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Improvement of Threadfin bream (*Nemipterus* spp.) surimi gel properties by electron beam irradiation

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Abstract The surimi gel of Threadfin bream (*Nemipterus* spp.) was irradiated with electrons from 0 to 5 kGy. When comparison to the non-irradiated gel, the gel treated at 5 kGy showed significantly maximum texture qualities (breaking force, deformation, and gel strength) as well as minimum in expressible water content ($p \leq 0.05$), resulting in smaller voids and a denser and compact network structure. The trichloroacetic acid-soluble peptide content of surimi gels were reduced after irradiation. The protein pattern from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed no significant changes in myosin heavy chain (MHC) intensity.

Keywords: E-beam, Microstructure, Protein gelation, SDS-PAGE, Threadfin bream surimi

Introduction

In Thailand, the threadfin bream (*Nemipterus* spp.) is an economically important fish species. It is used as a raw material for surimi manufacture due to its enormous production quantity and value (Yongsawatdigul *et al.*, 2010; Zhou *et al.*, 2014). Surimi quality is determined by a number of factors, one of which being gelation (Sutloet *et al.*, 2018b). The texture properties and sensory characteristics of surimi-based products are both a result of these physiochemical properties. The surimi processing business faces a challenge in improving this crucial parameter. Physical approaches such as microwave heating (Liu *et al.*, 2018), high pressure (Wang *et al.*, 2019), and irradiations have been used to improve this ability.

Ionizing radiation is an example of a non-thermal technology. Gamma irradiation, electron beam (Ebeam) irradiation, or X-ray technology are all options (Pillai and Shayanfar, 2018). However, due to its insoluble qualities,

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the use of cobalt-60 as a source of gamma irradiation can pollute water (Ashraf *et al.*, 2019). To provide very short (812 meters) wavelengths with high frequency (1016-1020 Hz) X-ray radiation necessitates substantial expenses (Adhikari, 2012). E-beam, which uses regular electricity as a source (Jaczynski and Park, 2003), has a low cost, safe, and effective operation when compared to other irradiation technologies.

One of the physical ways that has been used to improve gel-forming capabilities is electron beam irradiation. This approach produces free radicals such as H• and OH• (Acheson and Steele, 2001; Indiarto and Qonit, 2020), which impair protein cross-linking or polymerization (Lv *et al.*, 2018). Some physicochemical and chemical properties of gel samples may alter as a result of these impacts (Jaczynski and Park, 2003; Lin *et al.*, 2015a; Lin *et al.*, 2015b). According to Yang *et al.* (2014), dosage levels in the range of 0.5-3 kGy had no effect on nutritional value. To our knowledge, numerous fish species and fisheries products have been investigated to see how this affects them including Atlantic salmon fillet (Yang *et al.*, 2014), fresh Atlantic salmon (Yagiz *et al.*, 2010) frozen hairtail surimi (Lin *et al.*, 2015b), frozen *Collichthys lucidus* surimi (Deng *et al.*, 2017) and surimi seafood crabsticks (Jaczynski and Park, 2003). Furthermore, there is no information of electron irradiation on the threadfin bream surimi sol. To provide more information, the objective of this study was to investigate the effect of electron beam irradiation on the Threadfin bream (*Nemipterus* spp.) surimi gel properties.

Materials and methods

Materials

Frozen Threadfin Bream surimi (*Nemipterus* spp.) grade FA was purchased from Andaman Surimi Industries Co., Ltd., Samut Sakhon province, Thailand, and kept at -18 ℃ until used.

Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Gel electrophoresis reagents were purchased from Bio-Rad (Hercules, CA, USA).

