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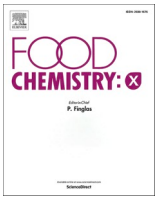

Effects of electron beam irradiation on protein oxidation and textural properties of shrimp (*Litopenaeus vannamei*) during refrigerated storage

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ABSTRACT

Protein oxidation leads to changes in shrimp texture, which affects sensory profile and consumer acceptability. This study aimed to evaluate the impact of electron beam irradiation (EBI) on protein oxidation and textural properties of *Litopenaeus vannamei* during refrigerated storage. Results revealed that EBI treatment and storage increased the protein oxidation level of shrimps. Shrimps irradiated with ≥ 7 kGy exhibited remarkably higher ($P < 0.05$) reactive oxygen species, turbidity, and carbonyl contents, and remarkably lower ($P < 0.05$) Ca^{2+}-ATPase activity, surface hydrophobicity, solubility, and total sulfhydryl contents compared to the control group (0 kGy) on the 7th day of storage. Shrimps irradiated with 3 and 5 kGy exhibited remarkably higher ($P < 0.05$) hardness, springiness, and chewiness compared to the control group (14.99 N, 1.26 mm, and 3.19 mJ). Collectively, suitable EBI doses of 3–5 kGy were recommended in shrimp preservation to inhibit texture softening by inducing moderate protein oxidation.

Introduction

To retard the quality loss of fresh shrimp during post-harvest storage, multiple methods have been proposed, such as low-temperature preservation, modified atmosphere packaging, chemical agent treatment, irradiation, and high hydrostatic pressure processing. Notably, electron beam irradiation (EBI) has recently attracted considerable attention to the preservation of shrimps as it possesses the capacity to eliminate microbial contamination (Arvanitoyannis et al., 2009; Gautam & Venugopal, 2021; Wang et al., 2023; Yu et al., 2022).

EBI exhibits a substantial capability to enhance food safety and prolong the commercial shelf life of food products based on its effectiveness in deactivating foodborne pathogens and spoilage microbes (Rosario et al., 2021). The antimicrobial performance of EBI is associated with its direct or indirect action on microorganisms. Direct destruction of genetic material (DNA and RNA) and physiological metabolism of microorganisms by electron beam can result in injury or death of microorganisms. Meanwhile, the indirect damage of microbial growth was triggered by free radicals generated through the radiolysis of water (Lung et al., 2015). While it is noteworthy that free radicals can also cause changes in food composition, such as the proteins in muscle. Due to the high abundance of proteins within cells and the high rates constant of oxidative modification, proteins are one of the preferred targets of these free radicals (Zhang et al., 2022).

In muscle-based foods, free radicals produced during processing and storage react efficiently with proteins, which is beneficial to various forms of protein oxidation, such as protein cross-linking, amino acid side chain modification, and protein fragmentation (Nawaz et al., 2022). Protein oxidation is vital in understanding the changes in other physical and chemical properties, especially the textural characteristics. Oxidative modification generally induces texture changes by affecting the structure of muscle proteins and their spatial arrangement (Bao & Ertbjerg, 2019). As known, the generation of free radicals induced by EBI is highly evident, and protein oxidation readily occurs in irradiated muscle. Studies involving irradiation-induced protein oxidation have been reported, mainly focused on pork (Zhang et al., 2020), shellfish (Lv et al., 2018), and fish (Riebroy et al., 2007; Shi et al., 2021). Electron beam irradiation is very effective in extending the shelf life of shrimp, but its effect on protein characteristics of postmortem shrimp muscle still needs to be fully understood. To explain the texture changes of irradiated shrimps from the perspective of protein oxidation is extremely necessary. Thus, we aim to explore the impact of EBI on protein oxidation of shrimp and textural development during further chilled storage by determining the ROS contents, carbonyl contents,

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total sulfhydryl contents, surface hydrophobicity, Ca^{2+}-ATPase activity, zeta potential, solubility, turbidity, and texture. The results can serve as a theoretical foundation for utilizing irradiation technology in shrimp preservation.

