 5'-tetramethylbenzidine (TMB), 1-anilino-8-naphthalene-sulfonate (ANS) and bovine serum albumin (BSA), were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The precast gels, nitrocellulose membranes (0.45 µm), sample and running buffers and horseradish peroxidase (HRP)-conjugated goat anti-human IgE antibodies were obtained from Thermo Fisher Scientific Inc. (Invitrogen™, Carlsbad, CA, USA). Goat anti-rabbit IgG conjugated to HRP was procured from Sangon Biotech (Shanghai) Co. Ltd. (Shanghai, China). The protein standard (PageRuler™ prestained protein Ladder), costar™ 96-well microtitre plates and enhanced chemiluminescent (ECL) kit was bought from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Only chemical reagents of analytical grade were used in this study, except otherwise stated.

2.2. Serum samples

Human sera from patients with a documented history of prawn

allergy were generously provided by Guangzhou Provincial Hospital of Traditional Chinese Medicine (Guangzhou, China). Serum with the specific prawn TM IgE levels > 0.35 kU/L, as characterized using an ImmunoCAP (Phadia AB, Uppsala, Sweden) was selected. None of the recruited subjects had any other forms of allergies. The serums were stored at −20 °C until analyses. The study was approved by the ethics committee of Guangzhou Provincial Hospital of Traditional Chinese Medicine with the aim of protecting the interests of the human subjects. Samples from non-allergic people were used as a negative control.

2.3. Extraction and preparation of prawn TM

Prawn TM was recovered according to the protocol of Wang et al. (2012). Briefly, prawn muscles recovered from the whole prawns was blended using a meat mincer (YLS-86A, Zhongshan Yalesi (Rileosip) Electric Industrial Co., Ltd., Zhongshan, China) and homogenized (1:10, w/v) in a buffer containing 20 mM KCl, 1 mM KHCO₃, and 0.1 mM 1,4-dithiothreitol for 10 min, using a laboratory homogenizer (FJ200, Shanghai Specimen Model Factory, Shanghai, China). The mixture was centrifuged at 10,000 × g for 10 min, and the precipitates were transferred into the above same buffer. The precipitate left was washed in 10-fold of 95% ethyl alcohol for three consecutive times and the resulting residues were treated with diethyl ether (v: v = 1:1) and dried in a fume cupboard. The protein powder was transferred into a solution containing 1 M KCl, 0.5 mM DTT, and 20 mM Tris-HCl (pH 7.5) at 4 °C and extraction was carried out with constant stirring overnight. The solution was subsequently filtered using a filter paper and isoelectrically precipitated at pH 4.5 using 1 M HCl. The mixture was allowed to stand in a refrigerator at 4 °C for 1 h and centrifuged at 10,000 × g for 10 min. The collected precipitate was dissolved in 0.01 M phosphate buffer saline (PBS, pH 7.4) and the pH was adjusted to 7.6 using 1 M NaOH. Fractionation was performed using 40% ammonium sulfate solution and dialyzed for 24 h with 0.01 M PBS. PBS was changed 3 times. The solution was subsequently boiled for 10 min and the denatured protein was removed by centrifugation. The final protein extracts was subjected to electrophoresis for identification. TM solution was stored at −20 °C until use.

2.4. Cold argon plasma jet treatment (CAPJ)

The plasma jet set-up used in this study was somewhat identical to the one described in our previous study (Ekezie et al., 2019). The plasma was ignited in a premixed gas containing 98% argon and 2% oxygen, blown through a quartz tube at 20 standard liters per min (slm). The plasma produced had a diameter of about 8 mm with a length of about 15 mm. Then, 20 mL of the TM solutions (adjusted to a concentration of 2 mg/mL) were exposed to the ionized gas for 3, 6, 9, 12 and 15 min time intervals, accompanied by constant shaking. Distance between the nozzle exit and the liquid surface was approximately 30 mm. Samples were treated in triplicate for each processing time. After completion of treatment, the samples were stored at 4 °C until use. TM without treatment was used as control. Meanwhile, Optical emission spectroscopy (OES) was used to qualitatively characterize the different active species present in the Ar-O₂ mixture plasma by using a computer controlled spectrometer (Model HR + 2000, Ocean Optics, Inc., FL, USA). The peaks were identified using the NIS Atomic Spectra Database by comparison.

2.5. Antigenicity analyses

2.5.1. Sodium-dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of the CAPJ-treated and untreated samples were evaluated using sodium SDS-PAGE. TM solutions were mixed with 7.5 µL NuPAGE 4 × loading buffer. The samples were rapidly heated at 70 °C for 10 min, followed by centrifugation at 10,000 × g for 5 min. The

electrophoresis was conducted at 200 V for 35 min with a Novex™ XCell SureLock™ Mini-Cell (Invitrogen™, Thermo Fisher Scientific Inc., Carlsbad, CA, USA) using NuPAGE™ 4–12% Bis-Tris precast gels and 1 × NuPAGE™ MES-SDS running buffer (Invitrogen™, Thermo Fisher Scientific Inc., Carlsbad, CA, USA). After electrophoresis, the gels were stained all-night with Coomassie Brilliant Blue R-250 and destained the following day. Gel images were subsequently obtained using a GS-800 densitometer (Model GS-800, Bio-Rad Laboratory Chemicals, Hercules, CA, USA).

2.5.2. Western blot analysis

Western blotting was performed to assess the IgG/IgE binding potentials of all the samples, according to the protocol described by Yuan et al. (2017). The CAPJ-treated and untreated TM protein samples were first resolved by electrophoresis using NuPAGE Gel System (Invitrogen™, Thermo Fisher Scientific Inc., Carlsbad, CA, USA). The pre-run gel was then transferred onto a 0.45 µm nitrocellulose membrane (Thermo Scientific Inc., Rockford, IL, USA) for 1 h at 30 V using a transfer buffer in an XCell II Blot Module (Invitrogen™, Thermo Fisher Scientific Inc., Carlsbad, CA, USA). Tris-buffered saline (0.1 M Tris, 0.15 M NaCl, 0.05% Tween-20, pH 7.5) served as the washing buffer, which also served as blocking buffer with the addition of 5% BSA. After blocking for 2 h, blots were incubated overnight at 4 °C with either IgE (pooled human sera 1:8 or IgG rabbit sera raised against tropomyosin diluted 1:2000), accompanied by mild shaking. The blots were washed 5 times for 15 min each and incubated with their corresponding secondary antibodies, i.e., HRP-labelled goat anti-human IgE (1:200 dilution in blocking buffer) or anti-rabbit IgG (1:5000 dilution in blocking buffer) for 1 h at room temperature of 25 °C. After extensive washing with TBST, the membranes were then photographed using enhanced chemiluminescence by a chemiluminescent imaging system (Tanon-4200SF, Tanon Science & Technology Co., Shanghai, China) and the optimal image was selected.

2.5.3. Indirect enzyme linked immune sorbent assay (ELISA)

The IgG/IgE binding capacities of the solutions were further evaluated using indirect ELISA following the method previously described by Lv, Lin, Li, Ahmed, and Chen (2017). The concentrations of both CAPJ-treated and untreated TM samples were first adjusted to 100 µg/mL using 50 mM carbonate buffer (pH 9.6). The polystyrene 96 well ELISA plates were loaded with 100 µL per well of the samples and incubated overnight at 4 °C. Thereafter, the plate was rinsed 5 times with TBST (20 mM Tris-HCl, pH 7.5, 0.145 M NaCl, 0.05% Tween-20) and blocked with 5% BSA in TBST (200 µL per well) at 37 °C for 2 h. After blocking, the plates were washed again as described above. The plates were then incubated at 37 °C for 2 h with either 100 µL of human sera (1:50 dilution) from the 5 prawn allergic patients or rabbit sera (1:2000 dilution). After washing, the plates were incubated again for 2 h with either HRP-labelled goat anti-human IgE (1:1000 dilution in blocking buffer) or anti-rabbit IgG (1:5000 dilution in blocking buffer). The plates were then developed with a substrate solution containing TMB (100 µL per well) for 20 min with subsequent addition of 2 M sulfuric acid (50 µL) to stop the reaction. A microplate reader (Multiscan 1 MK3, Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to measure the optical density (OD value) at a wavelength of 450 nm.