Preparation of surimi sol

Frozen surimi was partially thawed for 16-18 hours at 4±2 ℃ before being chopped into 1 x 1 x 1 cm pieces. Surimi was chopped at a low speed for 1 minute in a silent cutter (M18N, Hohentengen, Germany), then sodium chloride (2.5 % by surimi weight) was added to solubilize the myofibrillar proteins and chopped for another 2 minutes. We used iced water to get the

moisture level of the mixture to 80% during grinding. For another minute, the mixture was chopped. Throughout the process, the temperature of the mixture was kept below 10 ℃. Using a stuffer, the sol was filled into a cellulose casing (2.5 cm in diameter and 12 cm in length) and both sides were sealed tightly. The samples were vacuum-packed in polyethylene bags and preserved on ice.

Irradiation of surimi sol

The surimi sols (thickness less than 25 mm) were transported to the E-beam facility (Thailand Institute of Nuclear Technology, Pathum Thani, Thailand) in an ice cooler. Surimi sols were placed on a linear accelerator conveyer belt in an open iron box. At ambient temperature (30 ℃), samples were subjected to an accelerated electron beam with a maximum power of 150 kW and an electron energy of 10 MeV at 1-5 kGy doses (MB10-5, Mevex Corporation LTD., Canada). The dose that was absorbed was within 1.5 percent of the desired dose. After irradiation, samples were transferred to the laboratory room using ice cooler before heating.

Preparation of surimi gel

The irradiation samples were heated in two steps to produce the cooked gel: first at 40 ℃ for 30 minutes, then at 90 ℃ for 20 minutes. All gel samples were promptly cooled with chilled water after heating until the core temperature of the sample dropped below 4 ±2 ℃, and then stored at 4 ±2 ℃ before analysis. As a control, a gel that had not been irradiated was used.

Determination of texture analysis

Before analysis, the gels were equilibrated at room temperature (about 30 ℃) for 1 hour and then cut into 2.5 cm pieces. A texture analyzer (Model TA-XT2i, Stable Micro Systems, England) equipped with a spherical plunger of 5 mm diameter (P/5s) was used to determine the breaking force (g) and deformation (cm). The gel strength was calculated as g.cm by multiplying the breaking force by the deformation (Sutloet *et al.*, 2018b).

Determination of expressible water content

The expressible water content of the surimi gels was measured using the method of Sutloet *et al.* (2019).

Determination of whiteness

A colorimeter (HunterLab, ColorFlex CX2687, USA) with a D65 illuminant was used to determine the whiteness of the surimi gels. L* (lightness), a* (redness: green to red) and b* (yellowness: blue to yellow) were measured and the whiteness was calculated using the following equation:

$$\text{Whiteness} = 100 - [100 - L^*) 2 + a^*2 + b^*2]^{1/2}$$

Determination of TCA-soluble peptide content

The content of TCA-soluble peptide was determined following by the method of Sutloet *et al.* (2018b). The amount of soluble peptides in the supernatant was measured using the Lowry *et al.* (1951) method and expressed as mol tyrosine/g sample.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The protein patterns of the gel were analyzed using the method of Laemmli (1970). The protein concentration was determined by Lowry *et al.* (1951), using bovine serum albumin (BSA) as a standard protein. Preparation of solubilized samples, stacking and separating gels, and analyzing of protein patterns were carried out following the method of Sutloet *et al.* (2019). Using an electrophoresis apparatus, the samples were separated by electrophoresis at a constant current of 20 mA per gel (Mini-Protean II; Bio-rad Laboratories).

Microstructure of surimi gel

The microstructure of the gels was determined using a scanning electron microscope and energy dispersive X–ray spectrometer (JSM–IT500HR and JED–2300, JEOL, Japan). Gels were fixed with 2.5% glutaraldehyde in phosphate buffer solution (pH 7.2) at 4 ℃ for 24 hr. The samples were washed in phosphate buffer solution for 2 cycles (10 min/cycle) then in distilled water for 10 min. The samples were dehydrated in a gradient ethanol series of 30%, 50%, 70% and 95% (v/v) for 3 cycles (10 min/cycle). Using a critical point dryer (CPD 020, Balzers, Germany), the samples were dried to the critical point. The dried samples were sputter-coated with gold, and the specimens were examined using a scanning electron microscope (SEM) at an acceleration voltage of 15 kV (Sutloet *et al.,* 2019).

