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Effects of carrageenan oligosaccharides prepared by electron beam irradiation on frozen shrimps during storage

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Abstract: 1% carrageenan oligosaccharide solutions obtained by electron beam irradiation at the radiation doses of 9 kGy (CO-9) and 30 kGy (CO-30) were applied for shrimps before frozen, and their effects on quality of frozen shrimps were investigated by observing the microstructure of shrimp's muscle tissue, measuring their weight loss (%), hardness and pH after thawing. Comparison with the shrimps treated by 1% tetrasodium pyrophosphate solution ($Na_4P_2O_7$, positive control) and distilled water (negative control), the shrimp samples soaked in CO before frozen kept their initial properties, and the shrimps treated by CO-30 solution showed better quality than others after 3 weeks of frozen storage. Specifically, the mean distances of muscle bundles and muscle fibers in the shrimps soaked in CO-30 solution were 23.9 µm and 11.1 µm, respectively, much smaller than 37.9 µm and 14.8 µm in the shrimps treated by $Na_4P_2O_7$, and 46.7 µm and 19.8 µm in the shrimps soaked in distilled water only. Our results also revealed that the weight loss after thawing and pH of the shrimps soaked in CO-30 solution were 1.55% and 6.40, much lower than that of the positive control sample (2.59% and 6.82) and negative control sample (4.83% and 6.58), respectively. In contrast, the mean of hardness of the sample soaked in CO-30 solution was 20.4, about 20.7 and 36.0% higher than those of positive control (16.9) and negative control (15.0). These results suggested that CO-30 is effective to inhibit the denaturation of shrimp during frozen storage and can be applied to prolong the shelf life and keep the quality of frozen shrimp products.

Keywords: *Frozen storage, carrageenan oligosaccharides, irradiation, electron beam.*

I. INTRODUCTION

Carrageenan oligosaccharide (CO) is oligo-saccharide with biological activity, obtained from carrageenan by radiation degradation (gamma ray, electron beam), or chemical and biochemical hydrolyses. The average molecule weight (Mw) of oligo-saccharide is below 10kDa, and the CO with Mw of below 5.000 Da generally are constituted from approximately 10 units of D-galactose linked alternately with β(1,3)-D-galactose-4-sulfate and α(1-4)-3,6-anhydro-D-galactose [1,2,3]. Radiation scission of carrageenan in powder or solution forms by electron beam or gamma Co-60 ray irradiation can obtain fragments with M_w varying from 2.500 Da to 54.000 Da, depending on irradiation dose [1]. The method is considered to be simple, effective and environmentally friendly [1, 2]. Several properties of CO are

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widely exploited in a number of applications; for example, in agriculture (plant growth stimulation), in biomedicine (anti-oxidation, anti-ulceration, anti-microbiology, anti-tumor, and anti-cancer by promoting immune system) [4], in food technology (inhibition of the formation and development of ice crystals, and minimization of the physical damage of frozen seafood products). Furthermore, the protein-antioxidant activity of CO has significant effects on stabilizing muscle-protein structure and inhibiting the degeneration and oxidation of muscle-proteins, efficiently eliminating the weight-loss of the cold storage product during freezing and defrosting processes [5]. CO was also experimented in Surimi production from fish; herein, 0.2% addition of CO was proven to improve the quality and the stabilization property, which restricted the quality degradation of surimi during frozen storage [6]. CO can also inhibit the development of ice crystals, showing better preservation of naked white leg shrimp compared to tetrasodium pyrophosphate treatment after 8 weeks of frozen storage. In addition, CO can be utilized as an additive to enhance the hardness and the elasticity of meat rolls, or as a preservative additive to inhibit the growth of harmful microorganism in meat products without any change of color and flavor [8]. Therefore, CO is considered as an anti-denaturation additive during frozen storage, and it belongs to the cryoprotectant group that is accepted in many countries [7]. This study demonstrates the anti-denaturation properties of CO synthesized from kappa carrageenan for the preservation of frozen tiger shrimp. Herein, the electron beam irradiation was utilized.