2.6. Conformation stability

2.6.1. Amino acid analysis

The amino acids (AAs) contents of the TM samples were analyzed according to the procedure of Xia, Niu, Wu, and Zhou (2016) with little modification. An amount of 4 mL of the sample solution was transferred into a centrifugal test tube and mixed evenly with equal volumes of 15% sulfosalicylic acid. The samples were then refrigerated at 2–4 °C for 60 min and centrifuged at 10,000 rpm for 15 min. The supernatant was collected and centrifuged again at 10,000 rpm for 15 min. The

samples were then diluted and filtered with a 0.22–0.45 µm filter, and then transferred into reagent bottles for analysis using an automated amino acid analyzer (L-8900, Hitachi Ltd., Tokyo, Japan).

2.6.2. UV absorption and intrinsic fluorescence analysis

A UV–Vis-NIR spectrophotometer (UV-1800, Shimadzu Co., Kyoto, Japan) was used to obtain the absorption spectra of the CAPJ-treated and untreated TM solutions (1 mg/mL). Samples were scanned within a wavelength range of 220 to 330 nm. The intrinsic fluorescence of the samples were acquired using a fluorescence spectrophotometer (RF-6000, Shimadzu Co., Kyoto, Japan) at an excitation wavelength of 280 nm and between 300 and 400 nm for emission. The data were obtained using a 3 nm constant slit width at a temperature of 25 °C. A 1 cm path-length rectangular quartz cuvette was used in this experiment. Measurements were taken in triplicates.

2.6.3. Surface hydrophobicity

Surface hydrophobicity (H_0) of the protein samples was estimated using 8-anilino-1-naphthalene sulphonic acid (ANS) as the fluorescence probe by the method of Kato and Nakai (1980). Protein dispersions were diluted in series (0.75, 0.5, 0.25 and 0.125 mg). The slope of the plot of fluorescence intensity against protein concentration, as derived by linear regression, was used as an index of H_0 and referred to as S_0 ANS.

2.6.4. Total sulfhydryl (SH) groups

The total free sulfhydryl groups (SH) was estimated as follows: an amount of 4 mL of buffer containing 0.086 M Tris-HCl, 0.09 M glycine, 0.004 M EDTA, 8 M urea (pH 8.0) and 40 µL of Ellman's reagent (10 mM 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) in 0.1 M sodium phosphate buffer, pH 7.0) was used to solubilize 2 mL aliquot of the samples. The mixture was mixed and kept in the dark for 25 min and absorbance was read at 412 nm. The SH contents were estimated using the Ellman's molar absorptivity constant of 13, 600 and expressed in µmol/g.

2.6.5. Circular dichroism spectroscopy

The changes in the secondary structure of the TM samples (1 mg/mL) were determined using a spectropolarimeter (Chirascan-plus, Applied Photophysics Ltd., Surrey, UK) at a spectral range of 190–260 nm. The secondary structures content (%) was estimated from the data using CDNN software (Böhm, Muhr, & Jaenicke, 1992).

2.6.6. Particle size

Modifications in the particle size distribution of the control and CAPJ-treated TM samples (1 mg/mL) were analyzed at a temperature of 25 °C using a Zetasizer Nanosystem (Nano-ZS90, Malvern Instruments Ltd., Worcestershire, UK), after injection into a clear zeta cells. The particle size distribution was subsequently reported automatically using Malvern standard operation procedure (SOP) software (Malvern Instruments Ltd., Worcestershire, UK).

2.7. Statistical analysis

One-way analysis of variance (ANOVA) alongside Duncan's multiple range was used for analyzing the significant differences among the means of the samples, represented as mean ± standard deviation (SD), using SPSS software (SPSS Version 19.0, SPSS Inc., Chicago, USA). Only a significant difference ($p < 0.05$) was considered, unless otherwise indicated.

3. Results and discussion

3.1. Emission diagnostics

OES was used to diagnose the plasma gas in order to determine the type of reactive species liable for structural modifications of prawn TM

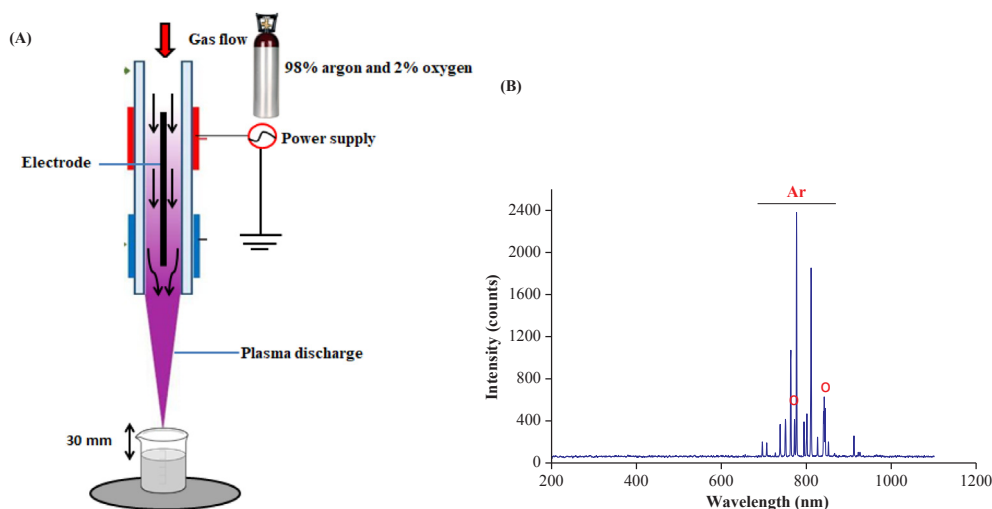


Fig. 1. (A) Diagrammatic and schematic representation of experimental set-up of the plasma jet system, (B) Typical optical emission spectrum of excited species generated in argon-oxygen gas.

and its associated allergenicity response. These active species may include both free radicals and UV-emitting particles. Fig. 1 depicts the typical emission spectra of the cold argon-oxygen plasma (CAPJ) scanned between a wavelength range from 190 to 2000 nm. As shown, no visible peaks could be seen around the shorter wavelength regions, i.e., UV-C and UV-B regions. However, the region between 650 and 850 nm excited states of argon atoms in the 4p state, and the metastable singlet state of oxygen at around 777.1 nm were visualized. Surowsky, Frohling, Gottschalk, Schluter, and Knorr (2014) also confirmed the presence of argon atoms and single state oxygen within this wavelength range. The concentration of oxygen species and radicals produced depends on the amount of oxygen present in the feed gas, with higher oxygen concentrations generating more amounts of these radicals. In general, protein molecules comprise of linear chains of amino acids, which are prone to oxidation by atomic or metastable oxygen species. It was thus speculated that reactive and oxidizing species generated in the plasma could alter the properties or the characteristic epitope of allergenic proteins in various ways, depending on the magnitude produced.