Statistical analysis

A completely randomized design (CRD) was used in the experiment. ANOVA was used to perform analysis of variance on the data. At $p \leq 0.05$, a Duncan's New Multiple Range Test was employed to assess differences between sample means. All of the trials were carried out twice.

Results

The breaking force, deformation and gel strength of surimi gels treated with various dosages of electron beam irradiation represented in Figure 1. The breaking force, deformation and gel strength increased as the dose irradiation level increased ($p \leq 0.05$). In comparison to the control, the breaking force of surimi samples increased by 8.31%, 24.01%, and 33.27% in gel with 1, 3 and 5 kGy of E−beam, respectively. Furthermore, when compared to the control, the equivalent increases were 6.56%, 19.72%, and 25.00% for deformation and 13.96%, 39.14%, and 49.78% for gel strength.

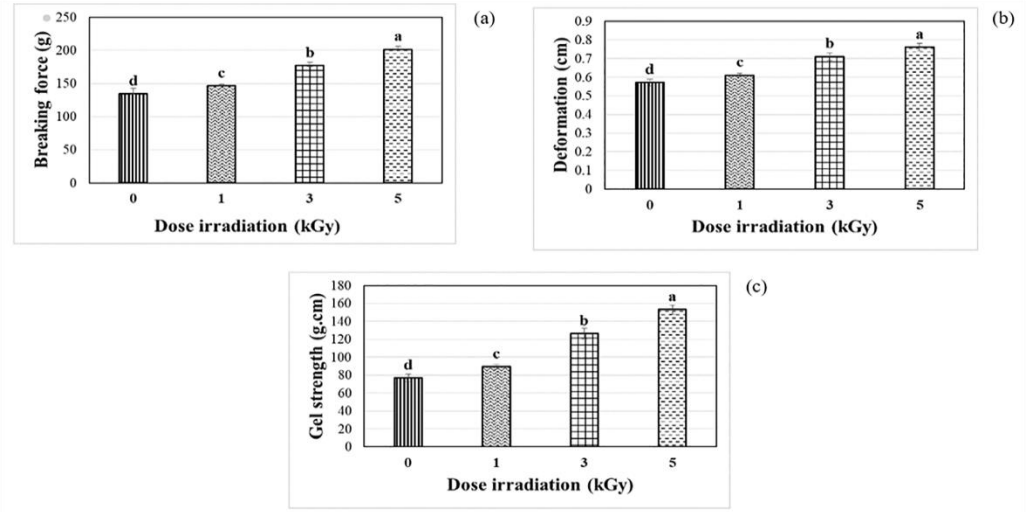

Figure 1. Breaking force (a), deformation (b) and gel strength (c) of surimi gels treated with various dosages of electron beam irradiation
Bars represent the standard deviation.
Different letters on each bar indicate significant differences ($p \leq 0.05$).
Control: surimi gels without treated with irradiation

The expressible water content of surimi gels treated with various doses of electron beam irradiation is shown in Figure 2. With dosage levels ranging from 1 to 5 kGy, a significant decrease in the expressible water content of

irradiated gels was detected ($p \leq 0.05$). The gel with 5 kGy irradiation had the lowest expressible water content ($p \leq 0.05$), which was 6.53% lower than the control, non-irradiated gel samples.

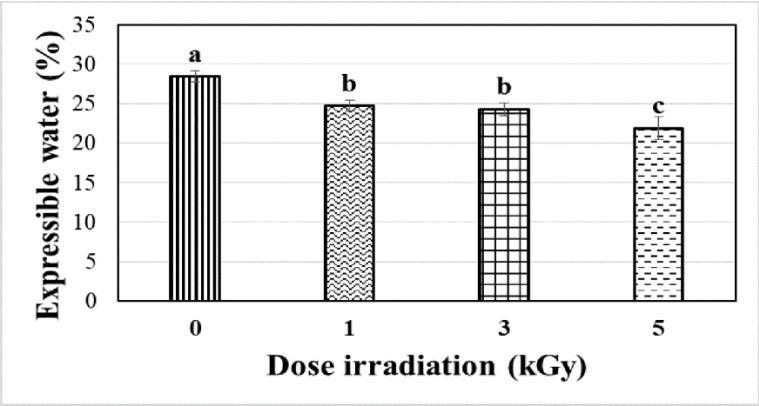

Figure 2. Expressible water content of surimi gels treated with various dosages of electron beam irradiation
Bars represent the standard deviation.
Different letters on each bar indicate significant differences ($p \leq 0.05$).
Control: surimi gels without treated with irradiation