Materials and methods

Sample collection and EBI treatment

Fresh shrimps (*Litopenaeus vannamei*, 21.37 ± 1.42 g) were purchased from an aquatic market in July 2021 (Tianjin, China), followed by washing, draining the surface water, and then sealing in low-density polyethylene bags (127 x 203 mm) with six shrimps per bag. Then, all shrimps were arbitrarily classified into six groups, subjected to irradiation at doses of 0, 1, 3, 5, 7, and 9 kGy, respectively. Shrimps without irradiation (0 kGy) were set as the control groups. A type of 10 MeV electron linear accelerator (VF-ProAcc-10/20, Tianjin LANFU Irradiation Technology Co., Ltd., China) was employed to perform EBI, with a dose rate of 25 kGy/min. After that, the processed shrimp were promptly transferred with crushed ice to the laboratory (Baoding, China) within 4 h and placed in the 4 °C refrigerator for 7 days. Samples with different storage times (0, 1, 3, 5, and 7 d) were collected for subsequent analysis.

Extraction of myofibrillar protein (MP)

Shrimp MP was extracted as presented by Lv et al. (2018) with slight modifications. The shrimp mince (2 g) was mixed with 4 volumes (w/v) of the 10 mM potassium phosphate buffer (PSB, pH 7.0) comprising 0.1 M NaCl, 2 mM $MgCl_2$, and 1 mM ethylenediaminetetraacetic acid (EDTA), then homogenized (7,000 rpm, 30 s) utilizing a homogenizer (Scientz-10, Scientz Biotechnology Co., Ltd., China). The homogenate was subjected to centrifugation (5,000 g, 4 ℃, and 15 min) to obtain a deposit. This process was repeated two times, and the deposit was collected. The recovered deposit was further extracted with 10 volumes of 10 mM, pH 7.4 PSB comprising 0.6 M KCl for 60 min at 4 ℃, followed by centrifugation (4 °C, 15 min, and 10,000 rpm). The resulting supernatant was obtained and regarded as MP. The BCA Protein Assay Kit (PT0001, Beijing Leagene Biotechnology Co., Ltd., China) was used to measure the protein concentration (mg/mL) of MP.

Determination of reactive oxygen species (ROS)

ROS contents were measured as described by Zhang et al. (2020) with slight modifications. The shrimp mince (0.5 g) was homogenized (10,000 rpm, 1 min) with 3 mL of 10 mM, pH 7.4 Tris-HCl comprising 10 mM sucrose, 0.1 mM EDTA-2Na, and 0.8 % [w/v] NaCl. The mixture was subjected to centrifugation (3000 g, 4 ℃, and 15 min) to obtain supernatant, determining the protein concentration (mg/mL) of supernatant by BCA Protein Assay Kit. A 100 μL supernatant of each sample and a 100 μL buffer solution (10 mM Tris-HCl, 10 mM sucrose, 0.1 mM EDTA-2Na, 0.8 % [w/v] NaCl, 10 μM DCFH-DA, pH 7.4) were mixed and kept at 37 ℃ for 0.5 h. Finally, the fluorescence intensity of the resulting solution before incubation and after incubation was detected using a microplate reader (BioTek Synergy 2, Gene Biotechnology International Trade Co., Ltd., Shanghai, China), utilizing an excitation wavelength of 485 nm and an emission wavelength of 528 nm. The ROS content was calculated as follows:

$$\text{ROS content (fluorescence/mg/mL/min)} = (F_2 - F_1) / c / T$$

where F_1 and F_2 represent fluorescence values before and after incubation, respectively; c means the protein concentration of the sample; T means the incubation time, 0.5 h.