3.2. SDS-PAGE analysis of CAPJ-treated TM

Tropomyosin is known as the major allergen in prawn for its essential role in muscle function and its highly conserved primary structure. As shown in Fig. 2, the band of native tropomyosin occurred around the protein marker of 36.5–40 kDa. The results showed that in the presence of reducing DTT, the band intensity of native TM remained almost parallel even at the maximum treatment duration of 15 min. In addition, no newly generated band emerged at all treatment times, signifying the absence of protein crosslinking. A corresponding result was obtained by Tammineedi, Choudhary, Perez-Alvarado, and Watson (2013), who observed no visible changes in the protein profile of α -casein, the major allergen in milk when compared with the untreated sample. On the other hand, in the absence of the reducing agent DTT, band denseness appears to slightly fade upon prolonged exposure to CAPJ. This may be due to increased protein-to-protein interactions derived during protein unfolding. Literature focused on examining the effect of cold plasma on allergenic proteins is relatively scarce, thus detailed comparison of results may be relatively difficult at this stage. It was concluded in the current study that no significant changes or biodegradation of protein profiles were observed but this tendency appeared to vary with nature of the allergen studied, power or voltage level, mode of exposure and the degree of structural modification, among others. Most importantly, the specific operating conditions

employed considerably determine the amount of active species such as free radicals, and having one or more unpaired electrons (e.g. hydroxyl and hydroperoxyl radical) should be very instrumental in biomolecule degradation by disruption or cleavage of TM molecules.

3.3. Antigenicity and allergenicity response of CAPJ treated TM

The antigenicity and allergenicity response of prawn TM was investigated via western blotting (Fig. 2). Similar to ELISA results, for the IgE binding capacity, which was determined using sera pooled from prawn allergic patients, no obvious difference in denseness between the native TM bands and the CAPJ-treated TM samples was observed within the first few min of treatment, except at longer treatment times of 12 and 15 min. Likewise, for IgG a similar trend was witnessed in the band width of native TM and samples exposed to CAPJ treatment at different time intervals (0, 3, 6, 9, 12 and 15 min). With the increase in CP treatment time, the intensities of the TM bands remained nearly parallel but changed slightly at the maximum treatment duration, which signifies lower IgG binding ability. This slight reduction in IgG binding potential may be due to the modification of conformational structure and release of hydrophobic or hydrophilic residues which permit re-orientation antigenic epitopes. The properties of allergenic proteins and conformational stability are quite intertwined. For the latter, it might be possible to achieve modification of conformational and linear epitopes concurrently. Conformational epitopes are modified as a result of formation of insoluble aggregates or crosslinking of proteins due to decline in protein solubility, while linear epitopes are influenced by fragmentation (Meinlschmidt et al., 2016). In addition, ample reactive oxygen and nitrogen species (ROS and RNS) are required since they may be able to attack oxidation-prone amino acids, thereby destroying the binding sites or limiting the accessibility for antibodies due to compact structure or re-association of protein molecules.

In addition, the antigenicity response of prawn TM following CAPJ treatment at different time intervals was determined with indirect ELISA using rabbit polyclonal antibodies (IgG) and serum pooled from allergic patients (IgE). As shown in Fig. 3, no significant difference in IgG was observed between the control and the TM samples were recorded at different time durations (0, 3, 6, 9 and 12 min), except for prolonged exposure of 15 min where up to 26% reduction was recorded. For IgE, a similar trend was visualized but upon prolonged exposure, it appeared that prawn TM showed nearly about 17.6% significant reduction in IgE-binding capacity (Fig. 3). Thus from the ELISA results, it could be deduced that TM experienced some degree of unfolding or aggregation due to oxidation. Previous literature indicated

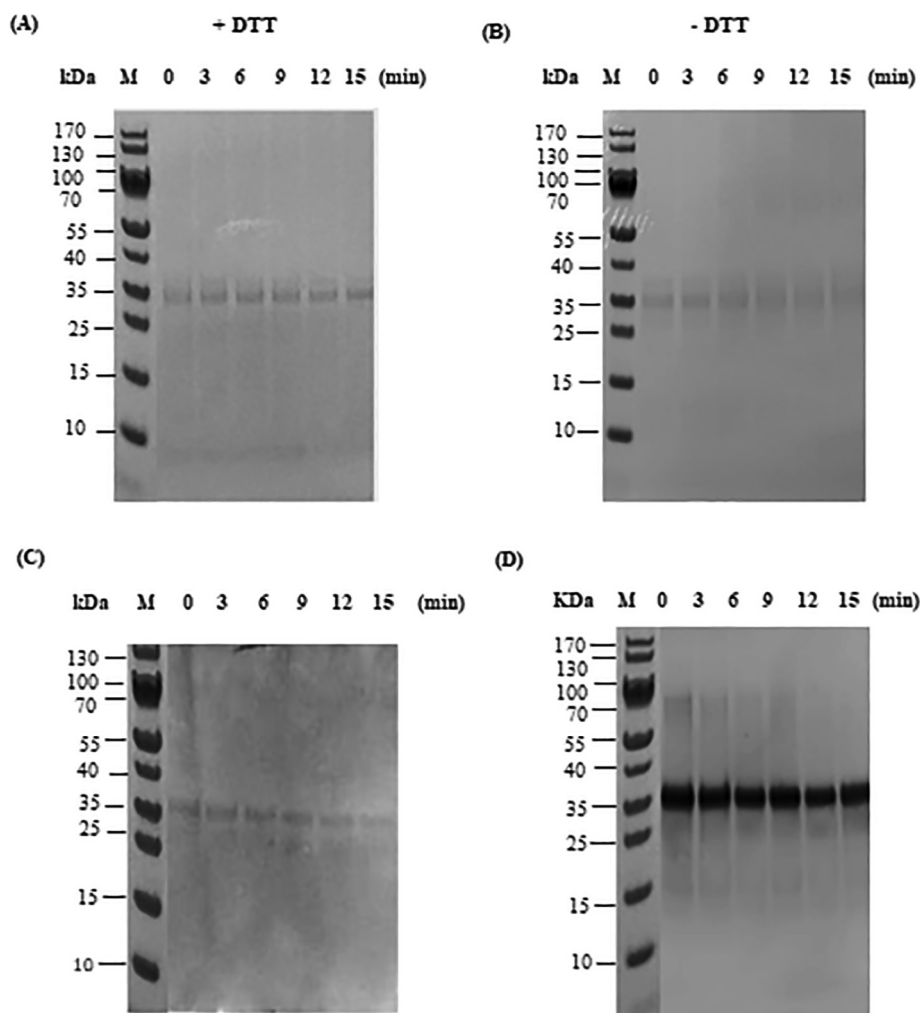


Fig. 2. SDS-PAGE profiles and western blot analysis of untreated and CAPJ-treated prawn TM subjected to different exposure times. SDS-PAGE (A) + dithiothreitol, and (B) – dithiothreitol. Western blot (C) IgE binding potential for detecting changes in the antigenic activity of treated prawn TM using pooled sera from patients allergic to prawn, and (D) IgG binding potential for detecting changes in the antigenic activity of treated prawn TM using rabbit polyclonal antibodies. M represents the standard protein marker. Lane 1: Protein marker; Lane 2–7: Prawn TM (control) and samples treated for 3, 6, 9, 12, 15 min, respectively.

that nonthermal plasma under specific conditions was effective in reducing the allergenicity of soy protein isolate (Meinlschmidt et al., 2016) and wheat allergens (Nooji, 2011). For instance, a voltage of 30 kV and frequency of 60 Hz were used to achieve attenuation of wheat allergenicity using DBD plasma. On the other hand, Tammineedi et al. (2013) reported the absence of significant differences ($p > 0.05$) in IgE binding abilities derived from Ci-ELISA analysis, between allergenic milk proteins subjected to plasma treatment and their control counterparts, although an indirect plasma source was used in their experiment. The slight changes in IgG and IgE binding abilities recorded in the current study might have occurred as a result of the type of active species present in the argon plasma discharge and their interaction with prawn TM. Nevertheless, it was speculated that higher argon plasma density and longer treatment duration have prospects for adequate disruption or masking of antibody binding epitopes, which may yield significant attenuation of prawn TM allergenicity.

