RESEARCH ARTICLE

Effects of electron beam irradiation on quality of weever fillets during refrigerated storage

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Abstract
The effects of electron beam irradiation doses (1–7 kGy) on the quality of weever (Lateolabrax japonicus) were investigated during refrigerated storage. The obtained results showed that lower irradiation dose (1–3 kGy) could dramatically extend shelf-life of weever fillets, guarantee hardness, and chewiness during storage. Biochemical properties of myofibrillar protein (MP) indicated that higher irradiation dose (5 and 7 kGy) significantly accelerated MP oxidization by the increase of carbonyl contents, reducing the sulfhydryl contents and Ca2+-ATPase activity during storage. Thus, low irradiation dose in combination with refrigerated storage could delay protein oxidation and be more suitable for fresh-keeping of weever meat. This may provide possible reference for the application of irradiation technology in weever preservation.

KEYWORDS
biochemical properties, electron beam irradiation, L. japonicas, myofibrillar protein, quality

1 | INTRODUCTION

Weever (Lateolabrax japonicus) has become one of the main aquaculture fish with commercial value in China and Southeast Asia due to its high nutritional value and delicious taste (Liu et al., 2010; Yang et al., 2014). However, weever deteriorates easily because of its high fat content, soft texture, as well as enzymatic and microbiological activities (Ju et al., 2017), the weever meat is perishable and its shelf life is only 6–8 days during refrigerated storage (Boyd et al., 1992; Cai et al., 2015; Kostaki et al., 2009), and hence, innovative preservation techniques are extremely necessary to be applied to guarantee its safety and maintain its quality for human consumption.

Fresh-frozen or chilled storage technologies are convenient methods of preserving fish, which can prolong their shelf life during storage by inhibiting the growth of pathogenic microorganisms. Nevertheless, the main problems of these techniques are that they fail to completely inhibit biochemical reaction and bacteriological activity, negatively affect the odor, flavor, texture, and nutritional values of fish (Liu et al., 2010). Electron beam irradiation technology, process foods with ionizing energy, can be called as “cold pasteurization” (Riebroy et al., 2007), it has been widely used as a versatile processing technology to control the quality of food and extend the shelf life by the reduction of microbial contaminants (An et al., 2017; Huang, Tu, Shangguan, Sha, 2019).

Food texture is a crucial criterion for sensory acceptance and rejection. For stored fish, the hydrolysis or physical disruption of myofibrils and protein oxidation have been considered as important factors affecting fish texture. Myofibrillar proteins (MPs), which account for...
55%–60% of all muscle protein, are mainly responsible for certain structural and physicochemical characteristics of muscle food (Wang, Miyazaki, Saitou, 2017; Wu et al., 2014). The biochemical properties of MPs are closely relevant to their structural integrity and act a pivotal part in the texture and quality of fish meat (Lv et al., 2018). Oxidative stresses of MP caused by irradiation (Deng et al., 2017; Huang et al., 2019; Lv et al., 2018; Özkan et al., 2007) and preservation methods (Benjakul et al., 2003; Xiong et al., 2009) are very obvious in fish products.

It had been proved that proper doses of electron beam (EB) irradiation (≤10 kGy) did not cause any changes with respect to quality and nutritional properties of aquatic food products (Lv et al., 2018; Yang et al., 2014). However, unlike other food materials or fish products, there is not enough investigation about effects of EB irradiation on the quality of weever fillets meat from the view points of texture, biochemical properties, and sensory attributes during refrigerated storage. Thus, the objective of this research was to assess the effects of EB irradiation on the texture changes of weever (*L. japonicus*) meat and MPs oxidation during refrigerated storage period. It was anticipated that this study could provide a better understanding of the effects of EB irradiation on the quality of *L. japonicus* meat during refrigerated storage.

### 2 MATERIALS AND METHODS

#### 2.1 Materials

Cultured *L. japonicus* (average weight and length of 650 g and 350 mm, respectively) was obtained from a local aquatic market in Ningbo, China. The live fish were kept in seawater and oxygen, then transferred to the laboratory within 30 min. The live fish were kept in ice for 45 min to be faint, immediately the fish was peeled, and its head and tail were cut off, and cut into two halves along the spine; the obtained fresh fillets were cut into pieces of 100 g and packed in polyethylene bag, following by storing in 4°C of refrigerator. All chemicals used were of analytical reagent grade.