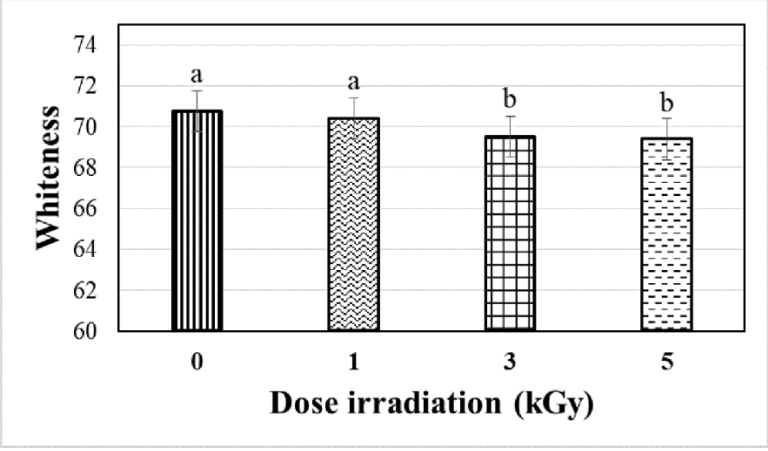

Figure 3. Whiteness of surimi gels treated with various dosages of electron beam irradiation
Bars represent the standard deviation.
Different letters on each bar indicate significant differences ($p \leq 0.05$). Control: surimi gels without treated with irradiation

The whiteness of sample gels tends to diminish as dose levels increase, as illustrated in Figure 3. The whiteness of gel samples ranged from 69.39 to

70.41, compared to 70.77 for the non-irradiated gel (control). The L* value of gel was the same way (data not shown). With dose levels more than 3 kGy, the b* value increased significantly (data not shown). The whiteness of the 5 kGy irradiation gel was the lowest ($p>0.05$).

The TCA-soluble peptide content of irradiated surimi gels at various levels of electron beam irradiation is shown in Figure 4. The TCA-soluble peptide content of gels tended to decrease as the irradiation dose was increased. The gel treated with 5 kGy had the lowest TCA-soluble peptide content ($p>0.05$), which was 7.50% lower than the control.

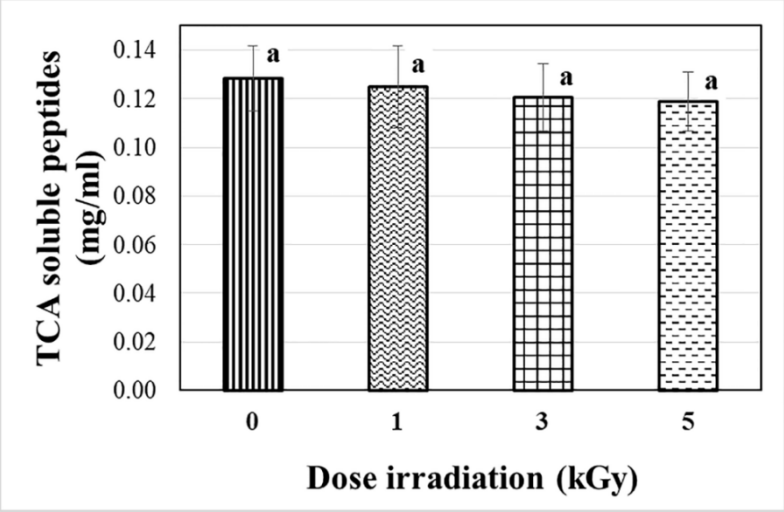

Figure 4. TCA-soluble peptise content of surimi gels treated with various dosages f electron beam irradiation

Bars represent the standard deviation.