3.4. Effect of CAPJ treatment on conformational stability of prawn TM

3.4.1. Amino acid profile

Changes in the primary structure of proteins can be inferred by using alterations in amino acid sequence. Tables 1 reflect the content of AAs in prawn TM exposed to CAPJ at different time intervals. The results indicate that CAPJ treatment showed a greater effect on AAs

profiles upon prolonged exposure. While all amino acids did not respond analogously to the different treatment times, compared with the control, the contents of most AAs increased, whereas sulfur-bearing AAs, i.e., cysteine and methionine, which have been described as particularly susceptible to ROS showed significant decrease ($p < 0.05$) from midway between the treatment intervals (6 min). It is possible that the side chains of the amino acids were oxidized preferentially to form various products. The interaction of plasma reactive species with sulfhydryl-containing amino acids were also reported in previous studies (Pal et al., 2016). However, in our study, an interesting observation was that the pronounced changes appeared to be associated with an increase in the concentration of the aromatic AAs (phenylalanine and tyrosine). Earlier investigations showed that both essential and non-essential amino acids in rice grain increased with ozone concentrations (Zhou et al., 2015). In general, the sequence of susceptibility of amino acids to active species is believed to start with sulfhydryl containing and aromatic AAs (Takai et al., 2014). Alterations in protein secondary structure and liberation of amino acids in plasma-protein interactions were described in literature (Surowsky, Fischer, Schlueter, & Knorr, 2013).

3.4.2. Effect of CAPJ on the tertiary structure of prawn TM

It is known that disulfide bonds formed by SH groups is important in defining protein tertiary structure and contributes to the stability and

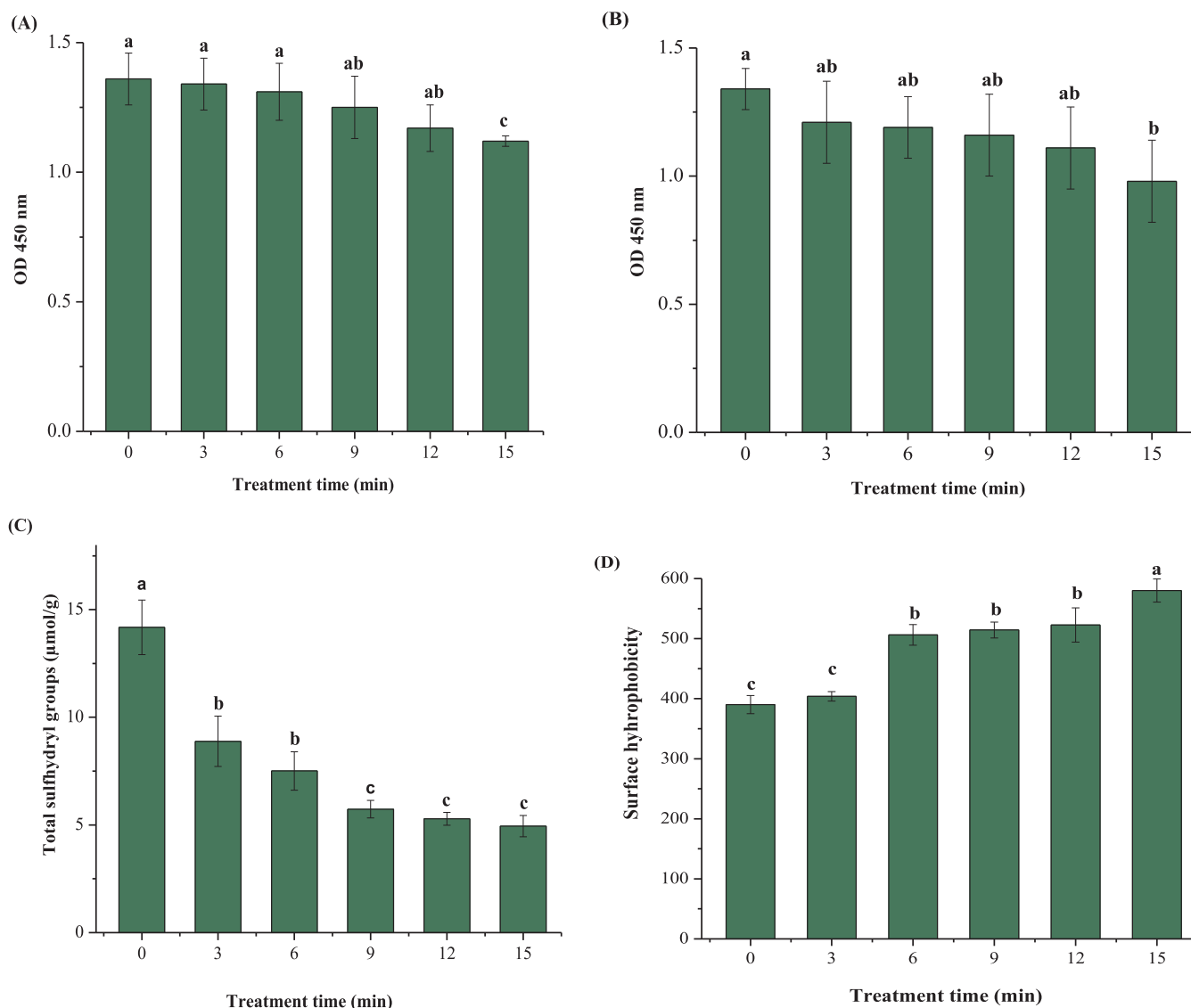


Fig. 3. Indirect ELISA response of tropomyosin subjected to different CAPJ treatment times against (A) IgE binding activity using pooled sera from allergic patients, and (B) IgG binding activity using rabbit polyclonal antibodies and changes in (C) Sulfhydryl groups and (D) Surface hydrophobicity of TM. *Values were means $\pm$ SD. Values in the same column with different letters were significantly different ($p < 0.05$).

function of protein molecules. Findings from CAPJ induced effects on the total sulfhydryls contents of prawn tropomyosin dilutions are displayed in Fig. 3c. In general, thiol groups are easily oxidized to disulfide bonds and a reduction in SH is an indicator of protein oxidation. In our study, the T-SH of the untreated prawn TM was $14.19 \pm 1.27 \mu\text{mol/g}$ proteins. As treatment progressed, a significant ($P < 0.05$) drop in T-SH was noticed until 15 min of plasma exposure where a minimum of $4.95 \pm 0.49 \mu\text{mol/g}$ protein was recorded. The remarkable decline in sulfhydryl contents was apparently due to oxidation of SH to form disulfide bonds during plasma application. The reactive free radicals, ions and neutrals present in the plasma gas might cause oxidation of susceptible free-SH groups, leading to the formation of S-S bonds and a decrease in total SH contents. Lower free SH contents upon plasma treatment were also reported in other works (Dong et al., 2017; Dong, Gao, Xu, & Chen, 2016; Ji et al., 2017).

In order to further understand the conformational changes, changes in surface hydrophobicity was used employed. As shown in Fig. 3d, the SoANS of samples processed from 6 to 15 min was significantly ($P < 0.05$) higher than that of control, with the maximum SoANS at the longest treatment time. Specifically, SoANS increased from an initial value of 390 ± 15.11 for untreated sample to 579.93 ± 19.21

after 15 min of CAPJ exposure. This implied that CAPJ treatment induced protein unfolding and caused hydrophobic portions initially buried in the interior to be exposed to the surface, resulting in an increase in SoANS. At longer treatment durations, the liberation of hydrophobic residues might enhance protein-protein interactions, leading to more re-association or aggregation among the unfolded proteins. Similar results were obtained by Segat et al. (2015), who found that CP treatment resulted in gradual increases of SoANS in whey protein isolate allergen as treatment time progressed, with stronger impacts at 30 and 60 min.