Measurement of surface hydrophobicity (So)

The measurement of So was performed using the method of Lv et al. (2018) and was slightly modified. The mixture of bromophenol blue (BPB, 200 μL, 1 mg/mL) solution and MP solution (1 mL, 2 mg/mL) was kept at 25 ℃ for 10 min. MP was replaced by 10 mM, pH 7.4 PSB comprising 0.6 M KCl as a blank group. All samples were subjected to centrifugation (3000 rpm, 15 min). The obtained supernatant was then diluted 10-fold and used for the absorbance determination at 595 nm. The So was estimated by calculating the amount of bound BPB, as given below:

$$\text{The amount of bound BPB (μg)} = 200 \times (A_0 - A_1) / A_0$$

where A_0 and A_1 represent the absorbances corresponding to blank and MP samples, respectively.

Measurement of carbonyl content

The carbonyl content was determined as presented by Zhang et al. (2018) with slight modifications. The mixture of MP solution (1 mL, 2 mg/mL) and 1 mL, 10 mM 2,4-dinitrophenyl hydrazine (DNPH, dissolved in 2 mol/L HCl) solution was kept in the dark for 60 min at room temperature, accompanied by intermittent vortex every 15 min. DNPH was replaced by 2 mol/L HCl as a blank group. After that, precipitate protein by adding 20 % trichloroacetic acid (TCA, 1 mL) solution. All samples were subjected to centrifugation (10,000 g, 5 min) to obtain deposits. Then, to eliminate excess DNPH in the deposit by washing thrice with ethanol/ethyl acetate (1:1, v/v). Guanidine hydrochloride (6 M, 3 mL) was added to dissolve the deposit. The resulting samples were further centrifuged (10,000 g, 5 min) after being kept at 35 ℃ for 15 min. The absorbance of obtained supernatants was detected at 370 nm, and the carbonyl contents (nmol/mg protein) of MP were estimated with an extinction coefficient of 22,000 mol^{-1}• cm^{-1}•L.

Detection of total sulfhydryl (SH) content

Detection of total SH content was performed as presented by Lv et al. (2018) with some changes. The mixture was prepared firstly by adding MP solution (1 mL) to 9 mL, 0.2 M PSB solution (comprising 8 M urea, 1 % sodium dodecyl sulfate, and 10 mM EDTA, pH 8.0). MP was replaced by 10 mM, pH 7.4 PSB comprising 0.6 M KCl as a blank group. After that, the reaction was initiated by adding the mixture (4 mL) to 0.2 M PSB solution (0.5 mL, pH 8.0) comprising 10 mM 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB). The absorbance of the reacting solution was detected at 412 nm after being incubated at 40 ℃ for 25 min. Total SH content was estimated as given below:

$$\text{Total SH content}\left(\text{mol}/10^5\text{g protein}\right) = (A \times n) / (\varepsilon \times c) \times 10^6$$

where A represents the absorbance; n means the dilution factor of MP; ε refers to the extinction coefficient (13,600 mol^{-1}• cm^{-1}•L); c means the protein concentration of MP.

Detection of Ca^{2+}-ATPase activity

Detection of Ca^{2+}-ATPase activity was executed according to the manufacturer's instructions of the $Ca^{++}Mg^{++}$-ATPase kit (CMATP-1-Y, Suzhou Comin Biotechnology Co., Ltd., China) based on quantifying phosphorus. 0.5 μmol/mL of phosphorus standard solution was provided. Ca^{2+}-ATPase activity was given as the release of 1 μmol inorganic phosphate (pi)/mg protein/h and calculated as follows:

$$Ca^{2+} - \text{ATPase activity (μmol pi/mg pro/h)} = 7.5 \times \Delta A_{(\text{determination})} \div \Delta A_{(\text{standard})} \div c$$

where $\Delta A_{(\text{determination})}$ is the difference between absorbance at 660 nm of

the determination tube and control tube; $\Delta A_{(standard)}$ is the difference between absorbance at 660 nm of the standard tube and blank tube; c refers to the protein concentration of the sample.

Detection of zeta potential

The ζ-potential of MP solutions (1 mg/mL) was detected at room temperature based on the technique of electrophoretic light scattering (Zetasizer Nano ZS90, Malvern Panalytical, Malvern, UK).