Different letters on each bar indicate significant differences ($p\leq0.05$). Control: surimi gels without treated with irradiation

SDS-PAGE represents the molecular weight of protein solution extracted from surimi gels exposed to various levels of electron beam irradiation (Figure 5). There were no differences in MHC intensity between irradiated and non-irradiated gels. As the dose levels increased, the intensity of the degraded protein with molecular weights of 97-116 kDa reduced slightly. There was no difference in actin intensity amongst the samples.

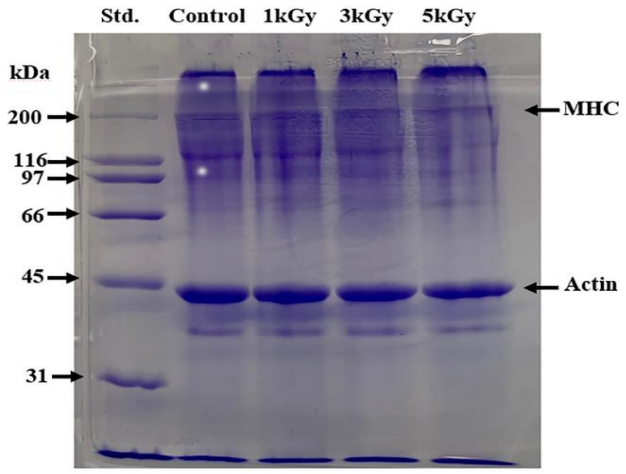

Figure 5. SDS- PAGE patterns of surimi gels treated with various dosages of electron beam irradiation
Bars represent the standard deviation.
Different letters on each bar indicate significant differences ($p \leq 0.05$).
Control: surimi gels without treated with irradiation

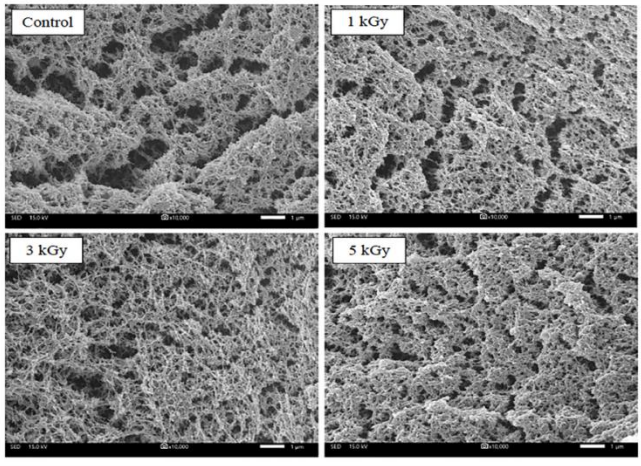

Figure 6. SEM micrographs of surimi gels treated with various dosages of electron beam irradiation
Bars represent the standard deviation.
Different letters on each bar indicate significant differences ($p \leq 0.05$).
Control: surimi gels without treated with irradiation

The microstructure of irradiated surimi gels at various dosages of electron beam irradiation (10,000 x magnification) is shown in Figure 6. Protein

aggregation were visible in the white and grey sections, while network holes were visible in the black. A fibrous network with associated protein clusters was visible on the non-irradiated gel. The non-irradiated gel structure had large voids, whereas the irradiated gel structure had smaller cavities. As a result, the density of the structure in the irradiation gel was remarkable. Irradiation at a greater dosage level produced a fine fibrous network with small reticular zones scattered throughout, resulting in a denser and compact network structure.