Intrinsic fluorescence of the CAPJ-treated samples was also obtained to ascertain the extent of conformational changes. In general, the intrinsic fluorescence spectra of proteins are chiefly ascribed to fluorescence emission by aromatic amino acid residue. The result of this assay is also presented in Fig. 4a. As treatment time progressed from 0 to 3 min, an initial increase in the maximum fluorescence intensity was observed, followed by a decline until the fluorescence intensity of prawn tropomyosin reached a minimum of 1864.20 ± 37.09 after 15 min of CAPJ exposure. In addition, a slight red shift in the maximum emission wavelength (from 338 to 339 nm) was also observed, corresponding to approximately 16% decline in the fluorescence intensity

Table 1
Free amino acid profiles of TM solutions subjected to cold argon plasma.

Amino acid (nmol/20 μ L)	Treatment time (min)					
	0	3	6	9	12	15
Aspartic acid	0.091 ^a	0.090 ^a	0.085 ^a	0.077 ^b	0.040 ^c	0.037 ^c
Threonine	0.093 ^a	0.101 ^a	0.131 ^a	0.142 ^a	0.178 ^a	0.376 ^a
Serine	0.048 ^b	0.064 ^{ab}	0.065 ^{ab}	0.0685 ^{ab}	0.068 ^{ab}	0.080 ^a
Glutamic acid	0.241 ^b	0.256 ^b	0.245 ^b	0.246 ^b	0.277 ^{ab}	0.289 ^a
Alanine	0.273 ^a	0.190 ^b	0.178 ^b	0.172 ^b	0.136 ^{bc}	0.10 ^c
Cysteine	0.159 ^a	0.150 ^a	0.132 ^{ab}	0.139 ^{ab}	0.137 ^{ab}	0.104 ^b
Valine	0.109 ^b	0.167 ^{ab}	0.134 ^{ab}	0.161 ^{ab}	0.224 ^a	0.232 ^a
Methionine	0.235 ^a	0.175 ^b	0.151 ^a	0.148 ^{cd}	0.117 ^{cd}	0.089 ^d
Isoleucine	0.038 ^b	0.058 ^{ab}	0.068 ^{ab}	0.065 ^{ab}	0.74 ^a	0.78 ^a
Leucine	1.138 ^a	1.110 ^a	1.147 ^a	1.164 ^a	1.185 ^{aa}	1.190 ^a
Tyrosine	2.247^e	2.668^d	3.335^c	4.103^d	6.301^a	6.167^a
Phenylalanine	3.700^e	3.819^e	5.345^d	6.455^c	6.910^b	7.360^a
Lysine	0.612 ^c	1.360 ^b	1.310 ^b	1.718 ^a	1.648 ^a	1.835 ^a
Histidine	< 0.01	< 0.01	< 0.01	< 0.01	0.096 ^a	0.105 ^a
Arginine	1.811 ^a	1.589 ^b	1.4393 ^c	0.836 ^d	0.5 ^d	< 0.01

^aValues were means $\pm$ SD. Values in the same column with different letters were significantly different ($p < 0.05$).

The most significant changes are indicated by bold-faced values.

after 15 min of CAPJ exposure. The decrease in the fluorescence intensities confirmed that plasma inherent reactive species could modify the microenvironment of Trp residues due to oxidation (Lakowicz, 2006). The changes illustrated that modification of TM conformation led to aggregation/reassociation reactions owing to the promotion of hydrophobic and/or hydrophilic interactions. Additionally, insights into the conformational changes in prawn TM induced by CAPJ treatment was also deduced using UV absorption spectroscopy. As

mentioned earlier, for native protein, the UV absorption spectra are predominately determined by Trp, Try and Phe present in the side chain groups (Tong et al., 2012). The maximum UV absorbance peak of untreated prawn TM appeared at around 276 nm. As depicted in Fig. 4b, CAPJ treatment resulted in a minor shift in UV absorbance peaks from 276 to 274 after CAPJ treatment for 15 min due to changes in micro-environment and polarity of amino acids. This structural modification of TM with plasma treatment might be responsible for the slight attenuation in IgE/IgG binding capacities.

3.4.3. Effect of CAP on the secondary structure of prawn TM

CD spectral analysis is a routine method used to ascertain changes in the secondary structure of proteins. As represented in Fig. 4c, native TM spectra showed a characteristic positive peak at approximately 196 nm, indicating the presence of β -sheets and two negative peaks (~ 210 nm and 222 nm) and thus confirming the existence of α -helices. Therefore the prawn TM predominantly contained α -helices. With increasing CAPJ treatment time, the intensity of the negative peaks began to decline, denoting a decrease in the amounts of α -helices in TM. By calculation, values of α -helices declined, while β -sheet, β -turn, and disordered structures increased (Fig. 4d). These changes in both the positive and negative minima symbolized alterations in TM secondary structure owing to oxidative interaction of reactive species with proteins. Meng et al. (2016) also reported variations in secondary structures in milk proteins exposed to gamma irradiation. An ample amount of active species is required to oxidize the amino acid side chains of TM, leading to the disruption of van der Waal bonds and alteration in secondary structures. Such aggregation/re-association reactions, re-arrangement and disruption in protein secondary due to protein unfolding can ultimately lead to modification of antigen epitopes and attenuation of their binding capacities. This might also be liable for the

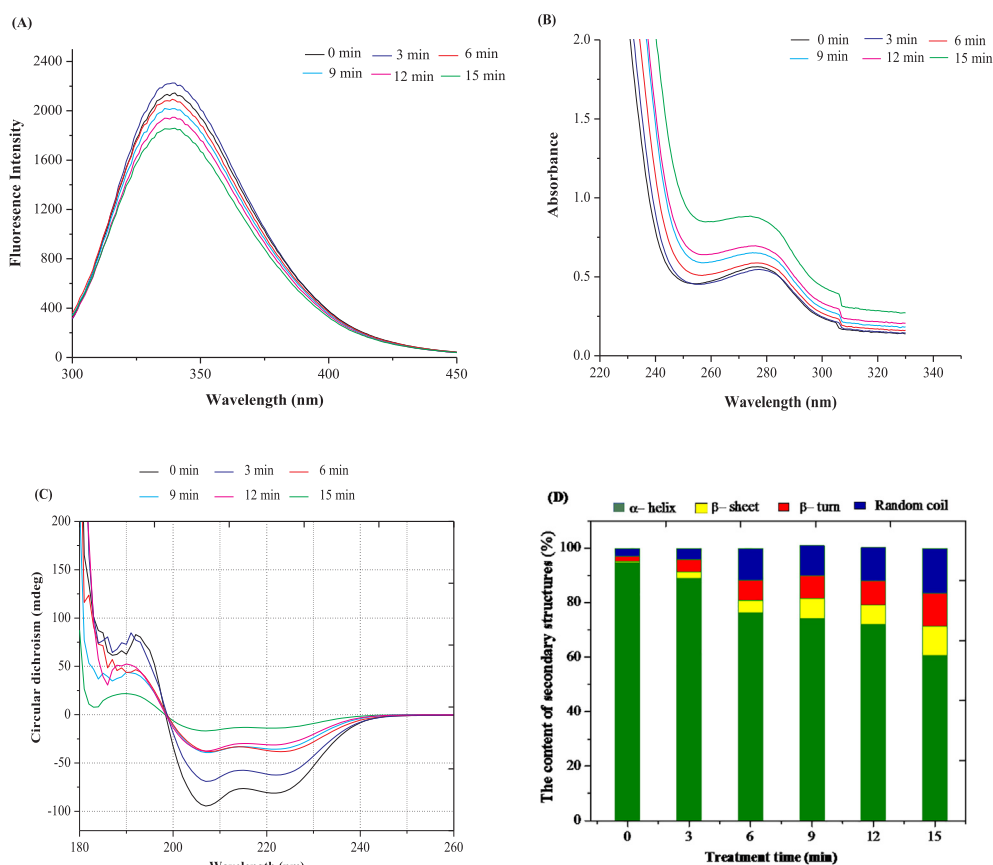


Fig. 4. Effects of increasing CAPJ treatment times on the intrinsic fluorescence (A) and UV absorption (B) circular dichroism spectra (C), and secondary structure content (D) of prawn tropomyosin.

profound 89–100% reduction in the allergenic potential of soy isolates recorded by Meinschmidt et al. (2016) using a DBD plasma (direct) and indirect (microwave plasma torch).