#### 2.2 Electron beam treatment

*L. japonicus* meat was exposed to an electron linear accelerator (NBL-1010, Ningbo Superpower High-Tech. Ltd., Ningbo, Zhejiang, China) with 10 MeV source strength. 1, 3, 5, and 7 kGy at a constant dose rate of 1 kGy/s were applied. Samples were arranged without overlapping each other in the well-proportioned area of irradiation field to avoid the differences in radiation dose as far as possible. The absorption dose was measured by a silver dichromate dosimeter, which was calibrated by National Institute of Metrology (Ningbo, China) and the deviation of absorbed dose is less than 3% of the target dose. The samples were then immediately transferred to a refrigerator (4°C) after irradiation. *L. japonicus* meat without irradiation were used as the control group.

### 2.3 Quality evaluation of *L. japonicus* during refrigerated storage

#### 2.3.1 Sensory analysis

Sensory analysis was evaluated according to the method of Yang et al. (2014) with some modification.

Two 20-member sensory panels (10 males/10 females, aged from 21 to 30 years) were recruited in Ningbo University. All panelists had previously participated in fish fillet sensory evaluation experiments and were familiar with fish fillet as sensory phenomena. Each panelist was randomly presented with the same sample thrice. Sensory attributes of the weever meat including appearance color, tissue structure, flavor, and taste were evaluated using a 9-point descriptive scale (1: dislike extremely to 9: like extremely). The last final sensory score is the sum of the weights for each indicator. Average score of each attribute was calculated and scores ≥4.0 were considered as acceptable.

#### 2.3.2 Textural analysis

The textural properties of weever meat during storage were evaluated according to the methods of Maqsood et al. (2015) with some modification. The meat was cut into uniform sized pieces (4 cm³). They were compressed to 30% of their original height with a constant speed of 1.0 mm/s using TA.XT Plus Texture Analyzer equipped with P50 probe. The texture parameters (hardness, chewiness, springiness, and resilience) were calculated for each sample.

### 2.4 Biochemical properties of MP from *L. japonicus* during refrigerated storage

#### 2.4.1 Extraction of MP

MP was extracted according to the procedure described by Xiong et al. (2009). Note that 10 ml of buffer A (20 mM Tris-maleate, 50 mM KCl, pH 7.0) was added to 1 g of fish meat and then homogenized. The homogenate was centrifuged at 10,000 r/min for 15 min. The obtained
precipitate was washed twice with buffer A, then homogenized with 10 times of volume of buffer B (20 mM Tris-maleate, 0.6 M KCl, pH 7.0). The homogenate was extracted for 60 min and centrifuged at 10,000 r/min for 15 min. The supernatant was the obtained MP solution. All operations were performed at 4°C.

2.4.2 Determination of total sulfhydryl content

Total sulfhydryl (SH) content of MP was determined as described by Benjakul et al. (1997) using the 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB). A 9 ml of 0.2 M Tris-HCl (pH 6.8, containing 8 M urea, 10 mM Ethylene diamine tetraacetic acid (EDTA) and 2% Sodium dodecyl sulfate (SDS)) were added to 0.5 ml MP solution. A 4 ml aliquot of the mixture was reacted with 0.4 ml of 0.1% (w/v) DTNB solution at 4°C for 25 min. The absorbance of the reaction mixture was assayed at 412 nm using a microplate reader (Spectra Max i3, Molecular Devices, America). The 0.6 M KCl (pH 7.0) was used as a blank. The molar extinction of 13,600 M–1 cm–1 was adopted to calculate the total SH content, and the results were expressed as mol/10^5 g protein.