Discussion

Food irradiators often use maximum (D_{max}) and minimum (D_{min}) doses to determine an absorbed dosage. The dose uniformity ratio is defined as the ratio of D_{max} to D_{min}. In food research applications, the dosage uniformity ratio should be near to 1 to ensure that the irradiation is very uniform (International atomic energy agency, 2002). Preliminary investigations revealed that gel samples treated with more than 5 kGy had a dose uniformity ratio of more than 1.5 (data not shown) and that the original gel sample flavor was changed (evaluated by a trained panel). As a result, only three doses of 1, 3, and 5 kGy were chosen for testing.

Gel characteristics are a crucial factor in determining the quality of a gel. The firmness of the surimi gel is represented by the breaking force, while the elasticity is represented by the deformation (Lin *et al.,* 2015a; Deng *et al.,* 2017). These two elements define the gel's strength. As the E-beam irradiation dose rose, all textural parameters increased as well. This could be concerned more chemical bonds occured during heating and electron irradiation, such as hydrophobic interaction and disulphide bond formation (Jaczynski and Park, 2004; Shi *et al.,* 2015; Deng *et al.,* 2017). The ordered structure of the protein may be altered by electron irradiation, exposing the SH groups (Shi *et al.,* 2015). During heating of surimi sol at 40℃: those exposed SH groups and unfold solubilized proteins interact covalently and noncovalently to form a fine, elastic, and translucent gel network (Lanier *et al.,* 1982; Liu *et al.,* 1982). In aggregation and coagulation, hydrophobic interaction are crucial (Liu *et al.,* 1982). The disulfide crosslinking of the myosin S-1 (head) region precedes the thermal unfolding and noncovalent interaction with the tail portion, resulting in the formation of a gel network (Samejima *et al.,* 1981). As aggregation continues, the elastic network of suwari is strengthened as gel samples are heated again at 90℃ (Roussel and Cheftel, 1990). At lower temperatures, the tail regions of myosin have been shown to be primarily engaged in crosslinking, however at temperatures above 60-70℃, the globular head part (HMM S-1) of myosin may play a major role (Liu *et al.,* 1982; Taguchi *et al.,* 1987; Sano *et al.,* 1989). On this premise, we suggest that following irradiation,

the surimi gel characteristics improved. Lin *et al.* (2015b) found that electron irradiation aided the production of disulphide linkages in a heat-set-cooked gel made from irradiated surimi. Lv *et al.* (2018) found that as the irradiation dose was increased, the total SH content of *Tegillarca granosa* myofibrillar protein decreased. Similar irradiations, such as gamma irradiation, resulted in SH group oxidation in fermented fish sausage (Riebroy *et al.*, 2007) and myofibrillar protein (Shi *et al.*, 2015), as evidenced by the reduced number of SH groups in irradiated samples. Furthermore, as seen by the TCA-soluble peptide content (Figure 4), which had the least proteolytic degradation at 5 kGy, the maximal textural qualities in this study corresponded with those for proteolytic activity.

The ability of protein to entrap water inside the three-dimensional network, as assessed by expressible water content, is determined by water-holding capacity (WHC). The increased WHC of the three-dimensional protein network was generally attributed for the decrease in expressible water content. The WHC of surimi gels rose as the E-beam irradiation dose increased, according to the findings. The findings corroborated those of Deng *et al.* (2017). They discovered that the WHC of gels increased as the irradiation dose increased in the range of 1-5 kGy, but no significant increase was observed as the dose levels were increased higher. The findings of this investigation matched the results of the textural properties. A denser protein gel network, which contains a higher amount of water inside the structure, could be linked to a decrease in expressible water content. Irradiation increases gel WHC by strengthening the gel network, according to the findings.

Whiteness is an important quality element that influences consumer choices. The whiteness of sample gels tends to diminish as the E-beam irradiation dose increases. It could be related to the Maillard process, which results in the production of a yellowish brown color (Lin *et al.*, 2015b). Furthermore, the result of expressible water content revealed that irradiation gels maintain a greater amount of water inside their structure than non-irradiated gels. As a result, more water was released from the non−irradiated gel and then the light could not be allowed to pass through, resulting in the whiteness of non−irradiated gel is higher than that of the irradiated gels. The outcome, however, did not concur with Deng *et al*. (2017). The irradiation gels had a higher whiteness than the non-irradiated gels, according to the researchers. Depending on the inherent color of the mince, irradiation may modify the gel color in a variety of ways.