Although no clear consensus have been reached on the exact mechanisms liable for the reduction in allergenicity by CAPJ treatment and further work is still necessary to identify the exact interactions that occur between the chemical species generated and the epitope sites in TM, studies shows that any process that can modify the structure of a protein may influence its ability to be recognized by IgE antibodies (Sathe, Liu, & Zaffran, 2016; Sicherer & Sampson, 2010; Song et al., 2015; Li et al., 2013; Yang, Mwakatage, Goodrich-Schneider, Krishnamurthy, & Rababah, 2012; Zhao et al., 2014; Zhu, Vanga, Wang, & Raghavan, 2018; Zhang, Zhang, Chen, & Zhou, 2018). Depending on the processing method, allergenicity may either increase or decrease due to re-orientation or destruction of epitopes or formation of new epitopes otherwise called ‘neoallergens’ which interferes with potential IgG/IgE binding capacity of an allergen (Sathe et al., 2016). These effects are thought to be due to modifications of secondary, tertiary, or even quaternary structures of polypeptides which lead to reorientation of characteristic epitopes present in the allergenic protein. In the case of plasma, studies have shown that its reactive species is capable of modifying the functional properties of proteins depending on process parameters such as the type of feed gas, power input, and extent of exposure to reactive species, mass transfer and structural complexity and stability of the protein (Bußler et al., 2015; Dong et al., 2016; Ji et al., 2017; Segat et al., 2015). Precisely, Bußler et al. (2015) observed an increase in the water and fat-holding capacities of pea flour following exposure to cold plasma for up to 10 min. The authors noted that the incidence of conformational alteration may be liable for the observed changes in the functionality of pea flour fractions. In addition, Segat et al. (2014) studied the impact of plasma on the techno-functionality of whey protein isolate at different time intervals. Their findings revealed an increment in surface hydrophobicity and foaming stability of the sample due to protein aggregation. Hence, the underlying mechanisms or possible pathways for the action of plasma on allergenic reactivity of TM may not be far-fetched from the norm. Plasma species may be capable of interacting with proteins and inducing structural modification, breakdown of peptide bonds and formation of protein–protein cross linkages or chemical modifications of the side chains by the action of the myriad of chemically active species such as by hydroxyl radicals ($\cdot\text{OH}$), superoxide anion radicals ($\text{O}_2\cdot$), hydroperoxy radicals ($\text{HOO}\cdot$) and nitric oxide ($\text{NO}\cdot$) constituting the plasma (Meinschmidt et al., 2016; Venkataratnam, Sarangapani, Cahill, & Ryan, 2019; Yang et al., 2018). Conformational epitopes can be modified via formation of insoluble aggregates or protein cross-linking due to decline in protein solubility, while linear epitopes are influenced by fragmentation caused by peptide bond cleavage and oxidation of amino acid side chains as a result of plasma-immanent species. Meanwhile, ROS and RNS also participate in protein oxidation and etching (Surowsky et al., 2013), by oxidizing amino acids, leading to destruction of binding sites, followed by a change in antibody binding capacity. These series of activities may have occurred in CAPJ-treated TM, thereby influencing the allergen’s recognition potential. A higher attenuation effect may be possible with plasma jet systems depending on feed gas employed, voltage level, plasma diameter and distance of the nozzle from sample.

3.4.4. Particle size distribution

The physicochemical and structural properties of proteins can transform mechanically during processing. It has been established that plasma active species, depending on the magnitude and process parameters employed, may cause partial denaturation of proteins, which significantly affects their properties (Ekezie et al., 2019; Ekezie, Cheng, & Sun, 2018a). The average particle size of TM samples under different time intervals of CP treatment are shown in Fig. 5. In comparison with the untreated TM, the average particle size of TM decreased obviously

from 685.7 nm to 524.1 nm within the first 6 min of CP application. The reduction in average size may be attributed to some level of protein miniaturization of existing TM agglomerates and aggregates. However, a critical look at the peaks of the polydispersed solution upon prolonged application of CP showed an increase in mean particle size from 524.1 nm (6 min) to 555.16 nm (15 min), indicating the occurrence of protein re-association owing to the bombardment of the TM solutions with high energetic ions (electrons, ions, and other active species), causing the exposure of active sites. The formation of larger aggregates or agglomerates was well noticeable at the longest treatment of 15 min (Peak 3). These results were in agreement with previous studies (Ji et al., 2017; Segat et al., 2015), in which an increase in particle size following application of CP to protein micelles was observed.

4. Conclusions

The current study was designed to assess the structural changes and allergenic response of prawn TM following exposure to CAPJ treatment. The current results showed that for IgE, nearly about 17.6% significant reduction in IgE-binding capacity was observed while for IgG binding, approximately 26.87% reduction occurred due to the fragmentation of conformational epitopes. By applying CAPJ treatment from 3 to 9 min, only slight changes in TM antigenicity are obtainable. At longer treatment times, surface hydrophobicity and total SH contents are altered considerably accompanied by changes in the content of secondary structures evident by a decline and increase in α -helix and β -sheets structures respectively. These modifications in tertiary and secondary structures may collectively influence the antibody-binding capacity of TM. Alteration of amino acids in the IgE binding regions could also be another main reason. Although it was possible to achieve some level of mitigation of TM allergenic potential using plasma jet system, to achieve better results, CAPJ can be combined with other methods such as enzymatic hydrolysis, interaction with phenolic compounds or even other emerging technologies. More specific treatment approaches should be designed for application in whole samples and complex food systems. This may include using a plasma-mediated process such as PAW (plasma activated water) or in-package treatment that proffers larger surface area. It is also recommended to conduct studies regarding plasma-treated TM’s digestibility by intestinal enzymes as well as clinical trials for validation. Further, developmental stages involving safety and toxicity assessments for understanding the potential health-related effects are needed. While the exact and precise mechanisms through which plasma alters allergenicity are still unclear; though it is possible that the reactive species induce alterations in the molecular structure and physical properties of the protein, thereby affecting allergenicity; further studies are needed for clarification. Various uncertainties regarding the industrial application of the current approach may exist; however, the work provides insights into the influence of various processing parameters on process efficacy, which can facilitate future process design and optimization. In general, plasma treatments may be potentially useful in commercial applications for reducing the allergenicity and extending the shelf-life of processed prawns and seafood products in general.

Declaration of Competing Interest

None.