2.4.3 Determination of Ca2+-ATPase activity

Ca2+-ATPase activity of MP was determined according to the methods of Thanonkaew et al. (2006) with some modification. The reaction mixture for Ca2+-ATPase assay contained 0.125 M, pH 7.0 Tris-maleate, 0.1 M kCl, and 0.005 M CaCl2. A 5 ml of the reaction mixture was kept at 25°C for 10 min, mixed with 0.25 ml of 20 mM ATP solution (pH 7.0) and 0.5 ml MP solution and then kept at 25°C for 5 min. After incubation, 2.5 ml of 15% Trichloroacetic acid (TCA) was added to the mixture and centrifuged at 6000 r/min for 15 min. The supernatant was collected to analyze the liberation of inorganic phosphate (Pi) by ammonium vanadate-molybdate spectrophotometric method. Using μM (Pi)/mg (pro)/min as the unit to express Ca2+-ATPase activity.

2.4.4 Determination of carbonyl content

Carbonyl content of MP was determined spectrophotometrically after a reaction with 2,4-dinitrophenylhydrazine (DNPH) described by Xia et al. (2009). A 1 ml MP was reacted with 1 ml of 10 mM DNPH in 2 M HCl for 1 h at room temperature in dark. The fractions were then precipitated with 10% TCA and centrifuged at 4000 r/min for 3 min. The pellet was washed three times with 4 ml of ethanol:ethyl acetate (1:1, v/v) mixture to remove free DNPH reagent. The MP was finally dissolved in 3 ml of 6 M guanidine hydrochloride in 20 mM potassium phosphate buffer (pH 2.3), and then centrifuged at 6000 r/min for 5 min. The absorbance of the supernatant was measured at 370 nm. The carbonyl content of MP was quantified by 22,400 M–1 cm–1 molar absorptivity, and nmol carbonyl/mg protein was used to express the results.

2.4.5 Determination of surface hydrophobicity

The surface hydrophobicity of MP was measured as described by Chelh et al. (2006) with some modification. The MP was diluted to 2 mg/ml with 20 mM phosphate buffer at pH 6.0. A 200 μl of 1 mg/ml bromophenol blue (BPB) sodium salt (in distilled water) was added to 1 ml of MP suspension. The mixture was agitated at 25°C for 10 min and centrifuged at 3000 r/min for 15 min and the supernatant was diluted 10 times with phosphate buffer. The absorbance of the diluted supernatant was then measured at 595 nm using a microplate reader, and phosphate buffer was used as the blank. The absorbance values were converted to the amount of BPB bound using the following formula:

\[ \text{BPB bound (mg)} = 200 \times (A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}} \]

where A is the absorbance at 595 nm.

2.5 Statistical analysis

Three independent experiments were followed a completely randomized design. Data analysis of variance and Duncan’s multiple range were performed using Origin 8.0 at p < 0.05 level. Results are expressed as the mean values ± standard deviation.

3 RESULTS AND DISCUSSIONS

3.1 Quality evaluation of L. japonicus during refrigerated storage

3.1.1 Sensory analysis

Fish samples were considered to be acceptable for human consumption until the sensory score reached 4. (Yang et al., 2014). As shown in Table 1, the sensory scores of samples presented a decreasing trend during storage, but the irradiated samples showed higher sensory scores, especially for 1 and 3 kGy treated samples. These suggested that irradiation could significantly guarantee weever meat quality and prolong shelf-life during refrigerated storage. With the treatments of EB irradiation, the generation of free radicals, for example H. and OH., can kill the present parasites, inactivate endogenous enzymes, and inhibit microbial growth in fish or fish products, and effectively extend the shelf-life of food (Lv et al., 2018; Özkan et al., 2007). Yang et al. (2014) found that irradiation reduced log10 cfu of Atlantic salmon fillets largely, while it rapidly increased counts in control samples during 4°C storage. Ju et al. (2017) used tea polyphenols combined with nisin to show that they could extend the shelf life of weever meat to 4 days based on sensory scores, which was significantly shorter than presented results (14–20 days) in similar storage condition. However, the generation of unacceptable “color fading” and “irradiation odor” during high-dose irradiation could greatly affect the sensory quality of fish fillets (Yang et al., 2014). Low doses of irradiation produced either negligible or very light effects in the visual aspect of fish fillets and also extended its shelf life (An et al., 2017; Yang et al., 2014). Therefore, 1–3 kGy was considered suitable to treat weever fillets.
Texture analysis