II. EXPERIMENTAL

Sample preparation

Carrageenan (from Kappa-carrageenan, Duy Mai TRA-JSCO, Vietnam) was irradiated in 1 % solution by electron beam at dose of 9 and 30 kGy to obtained oligocarrageenan (CO) with average molecular weight (M_w) were 5,71 x 10^3 Da (CO-9) and 2,57 x 10^3 Da (CO-30), respectively, as mentioned in our previous study [9]. Fresh black tiger shrimps with average body weight and length are 29,37 ± 1,35 g and 16,00 ± 1,15 cm in good conditions (natural color and flavor, heads sturdily joined to bodies, shining peritoneum, hard and elastic flesh that strongly attached to the shell), were purchased from Area B, Thu Duc Market, Ho Chi Minh City. The number of pared black tiger shrimps per weight was 31,00 ± 0,58 shrimps/pound, which was categorized as Type 1 [10, 11].

Methodology

- *Procedure of preserving frozen naked shrimps:* Fresh black tiger shrimps were cleaned in 50 ppm chlorine solution for 2-3 minutes, and then cleaned with fresh water. After being dried for 1 minute, the shrimps were weighted to categorize. The heads, the legs and the shells were removed, and the shrimps were then washed with cold water (5°C) mixed with 10 ppm chlorine solution. In the next step, the shrimps' bodies were slightly washed in distilled water (0-4°C) and dried for other 1 minute. The naked shrimps were then weighted and categorized [10, 11]. Next, naked shrimps were soaked in 1% tetrasodium pyrophosphate solution ($Na_4P_2O_7$, positive control), distilled water (negative control), CO-9 and CO-30 solutions (CO-9 and CO-30 samples) in 60 minutes, dried for 1 minute, then put in the Styrofoam trays and packed in zip PE bag. The trays were frozen at -25°C for 3 hours 20 minutes, then preserved in a freezer (VH230HY 230L freezer, Sanaky, Japan) at -18°C. During storage, the shrimps were examined at various time intervals.

- *Microstructure of the muscle tissue:* The formation and growth of the ice crystals in muscle tissue of the frozen shrimps were

observed with an optical microscope (magnification 10x) to assess the structural change of the muscle tissue just after frozen stage and 21 days of storage. The second segments of the shrimps were cut into three parts (one horizontal part and two vertical parts) and stabilized in Davidson sample [19] solution (ratio 1:9) in at least 24 hours at room temperature. Then, all the samples were sent to tissue laboratory at the Research Institute for Aquaculture II, Ho Chi Minh City to conduct the tissue processing and observation.

- *Determining the defrost weight loss (DWL) (%)* by weighting the shrimps before and after defrosting, according to the AOAC method, [20] and following the formula (1):

$$DWL\ (\%) = \frac{m_1 - m_2}{m_1} \times 100 \qquad (1)$$

Herein, m_1 and m_2 are the weights of frozen and defrosted shrimps, respectively (g).

Frozen shrimps were defrosted by microwave at high temperature for 10 seconds so that the central temperature of the shrimps was in the range of 5-7°C. The recorded result was the mean of three measurements.

- *Hardness measurement:* The shrimp was placed on the surface of the hardness testing machines (shore C Test Stand, HLX-AC, HANDPI, China). The probe was adjusted up and down from the 2nd to the 4th segment of the shrimp's body so that the needle was at 0. After that, the lever was pushed down and the data on the monitor was noted. The recorded result was the mean of six measurements.

- *pH measurement:* the shrimps were ground in a meat grinder to slury. The pH probe (pH 11, Horiba, Japan) was put into the pureed shrimp and the data on the monitor was noted. The recorded result was the mean of six measurements.