Acknowledgements

The authors are grateful to the National Key R&D Program of China (2018YFC1603404) for its support. This research was also supported by the Natural Science Foundation of Guangdong Province (2017A030310558), the S&T Project of Guangzhou (201804010469, 201610010104), the S&T Project of Guangdong Province (2017B020207002), the Agricultural Development and Rural Work of

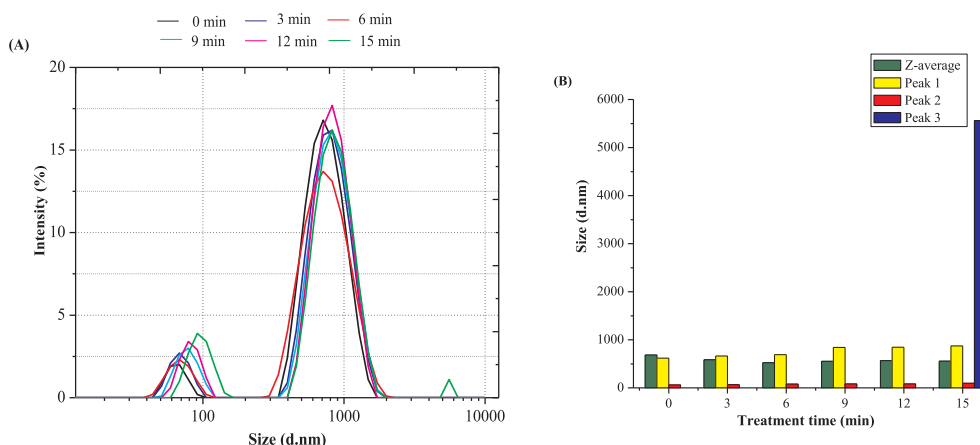


Fig. 5. Effects of CAPJ on the particle size distribution (A) and particle size (B) of prawn TM.

Guangdong Province (2017LM4173), the International and Hong Kong – Macau - Taiwan Collaborative Innovation Platform of Guangdong Province on Intelligent Food Quality Control and Process Technology & Equipment (2015KGJHZ001), the Guangdong Provincial R & D Centre for the Modern Agricultural Industry on Non-destructive Detection and Intensive Processing of Agricultural Products, the Common Technical Innovation Team of Guangdong Province on Preservation and Logistics of Agricultural Products (2016LM2154) and the Innovation Centre of Guangdong Province for Modern Agricultural Science and Technology on Intelligent Sensing and Precision Control of Agricultural Product Qualities. Flora-Glad Chizoba Ekezie is in receipt of financial support from China Scholarship Council for her PhD study.

References

- Böhm, G., Muhr, R., & Jaenicke, R. (1992). Quantitative analysis of protein far UV circular dichroism spectra by neural networks. *Protein Engineering, Design and Selection*, 5(3), 191–195.
- Buřler, S., Steins, V., Ehlbeck, J., & Schlüter, O. (2015). Impact of thermal treatment versus cold atmospheric plasma processing on the techno-functional protein properties from *Pisum sativum* 'Salamanca'. *Journal of Food Engineering*, 167, 166–174.
- Cheng, L., Sun, D.-W., Zhu, Z., & Zhang, Z. (2017). Effects of high pressure freezing (HPF) on denaturation of natural actomyosin extracted from prawn (*Metapenaeus ensis*). *Food Chemistry*, 229, 252–259.
- Chouljenko, A., Chotiko, A., Solval, M. J. M., Solval, K. M., & Sathivel, S. (2017). Chitosan nanoparticle penetration into shrimp muscle and its effects on the microbial quality. *Food and Bioprocess Technology*, 10(1), 186–198.
- Dai, X.-Y., Zhang, M.-X., Wei, X.-Y., Hider, R. C., & Zhou, T. (2016). Novel multifunctional hydroxypropylidone derivatives as potential shrimp preservatives. *Food and Bioprocess Technology*, 9(7), 1079–1088.
- Dong, S., Gao, A., Xu, H., & Chen, Y. (2016). Effects of Dielectric Barrier Discharges (DBD) cold plasma treatment on physicochemical and structural properties of zein powders. *Food and Bioprocess Technology*, 10(3), 434–444.
- Dong, S., Wang, J. M., Cheng, L. M., Lu, Y. L., Li, S. H., & Chen, Y. (2017). Behavior of zein in aqueous ethanol under atmospheric pressure cold plasma treatment. *Journal of Agriculture and Food Chemistry*, 65(34), 7352–7360.
- Ekezie, F.-G. C., Cheng, J.-H., & Sun, D.-W. (2018a). Effects of mild oxidative and structural modifications induced by argon-plasma on physicochemical properties of actomyosin from king prawn (*Litopenaeus vannamei*). *Journal of Agricultural and Food Chemistry*, 66(50), 13285–13294.
- Ekezie, F.-G. C., Cheng, J.-H., & Sun, D.-W. (2018b). Effects of nonthermal food processing technologies on food allergens: A review of recent research advances. *Trends in Food Science & Technology*, 74, 12–25.
- Ekezie, F.-G. C., Cheng, J.-H., & Sun, D.-W. (2019). Effects of atmospheric pressure plasma jet on the conformation and physicochemical properties of myofibrillar proteins from king prawn (*Litopenaeus vannamei*). *Food Chemistry*, 276, 147–156.
- Ekezie, F.-G. C., Sun, D.-W., & Cheng, J.-H. (2017). A review on recent advances in cold plasma technology for the food industry: Current applications and future trends. *Trends in Food Science & Technology*, 69, 46–58.
- Han, Y.-X., Cheng, J.-H., & Sun, D.-W. (2019). Activities and conformation changes of food enzymes induced by cold plasma: A review. *Critical Reviews in Food Science and Nutrition*, 59(5), 794–811.
- Ji, H., Dong, S., Han, F., Li, Y., Chen, G., Li, L., & Chen, Y. (2017). Effects of Dielectric Barrier Discharge (DBD) cold plasma treatment on physicochemical and functional properties of peanut protein. *Food and Bioprocess Technology*, 11(2), 344–354.
- Kato, A., & Nakai, S. (1980). Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochimica et Biophysica Acta (BBA)-Protein Structure*, 624(1), 13–20.
- Kaur, B. P., Kaushik, N., Rao, P. S., & Chauhan, O. P. (2013). Effect of high-pressure processing on physical, biochemical, and microbiological characteristics of black tiger shrimp (*Penaeus monodon*). *Food and Bioprocess Technology*, 6(6), 1390–1400.
- Lakowicz, J. R. (2006). Plasmonics in biology and plasmon-controlled fluorescence. *Plasmonics*, 1(1), 5–33.
- Lasekan, A. O., & Nayak, B. (2016). Effects of buffer additives and thermal processing methods on the solubility of shrimp (*Penaeus monodon*) proteins and the immunoreactivity of its major allergen. *Food Chemistry*, 200, 146–153.
- Li, Y., Yang, W., Chung, S.-Y., Chen, H., Ye, M., Teixeira, A. A., Gregory, J. F., Welt, B. A., & Shriver, S. (2013). Effect of pulsed ultraviolet light and high hydrostatic pressure on the antigenicity of almond protein extracts. *Food and Bioprocess Technology*, 6(2), 431–440.
- Lopata, A. L., Kleine-Tebbe, J., & Kamath, S. D. (2016). Allergens and molecular diagnostics of shellfish allergy. *Allergy Journal*, 25(7), 24–32.
- Lv, L., Lin, H., Li, Z., Ahmed, I., & Chen, G. (2017). Determining the effect of malondialdehyde on the IgE-binding capacity of shrimp tropomyosin upon in vitro digestion. *Journal of the Science of Food and Agriculture*, 97(13), 4588–4594.
- Meinlschmidt, P., Ueberham, E., Lehmann, J., Reineke, K., Schlüter, O., Schweiggert-Weisz, U., & Eisner, P. (2016). The effects of pulsed ultraviolet light, cold atmospheric pressure plasma, and gamma-irradiation on the immunoreactivity of soy protein isolate. *Innovative Food Science & Emerging Technologies*, 38, 374–383.
- Meng, X., Li, X., Wang, X., Gao, J., Yang, H., & Chen, H. (2016). Potential allergenicity response to structural modification of irradiated bovine α -lactalbumin. *Food & Function*, 7(7), 3102–3110.
- Nirmal, N. P., & Benjakul, S. (2012). Effect of green tea extract in combination with ascorbic acid on the retardation of melanosis and quality changes of pacific white shrimp during iced storage. *Food and Bioprocess Technology*, 5(8), 2941–2951.
- Nooj, J. K. (2011). *Reduction of wheat allergen potency by pulsed ultraviolet light, high hydrostatic pressure, and non-thermal plasma*. University of Florida.
- Pal, P., Kaur, P., Singh, N., Kaur, A., Misra, N., Tiwari, B. K., ... Virdi, A. S. (2016). Effect of nonthermal plasma on physico-chemical, amino acid composition, pasting and protein characteristics of short and long grain rice flour. *Food Research International*, 81, 50–57.
- Pan, Y., Cheng, J.-H., Lv, X., & Sun, D.-W. (2019). Assessing the inactivation efficiency of Ar/O₂ plasma treatment against listeria monocytogenes cells: sublethal injury and inactivation kinetics. *LWT - Food Science and Technology*, 111, 318–327.
- Sathe, S. K., Liu, C., & Zaffran, V. D. (2016). Food Allergy. *Annual Review of Food Science and Technology*, 7, 191–220.
- Savage, J., Sicherer, S., & Wood, R. (2016). The natural history of food allergy. *The Journal of Allergy and Clinical Immunology*, 4(2), 196–203.
- Segat, A., Misra, N. N., Cullen, P. J., & Innocente, N. (2015). Atmospheric pressure cold plasma (ACP) treatment of whey protein isolate model solution. *Innovative Food Science & Emerging Technologies*, 29, 247–254.
- Segat, A., Misra, N. N., Fabbro, A., Buchini, F., Lippe, G., Cullen, P. J., & Innocente, N. (2014). Effects of ozone processing on chemical, structural and functional properties of whey protein isolate. *Food Research International*, 66, 365–372.
- Sicherer, S. H., & Sampson, H. A. (2010). Food allergy. *Journal of Allergy and Clinical Immunology*, 125(2), S116–S125.
- Song, Y., Li, Z., Lin, H., Du, S., Hao, Z., Lin, H., & Zhu, Z. (2015). Effect of malondialdehyde treatment on the IgE binding capacity and conformational structure of shrimp tropomyosin. *Food Chemistry*, 175, 374–380.
- Surowsky, B., Fischer, A., Schlüter, O., & Knorr, D. (2013). Cold plasma effects on enzyme activity in a model food system. *Innovative Food Science & Emerging Technologies*, 19, 146–152.
- Surowsky, B., Frohling, A., Gottschalk, N., Schlüter, O., & Knorr, D. (2014). Impact of cold plasma on *Citrobacter freundii* in apple juice: Inactivation kinetics and mechanisms. *International Journal of Food Microbiology*, 174, 63–71.
- Takai, E., Kitamura, T., Kuwabara, J., Ikawa, S., Yoshizawa, S., Shiraki, K., ... Kitano, K. (2014). Chemical modification of amino acids by atmospheric-pressure cold plasma in aqueous solution. *Journal of Physics D: Applied Physics*, 47(28), 285403.
- Tammineedi, C. V. R. K., Choudhary, R., Perez-Alvarado, G. C., & Watson, D. G. (2013).