Texture is a predominant element of the acceptability and quality of food products (Huang et al., 2019). As shown in Table 2, the hardness, chewiness, springiness, and resilience values of all weever meat sharply decreased during refrigerated storage. Maqsood et al. (2015) also reported that long time of refrigerated storage decreased hardness, gumminess, chewiness, and springiness of camel meat by significantly increasing drip loss and decreasing water holding capacity. Compared with control group, irradiation significantly increased the hardness of fish fillets, 5 and 7 kGy irradiated weever meats normally had highest hardness (p < 0.05), and 1 and 3 kGy irradiated samples presented slow decreasing rates during storage. This might be caused by the generation of free radicals (e.g., H. and OH.) that can react with tyrosine and phenylalanine in protein, resulting in the cross-linking or polymerization of proteins (Huang et al., 2019). Furthermore, the higher molecular weight aggregates could be formed by the occurrence of hydrophobic and electrostatic interactions and the formation of disulphide bonds (Yang et al., 2014) also resulted in strengthening fish fillets' structure. All the irradiated samples had higher chewiness than non-irradiated samples during refrigerated storage.

### Table 1: Sensory evaluation of irradiated *L. japonicus* meat during refrigerated storage

| Samples | 0 day | 3 days | 6 days | 9 days | 14 days | 20 days |
|---------|-------|--------|--------|--------|---------|---------|
| 0 kGy   | 9.92 ± 0.20 Da | 9.17 ± 0.55 Da | 6.83 ± 0.61 Da | 3.75 ± 0.69 Da | 2.21 ± 0.31 Ba | 1.33 ± 0.41 Aa |
| 1 kGy   | 9.96 ± 0.21 Db | 9.83 ± 0.26 Dc | 9.33 ± 0.52 Bb | 8.75 ± 0.27 Cd | 6.55 ± 0.40 Bb | 5.42 ± 0.79 Bc |
| 3 kGy   | 9.92 ± 0.20 Eb | 9.22 ± 0.28 Dc | 7.85 ± 0.85 Dc | 4.13 ± 0.99 Bc | 6.63 ± 0.40 Bb | 5.23 ± 0.41 Ab |
| 5 kGy   | 9.75 ± 0.27 De | 9.67 ± 0.41 De | 9.00 ± 0.32 Dc | 7.83 ± 0.82 Bb | 5.96 ± 0.47 Bb | 4.88 ± 0.72 Ac |
| 7 kGy   | 9.08 ± 0.20 Da | 8.83 ± 0.52 Da | 8.81 ± 0.37 Db | 7.92 ± 0.38 Bb | 5.52 ± 0.46 Bb | 3.30 ± 0.63 Ab |

Values are mean ± SD; A—F: Different letters are significantly different (p < 0.05) for the same sample during storage days; a, b, c: letters are significantly different (p < 0.05) between different samples during the same storage day.

### Table 2: Effect of various EB irradiation doses on textural properties of *L. japonicus* meat during refrigerated storage

| 0 days | 3 days | 6 days | 9 days | 14 days | 20 days |
|--------|--------|--------|--------|---------|---------|
| Hardness | 2117.79 ± 20.53 Da | 2069.76 ± 34.21 Ca | 2062.03 ± 32.39 Ca | 1968.75 ± 24.79 Ba | 1876.61 ± 52.19 Aa | 1845.05 ± 49.73 Aa |
| Chewiness | 690.98 ± 12.41 Fa | 657.42 ± 4.34 Dc | 539.07 ± 11.63 Da | 440.47 ± 10.53 Ca | 309.85 ± 4.11 Ba | 237.61 ± 14.54 Ab |
| Springiness | 0.57 ± 0.00 Fa | 0.57 ± 0.00 Fa | 0.50 ± 0.01 Da | 0.45 ± 0.01 Ca | 0.38 ± 0.00 Bb | 0.37 ± 0.01 Aa |
| Resilience | 0.25 ± 0.01 Da | 0.22 ± 0.01 Ca | 0.20 ± 0.00 Ca | 0.18 ± 0.01 Ba | 0.15 ± 0.01 Aa | 0.14 ± 0.01 Aa |