Measurement of protein solubility

Measurement of MP solubility was performed as presented by Li et al. (2018) and was slightly modified. For sarcoplasmic protein solubility (SPS), the shrimp mince (1.0 g) was homogenized (6,500 rpm, 60 s) with 25 mM PSB solution (10 mL, pH 7.2) and kept at 4 °C overnight. The mixture was subjected to centrifugation (1,500 g, 4 ℃, 20 min). Then, a BCA Protein Assay Kit was applied to measure the protein concentration of the collected supernatant, which was regarded as SPS. For total protein solubility (TPS), the shrimp mince (1.0 g) was homogenized with 0.1 M PSB solution (20 mL, pH 7.2) comprising 1.1 M potassium iodide. The extraction and determination processes were performed as mentioned above, and TPS was obtained. The protein solubility (mg/g sample) of MP was calculated by the difference between TPS and SPS.

Measurement of turbidity

Turbidity of MP (2.0 mg/mL) was determined as described by Wang et al. (2013) with slight changes. The absorbance was measured at 350 nm by a microplate analyzer (Multiskan GO, Thermo Fisher Scientific, USA).

Detection of textural indicators

Textural profile analysis was conducted as presented by Shi et al. (2018), utilizing a TMS-Pro texture analyzer (Food Technology Corporation, US). The P/5 cylindrical probe was selected, and shrimps were compressed with 45 % deformation. Each assay was repeated twelve times for each group.

Statistical analysis

The analyses were run in triplicate if not additionally illustrated. SPSS 22.0 software (Chicago, IL, USA) was applied to perform the analysis of variance, and the data were presented as means ± standard deviations. The significant differences ($P < 0.05$) among mean values were observed through Duncan's multiple comparisons test.

Results and discussion

ROS contents

Changes in ROS contents of irradiated shrimps at different storage times were shown in Fig. 1A. On 0 d, compared to control sample (0 kGy), ROS contents of samples increased significantly after irradiation (≥1 kGy) and reached the highest level of 69.13 in 9 kGy treatment ($P < 0.05$). Then, the ROS contents of all shrimps increased with the variation of storage duration from 0 to 7 d. Shrimps treated with ≥ 3 kGy irradiation always exhibited higher ROS content than the control group ($P < 0.05$). The result revealed that irradiation treatment coupled with chilled storage contributes to elevated levels of ROS.

The presence of ROS in shrimps is inevitable. They are formed either from external (ionizing radiation) or internal (such as enzyme catalysis and metabolic process) factors. Firstly, EBI treatment induced the formation of ROS immediately after implementation. Energetic electrons interact with intracellular or extracellular atoms or molecules, especially water molecules, leading to the ionization, dissociation, and excitation of water molecules. The primary result produced by the radiolysis of water is the generation of free radicals and hydrated electrons (e^-_{aq}). Then, these reactive species will combine either with themselves or with other constituents, such as water molecules, oxygen, reaction products, and organic molecules (RH) within tissues or cells, to produce a range of ROS (such as hydroperoxyl radical, superoxide anion, hydrogen peroxide, and hydroxyl radical) (Lung et al., 2015). In addition, ROS is also formed enzymatically and chemically in the mitochondria, phagocytic organelles, endoplasmic reticulum, cytosol, and peroxisomes within cells (Zhang et al., 2022). Zhang et al. (2020) demonstrated the ROS accumulation of bovine meat with aging from 0 to 168 h. Thus, the ROS content of shrimp increased when subjected to irradiation and chilled storage.

Surface hydrophobicity (So) of MP

Changes in So of MP from irradiated shrimps at different storage times were shown in Fig. 1B. On 0 d, compared to control samples, the value of So enhanced significantly in samples after 3, 5, and 7 kGy irradiation treatment ($P < 0.05$). As the storage duration progressed, the So level of irradiated shrimps (1, 3, 5, 7, and 9 kGy) raised and peaked at 3 d, then tended to descend. The So of control samples showed a similar trend but peaked at 5 d.