- Determining the effect of UV-C, high intensity ultrasound and nonthermal atmospheric plasma treatments on reducing the allergenicity of α -casein and whey proteins. *LWT - Food Science and Technology*, 54(1), 35–41.
- Tong, P., Gao, J., Chen, H., Li, X., Zhang, Y., Jian, S., ... Liu, F. (2012). Effect of heat treatment on the potential allergenicity and conformational structure of egg allergen ovomucoid. *Food Chemistry*, 131(2), 603–610.
- Vanga, S. K., Singh, A., & Raghavan, V. (2017). Review of conventional and novel food processing methods on food allergens. *Critical Reviews in Food Science and Nutrition*, 57(10), 2077–2094.
- Venkataratnam, H., Sarangapani, C., Cahill, O., & Ryan, C. B. (2019). Effect of cold plasma treatment on the antigenicity of peanut allergen Ara h 1. *Innovative Food Science & Emerging Technologies*, 52, 368–375.
- Wang, S., Delgado, J. C., Ravkov, E., Eckels, D. D., Georgelas, A., Pavlov, I. Y., ... Wagner, L. A. (2012). *Penaeus monodon* tropomyosin induces CD4 T-cell proliferation in shrimp-allergic patients. *Human Immunology*, 73(4), 426–431.
- Wang, Y., Liu, L., Zhou, J., Ruan, X., Lin, J., & Fu, L. (2015). Effect of chitosan nanoparticle coatings on the quality changes of postharvest whiteleg shrimp, *Litopenaeus vannamei*, during storage at 4 °C. *Food and Bioprocess Technology*, 8(4), 907–915.
- Wang, J., Tang, J., Rasco, B., Sablani, S. S., Ovissipour, M., & Qu, Z. (2018). Kinetics of quality changes of shrimp (*Litopenaeus setiferus*) during pasteurization. *Food and Bioprocess Technology*, 11(5), 1027–1038.
- Wu, Y., Liang, Y., Wei, K., Li, W., Yao, M., & Zhang, J. (2014). Rapid allergen inactivation using atmospheric pressure cold plasma. *Environmental Science and Technology*, 48(5), 2901–2909.
- Xia, Q., Niu, M., Wu, C., & Zhou, R. (2016). Formation of ethyl carbamate in Goji wines: Effect of Goji fruit composition. *Food Science and Biotechnology*, 25(3), 921–927.
- Yang, R., Liu, Y., Meng, D., Wang, D., Blanchard, C. L., & Zhou, Z. (2018). Effect of atmospheric cold plasma on structure, activity, and reversible assembly of the phytoferritin. *Food Chemistry*, 264, 41–48.
- Yang, W. W., Mwakatage, N. R., Goodrich-Schneider, R., Krishnamurthy, K., & Rababah, T. M. (2012). Mitigation of major peanut allergens by pulsed ultraviolet light. *Food and Bioprocess Technology*, 5(7), 2728–2738.
- Yuan, F., Lv, L., Li, Z., Mi, N., Chen, H., & Lin, H. (2017). Effect of transglutaminase-catalyzed glycosylation on the allergenicity and conformational structure of shrimp (*Metapenaeus ensis*) tropomyosin. *Food Chemistry*, 219, 215–222.
- Yuan, G., Pan, Y., Li, W., Wang, C., & Chen, H. (2019). Effect of extrusion on physico-chemical properties, functional properties and antioxidant activities of shrimp shell wastes protein. *International Journal of Biological Macromolecules*.
- Zhang, Z., Zhang, X., Chen, W., & Zhou, P. (2018). Conformation stability, *in vitro* digestibility and allergenicity of tropomyosin from shrimp (*Exopalaemon modestus*) as affected by high intensity ultrasound. *Food Chemistry*, 245, 997–1009.
- Zhao, X., Yang, W., Chung, S.-Y., Sims, C. A., Otwell, S. W., & Rababah, T. M. (2014). Reduction of IgE immunoreactivity of whole peanut (*Arachis hypogaea* L.) after pulsed light illumination. *Food and Bioprocess Technology*, 7(9), 2637–2645.
- Zhou, X., Zhou, J., Wang, Y., Peng, B., Zhu, J., Yang, L., & Wang, Y. (2015). Elevated tropospheric ozone increased grain protein and amino acid content of a hybrid rice without manipulation by planting density. *Journal of the Science of Food and Agriculture*, 95(1), 72–78.
- Zhu, Y., Vanga, S. K., Wang, J., & Raghavan, V. (2018). Effects of ultrasonic and microwave processing on avidin assay and secondary structures of egg white protein. *Food and Bioprocess Technology*, 11(11), 1974–1984.