Values are mean ± SD; A—F: Different letters are significantly different (p < 0.05) for the same sample during storage days; a, b, c: letters are significantly different (p < 0.05) between different samples during the same storage day.
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FIGURE 1

Changes in the total SH content (a) and Ca\(^{2+}\)-ATPase activity (b) of myofibrillar protein from weever meat during refrigerated storage. Values are mean ± SD; A–F: Different letters are significantly different (p < 0.05) between storage days for the same sample; a, b, c: letters are significantly different (p < 0.05) between different samples during the same storage day.

3.2 | Biochemical properties of MP from L. japonicus during refrigerated storage

3.2.1 | Total SH content

As shown in Figure 1a, unirradiated sample had the highest SH contents, high irradiation dose (5 and 7 kGy), and significantly (p < 0.05) decreased SH contents of protein. These indicated that the higher irradiation dose might accelerate oxidation of protein during storage. High dose of irradiation changed the ordered structure of protein and exposed more contents of SH groups, then the exposure of SH could be further oxidized to form disulfide bonds (Lund et al., 2007). Additionally, the generated radicals also could accelerate the oxidation rate of SH (Li et al., 2013), contributing to the formation of denser structure of fish fillets with high hardness (Table 2). Similar changes in total SH contents of irradiated grass carp’s meat protein have previously been found by Shi et al. (2015). Besides, the total SH contents of all samples decreased gradually (p < 0.05) during refrigerated storage. After 20 days of storage, total SH content of the control was 1.26 ± 0.03 mol/10^5 g protein and decreased by 64.19%, while those of 1, 3, 5, and 7 kGy groups were 1.16 ± 0.05, 1.19 ± 0.02, 1.07 ± 0.08, and 1.04 ± 0.09 mol/10^5 g protein and decreased by 68.18%, 67.27%, 74.42%, and 78.48%, respectively, in comparison with their initial values. This might be because the oxidation of sulfhydryl groups of cysteine residues oxidized into disulfide bonds subsequently (Zhang et al., 2018). Similarly, Shi et al. (2014) also found the total SH decreased in silver carp fillets during refrigerated storage. Furthermore, 1 and 3 kGy irradiated samples had lower decreasing rates of SH contents than those of 5 and 7 kGy treated samples, demonstrating that low dose of irradiation had less influence on protein oxidation during storage. During refrigerated storage, the conformational changes of protein were prone to oxidation or disulfide interchange. And the formation of formaldehyde during storage could induce protein aggregation, which was also supposed to result in the decrease in free sulfhydryl groups available for determination (Benjakul et al., 2003).

3.2.2 | Ca\(^{2+}\)-ATPase activity

ATPase activity has been widely used in fish meat as a primary indicator in relation to the integrity of MP head region. Protein denaturation or degradation could lead to the decrease of the Ca\(^{2+}\)-ATPase activity (Donald & Lanier, 1994). The results presented that there were marked decline (p < 0.05) in the Ca\(^{2+}\)-ATPase activity of MP after irradiation, especially in 7 kGy group (Figure 1b), which suggested that irradiation accelerated a denaturation or aggregation of MP. The Ca\(^{2+}\)-ATPase activity of all samples declined rapidly with the increase of the storage period (p < 0.05). The active center of Ca\(^{2+}\)-ATPase is located in the myosin heavy chain, and the decrease of Ca\(^{2+}\)-ATPase activity may be due to the degradation of myosin heavy chain caused by high doses of irradiation. A similar mechanism was found by Zhou et al. (2006), who reported that Ca\(^{2+}\)-ATPase activity decreased when the oxidation occurred, demonstrating that irradiation could accelerate destruction and degradation of myosin. Moreover, the rearrangement of proteins via protein–protein interactions caused by decreasing water-retention ability might contribute to reducing Ca\(^{2+}\)-ATPase activity (Zhang, Li, Jia, Huang, & Luo, 2018). Here, the different reduction degree of sulfhydryl oxidation and Ca\(^{2+}\)-ATPase in samples during refrigerated storage was probably caused by the differences in susceptibility of MPs to oxidation, which masked the reactive SH structure of actomyosin molecules (Benjakul et al., 2003; Zhang, Fang et al. 2018). Interestingly, there were no significant differences between control and 1 kGy treated fish fillets, demonstrating that low dose of irradiation had less effect on protein oxidation during long periods of storage.