III. RESULTS AND DISCUSSION

Microstructure of shrimps' muscle tissue during frozen storage

Microstructures of muscle tissues of all shrimp samples were observed just after frozen storage. As one can see from Figures 1-3, the muscle bundles were arranged closely with the distance of 29.80 µm (Figure 2. A-1 and 3a) and the muscle fibers were parallel arranged, continuously extended and closely packed (Figure 2. A-2 and 3b), with a distance of 8.80 µm, which was the shortest distance compared with others. For the shrimps treated by CO-9 (Figure 1. B-1, B-2 and 3a, 3b), CO-30 (Figure 1 C-1, C-2 and 3a, 3b) and $Na_4P_2O_7$ (Figure 1 D-1, D-2 and 3a, 3b), the average distance between muscle bundles decreased, but the average distance between muscle fibers increased, compared to the fresh shrimps. The most significant decrease in distance between muscle bundles was 16.80 µm, recorded with shrimps treated by CO-9. Smaller reductions were 5.40 µm and 5.00 µm, recorded with the shrimps treated by CO-30 and $Na_4P_2O_7$, respectively. The distance between the muscle fibers showed the highest increase was 13.20 µm in the sample treated by $Na_4P_2O_7$ and the smallest increase was 6.4 µm in the sample treated with CO-9. Obviously, the shrimps treated with CO-9, CO-30 showed the shorter average distances between muscle fibers, the more closely-arranged muscle bundles; herein, the muscle fibers were still parallel and did not undergo significant cutting, compared to the samples treated with $Na_4P_2O_7$ and distilled water (Figure 1. E-1, E-2 and 3a, 3b). The CO solution minimized the physical damage of the muscle because of the bonding with the surface of growing ice crystals, which leaded to the formation of stable crystal lattices in the muscle structure, and restricted the growth of the ice crystals [7, 12, 13]. On the other hand, for the sample soaked in distilled water, the

distance between muscle bundles showed the most considerable increase to 52.00 µm, with the uniform expansion among the muscle bundles (Figure 1. E-1 and 3a). Furthermore, the muscle fibers were unparallelly and disorderedly arranged - they experienced cutting into several short fibers at random positions, and the average distance of the muscle fibers increased by 5.20 µm (Figure 2. E-2 and 3b) compared to the fresh shrimp sample. During the freezing process, water gradually freeze to be ice crystals, the increase of solute concentration in the cytosol denatures muscle proteins, leading to protein degradation and weakening of the binding force between muscle fibers [14]. In addition, because of the slow freezing, the ice crystals tended to grow as the freezing time, which destroyed the cell membrane, cut the muscle fibers and expanded the outer space among the bundles [11].

However, the muscle bundles were still arranged closely to each other (Figure 2. C-4), except the fibers that were vertically cut due to the growth of ice crystals (Figure 2. C-5). The observation was similar to the arrangement of muscle fibers in the sample $Na_4P_2O_7$ (Figure 2. D-5) when there is a minimal difference of 3.70 µm in the distance between the muscle fibers (Figure 3b).

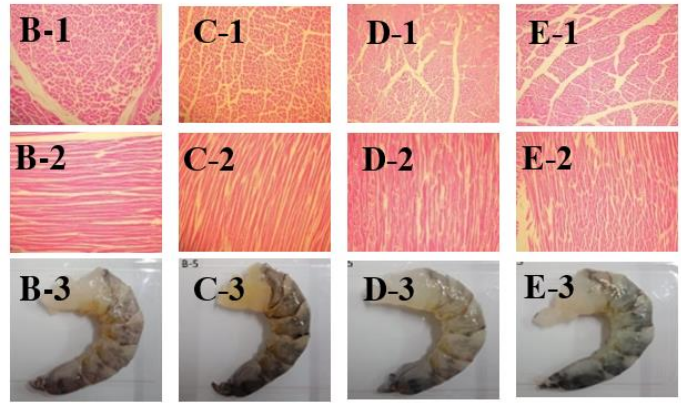

Fig.1. Muscle tissue samples at the second segment of the frozen shrimp (horizontally and vertically) and photos of shrimp samples: frozen shrimp CO-90: B-1, B-2, B-3 (0 day); frozen shrimp CO-30: C-1, C-2, C-3 (0 day); frozen shrimp $Na_4P_2O_7$ (positive control): D-1, D-2, D-3 (0 day); frozen shrimp distilled water (negative control): E-1, E-2, E-3 (0 day). Magnification of 10X.