The trichloroacetic acid (TCA)-soluble peptide content was utilized to measure the degree of protein hydrolysis caused by endogenous proteinase (Deng *et al.*, 2017). When compared to non-irradiated gels (control), irradiation tends to reduce the TCA-soluble peptide content of gels, implying that

degradation was reduced by the irradiation. The serine protease causes MHC degradation in threadfin bream gel at 65℃ (Toyohara and Shimizu, 1988). These enzymes are responsible for degrading myofibrillar proteins, particularly myosin, as well as preventing the formation of a three-dimensional gel network, which lowers the gel's quality (Morrissey *et al*., 1993; Benjakul *et al.,* 2003; Yongsawatdigul *et al.,* 2006). Endogenous enzyme activity can be induced in the 50-70℃ temperature range (Kinoshita *et al.,* 1990; An *et al.,* 1994), causing the gel structure to deteriorate (Jiang, 2000). Based on the findings of this investigation, it appears that after irradiation, the degradation of protein in irradiated gels, which is destroyed by protease activity, was prevented.

To determine the change in protein, the protein pattern by SDS-PAGE profile is utilized. Myosin is thought to be the most vulnerable MF. The reduction in myosin heavy chain intensity implies that myosin molecules are destroyed during processing, as seen by high molecular weight polymerization or degradation of damaged protein. Myosin heavy chains crosslink via disulfide and nondisulfide covalent bonds, resulting in the production of high molecular weight polymers and aggregates (Benjakul and Sutthipan, 2009). SDS-PAGE represents the molecular weight of protein solution extracted from surimi gels exposed to various levels of electron beam irradiation (Figure 5). When compared to the non-irradiated gel, there was no discernible difference in MHC and actin intensity (control). It's possible that electron irradiation has a minor effect on the molecular weight of protein bands (Yang *et al.,* 2014). Other species have shown similar results with 1-5 kGy electron irradiation, including Atlantic salmon fillet (Yang *et al.,* 2014), Hairtail surimi gel (Lin *et al.,* 2015a, b), and Alaskan pollock surimi gel (Jaczynski and Park, 2004). However, when the irradiation dose was greater than 5 kGy in Hairtail surimi gel (Lin *et al.,* 2015a) and 25 kGy in Alaskan pollock surimi, the MHC intensity decreased (Jaczynski and Park, 2004).

The changes in gel forming ability are investigated using the microstructure. The microstructure of irradiation surimi gels revealed a fine fibrous network with small reticular zones scattered throughout the network, as shown in Figure 6. As a result, the gel's structure was discovered to be denser and more compact. The increased degree of cross-linking between exposed free amino acids and proteins, as well as protein-to-protein aggregation in gel proteins, could explain these structural alterations (Lin *et al.,* 2015a; Deng *et al.,* 2017). Lin *et al.* (2015b) studied the influence of electron irradiation on the hairtail surimi gel properties at doses ranging from 1 to 9 kGy and found that 7 kGy irradiation resulted in a compact gel network and a regularly ordered network structure. Furthermore, Deng *et al.* (2017) who studied the effect of electron irradiation in the range of 1–9 kGy on the gel properties of *Collichthys*

lucidus surimi found that surimi gel treated with a dose of 5 kGy represents a denser, compact, and ordered gel network structure.

In conclusion, when electron irradiation at doses ranging from 1 to 5 kGy was used on Threadfin bream surimi, the maximum gel strength and water-holding capacity were reported as a result irradiation at 5 kGy. Irradiation at this level resulted in gels with less protein breakdown. The microstructure from SEM supported a denser and compact three-dimensional network structure. There was no significant different in MHC intensity degradation with SDS-PAGE. To improve surimi gel-forming ability, we recommend using a 5 kGy dosage of electron irradiation during processing and manufacturing.

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