3.2.3 | Protein carbonyl content

Carbonyl compounds are generated during protein oxidation and could be considered as a notable indicator of protein oxidation (Stadtman & Berlett, 1997). As shown in Figure 2a, compared to the control group, higher irradiation doses (3, 5, and 7 kGy) significantly increased the carbonyl contents of weever meats (p < 0.05), while there was no
pronounced difference ($p > 0.05$) between control and 1 kGy group. Moreover, carbonyls continually increased during the lapse of the storage period, and the highest carbonyl content was observed in 7 kGy group after 20 days storage. This rapid progress of protein carbonyl contents after irradiation may be attributed to the free radicals induced by irradiation (Riebroy et al., 2007). The free radicals are attacked on protein side chains of amino acids (lysine, histidine, arginine) or peptide bonds, leading to the accelerating generation of carbonyls, which make the MP more particularly prone to oxidation (Sante-Lhoutellier et al., 2007). As also illustrated by Zhang et al. (2018) and Pazos et al. (2013), myofibrillar carbonylations were possibly originated from alpha skeletal actin, glycogen phosphorylase, pyruvate kinase, or other proteins. Thus, high dose of irradiation could destroy the nutrition of weever meat by the accelerate of protein oxidization during refrigerated storage.

### 3.2.4 Surface hydrophobicity

Changes in surface hydrophobicity have been considered as an appropriate parameter to indicate conformational changes in protein structure (Zhang et al., 2018). After irradiation, the amounts of BPB bound of irradiated samples significantly increased ($p < 0.05$) at the dose of 1 and 3 kGy, but then decreased at the dose of 5 and 7 kGy ($p < 0.05$) (Figure 2b). These might be because low irradiation dose makes more hydrophobic groups inside exposed outside, while high irradiation dose accelerated protein aggregation through hydrophobic interactions (Wang et al., 2017). Moreover, the surface hydrophobicity of all samples increased steadily along with the prolonging storage time, and obviously at 3 kGy ($p < 0.05$). The increase in surface hydrophobicity might be related to the unfolding of proteins caused by irradiation (Santé-Lhoutellier et al., 2008). Irradiation treatment might bring some hydrophobic regions that were located within the interior of the molecules at first to the surface, such as the exposure of hydrophobic amino acid residues, resulting in the increase of surface hydrophobicity (An et al., 2012). Badii and Howell (2002) also revealed that the unfolding of proteins and exposure of hydrophobic aliphatic and aromatic amino acids contributed to the increase of hydrophobicity of proteins during refrigerated storage. Thus, high EB irradiation dose and refrigerated storage directly affected the conformational changes of protein, resulting in the loss in functionality as observed by the decrease gel forming ability and sensory scores.

### 4 CONCLUSION

The present study gives a better understanding of the effects of EB irradiation on the quality of weever meat by textural analysis and evaluating biochemical properties of MPs. It shows that low EB-irradiation dose (1 and 3 kGy) is an effective method to guarantee the quality of weever meat. High irradiation dose significantly accelerated protein oxidization by decreasing SH contents and Ca$^{2+}$-ATPase activity, showing high carbonyl contents during refrigerated storage. The surface hydrophobicity of MP increased first and then decreased, and the maximum value was obtained in the 3 kGy group. During storage, high irradiation treated samples had the highest declining rates of total SH contents and Ca$^{2+}$-ATPase activity and increasing rates of the carbonyl contents, suggesting that high irradiation dose could significantly destroy nutrition of weever meat by accelerating of protein oxidization. In conclusion, the lower dose (1–3 kGy) irradiation is more suitable for the preservation of weever. Besides, further studies are also needed to evaluate the applications of the combination low dose of irradiation with other preservation method such as biological film or coating on the quality of weever meat during long time of storage.

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### CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.
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