After 21 days of frozen storage (Figure 2 and 3a, 3b), microstructure of shrimp samples revealed a linear increase in distances between muscle bundles and fibers as observed in all treated samples. The effect of stabilizing muscle structure was clearly demonstrated in each soaked solution. The muscle bundles and fibers of the CO-9 sample still bonded strongly to each other without the large gap generated from the growth of the ice crystals on the cross-section of the muscle tissue (Figure 2. B-4, B-5). The shortest distances among the muscle bundles and muscle fibers in the CO-9 sample were 10.10 and 9.40 µm, respectively (Figure 3a, 3b). For CO-30 sample, the distances among the muscle bundles and muscle fibers were respectively 23.90 and 11.10 µm (Figure 3a, 3b). In the muscle structure, there were some big gaps within the muscle bundles and fibers;

However, $Na_4P_2O_7$ sample was more gaps formed in the horizontal cross-section compared to CO-30 sample increased the distance between the muscle bundles to 37.90 µm (Figure 2. D-4 and 3a). Previous studies [9] proved that extracellular

environments witness the faster growth of ice crystals compared to intracellular environments. The increase of solute concentration in the extracellular solution depleted water inside the muscle fibers, which reduced the intracellular freezing point and expanded the muscle bundles and fibers' distances due to the growth of ice crystals [7]. The destruction of muscle tissue's structure was thoroughly observed in the samples soaked in distilled water, since the formation and growth of ice crystals were supported by surrounding water environment. We observed the uniform distribution of gaps in both horizontal and vertical cross-section (Figure 2. E-4 and E-5). The muscle bundles and fibers were not closely packed as in the control; instead, their distances expanded significantly to 46.70 and 19.80 µm, respectively (Figure 3a, 3b), which were the longer distances among the samples.

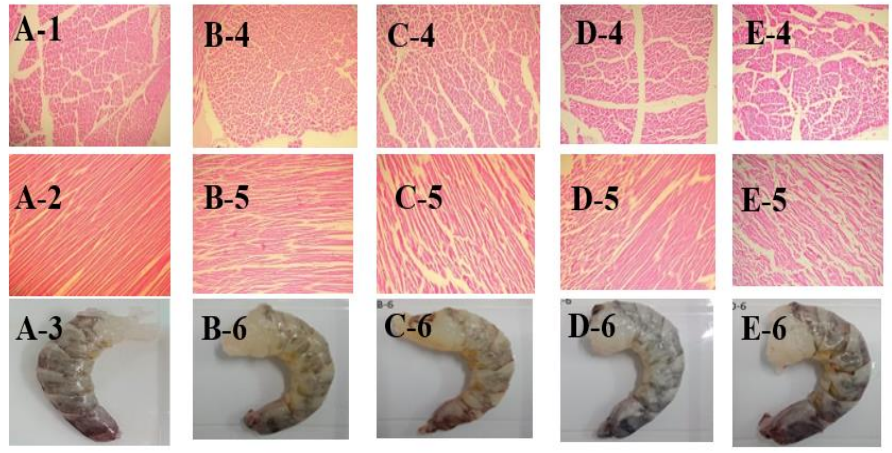

Fig.2. Microstructure of muscle tissues at the second vertebrae of the fresh shrimp (horizontally and vertically): A-1, A-2, A-3 (control). The shrimps after 21 days of frozen storage: B-4, B-5, B-6: frozen shrimp muscle tissue soaked in CO-9 solution; C-4, C-5, C-6: frozen shrimp tissue soaked in CO-30 solution; D-4, & D-5, D-6: frozen shrimp tissue soaked in 1% tetrasodium pyrophosphate solution (positive control); E-4,E-5, E-6: frozen shrimp tissue soaked in distilled water (negative control). Magnification of 10X.

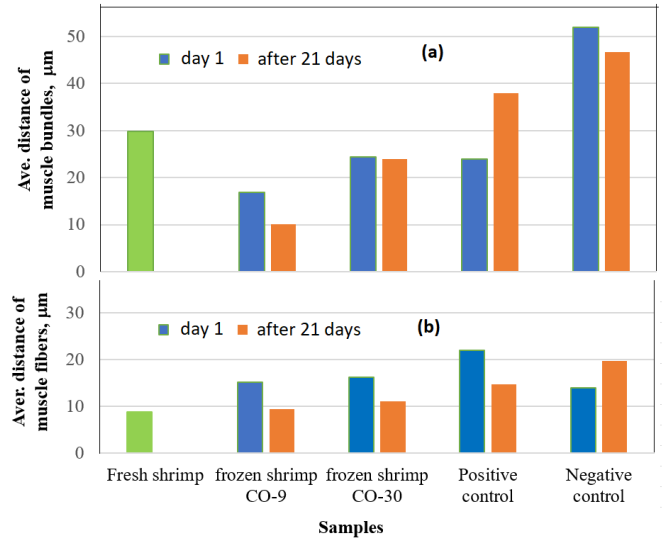

Fig.3. The average distance of muscle bundles (a) and muscle fibers (b) of fresh and frozen shrimps' tissue at days 1 and 21 of storage.

The hardness and weight loss of frozen shrimps after defrost

The hardness, elasticity and coherence of the shrimp's muscle structure decreased during defrosting process may due to some transformations in the freezing process [14]. When water crystallized and moved, the water was separated from the protein, which transformed the protein and reduced the number of hydrophilic centers in the muscle' structure [15]; hence, the shrimp muscle' capability of absorbing and retaining water decreased during defrosting. Figure 4 and 5 revealed that the average hardness of shrimp at day 1 of frozen storage was 4.7-30.8% lower, while the weight loss was 17.1-198.6% higher, compared to those after 21-days of frozen storage. It was due to stable growth of the ice crystals and formation of the crystal lattice, which interleaved among other components. During the defrost process, it was more time-consuming for the crystal lattice to be destroyed and the ice crystals melted; hence, the extract amount from the shrimps was lower compared to the shrimp sample just after treatment.

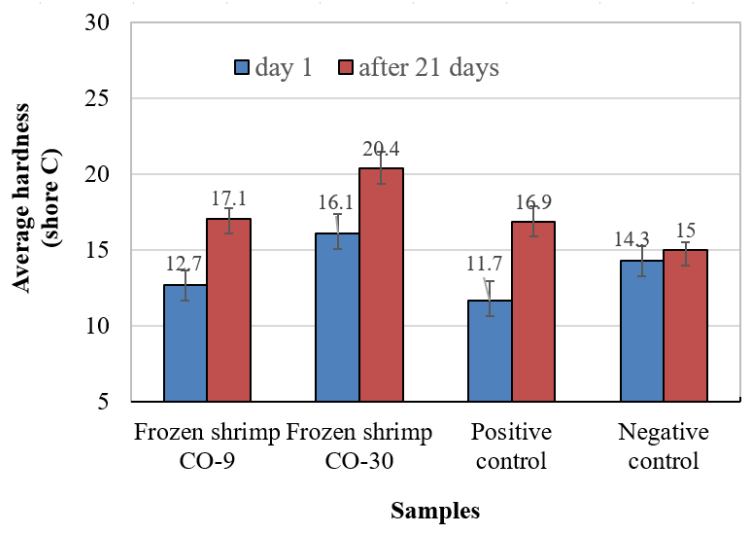

Fig. 4. Hardness variation of frozen shrimps after defrost (at days 1 and 21 of frozen storage)

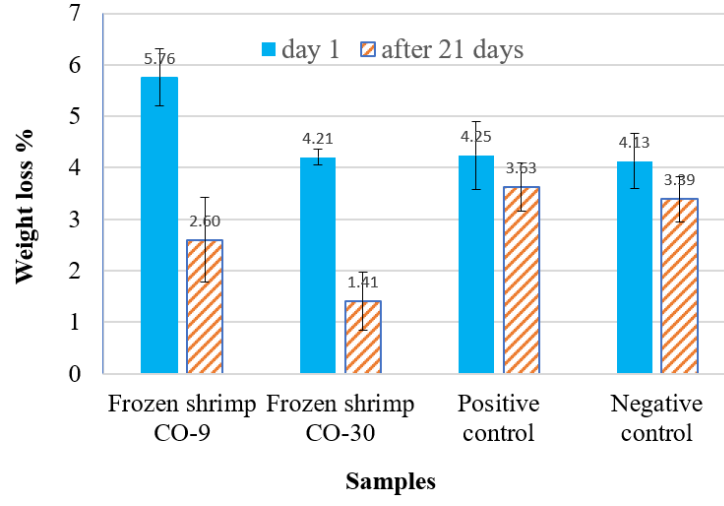

Fig. 5. Weightloss percentage of frozen shrimps after defrost (at days 1 and 21 of frozen storage)

After 21-day storage, the CO-9 and CO-30 shrimp samples showed the better hardness and the lower weight loss compared to the $Na_4P_2O_7$ and distilled water shrimp samples. It demonstrated the better preservative effects of CO-9 and CO-30 on the frozen shrimps. Weight loss of CO-30 shrimps was 1.41%, which was the lowest value. In addition, the hardness of CO-30 shrimp sample was 20.4, which was the highest value among other samples. The CO bonded with ice crystals generated the ice-oligosaccharide complex, forming the stable crystal lattice in the muscle structure, which inhibited the growth of ice crystals and minimized the negative impact of crystallization [7]; therefore, CO supported to reduce the weight loss of shrimp during defrosting. Besides, $Na_4P_2O_7$ also showed the positive effect as the interaction between the phosphate base and the muscle tissue enhanced the muscle's capability of retaining water, which minimized the extract amount and stabilized the shrimp's weight before and after defrosting [16]. The distilled water shrimp sample showed the worst hardness and weight loss, which can be attributed to the denaturation of the muscle protein in the environment with high solute concentration. The freezing of water and the quick evaporation during the freezing process led to the oxidation of muscle protein due to the free radicals, implying the stronger interaction among the protein molecules and the weaker water-protein interaction [17]. Figure 2. E-4 and E-5 revealed the entire destruction of the muscle tissue structure under the growth of ice crystals, which teared the muscle membrane, broke the endomysium [18]; therefore, the shrimp protein's capability of absorbing and retaining water decreased substantially during defrosting.

pH of frozen shrimps after storage

pH is an important factor that affects the microorganism's growth. Seafood with higher pH is more prone to spoil due to the microorganism's growth, reducing its shelf-life. After death, aquatic creatures generally undergo several transforming periods: slime secretion (pH~7), hardening (pH = 6.0-6.5) and self-decomposing by microorganism (rotting) (pH close to neutral) [14]. pH decrease is due to accumulation of acid lactic when glycogen was enzymatic decomposed, and the decrease of surface charge within the muscle tissue protein leads to the local denaturation and weakens the capability of muscle tissue' retaining water [14]. Table I did not indicate any change in the pH of CO-30 shrimp, with the values of 6.43 (0-day storage) and 6.40 (21-day storage), which were the lowest values among the other three samples. It means CO-30 shrimp was in the hardening period, while the other three samples were soften and seemed to be self-decomposed. This result confirmed that CO-30 shrimp was quite fresh, and it could recover to the initial condition after defrosting, implying the most suitability for frozen shrimp preservation.

Table I. pH of frozen shrimps after defrost (at days 1 and 21 of frozen storage).

Samples	pH	
	0 day	21 days
Frozen shrimp CO-9	6.80 ± 0.06	6.63 ± 0.10
Frozen shrimp CO-30	6.43 ± 0.09	6.40 ± 0.15
Positive control	6.95 ± 0.12	6.82 ± 0.07
Negative control	6.75 ± 0.09	6.58 ± 0.07

IV. CONCLUSIONS

κ-carrageenan oligosaccharide, obtained by EB radiation decomposition as in previous study, was utilized as an agent to prevent the degeneration of structure and quality of frozen shrimps. Our results revealed that the shrimps treated by carrageenan oligosaccharide before freezing still remained their best quality compared to the controls, as observed by their microstructure. This positive effect of CO treatment on the hardness, weight loss and pH of shrimp's muscles were also recognized. Thus, carrageenan oligosaccharide solution can be applied to extend the shelf-life and maintain the frozen shrimp products in good quality during storage.

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