The increase in So indicated that protein unfolding induced by EBI treatment and chilled storage results in the exposure of hydrophobic groups buried inside MP. Nevertheless, the So level was reduced when the samples were subjected to high-dose (9 kGy) irradiation and prolonged storage. This phenomenon could be explained by the extensive

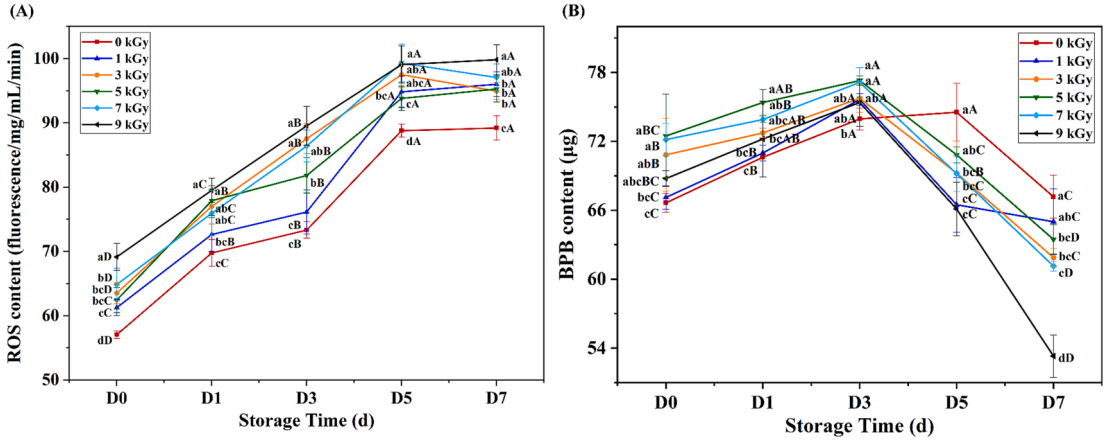

Fig. 1. The ROS contents (A), and surface hydrophobicity (B) of MP from *Litopenaeus vannamei* muscle irradiated with different doses at different storage times.

oxidation-induced development of protein aggregation and cross-linking (Li et al., 2021). In other words, exposed hydrophobic groups can aggregate through hydrophobic and electrostatic interactions. Shi et al. (2021) demonstrated the changes in So of MP from grass carp subjected to EBI treatment, finding that high irradiation doses (≥8 kGy) decreased the So and could be more prone to causing protein aggregation. The So of myofibrillar protein is closely associated with protein solubility. Increased hydrophobicity promotes protein–protein interactions while weakening protein-water interactions, reducing protein solubility (Xiong & Guo, 2020).

Carbonyl contents of MP

Carbonyl contents of shrimp MP were detected to reveal the extent of protein oxidation. The alterations in carbonyl contents of irradiated shrimps at various storage times were shown in Table 1. After irradiation with various doses, the carbonyl content showed a dose-dependent increase at 0 d storage. Whereas no noticeable distinctions in carbonyl contents were observed between the two groups that underwent 3 and 5 kGy irradiation, nor those exposed to 5 and 7 kGy irradiation ($P > 0.05$). As the storage duration progressed, the carbonyl contents in all treatments exhibited a gradual rise. Notably, the carbonyl values in irradiated groups with dose ≥ 5 kGy were higher ($P < 0.05$) than those in 0 and 1 kGy treatments at the same storage duration, which revealed that a noticeable aggravation of oxidative modification proceeded in samples as the irradiation dose increased.

Table 1
Changes in the carbonyl content and total sulfhydryl content of *Litopenaeus vannamei* muscle irradiated with different doses at different storage times.

	Dose (kGy)	Storage time (d)				
		0	1	3	5	7
Carbonyl content (nmol/mg protein)						
	0	0.35 ± 0.01^{eA}	0.40 ± 0.02^{cA}	0.60 ± 0.01^{cB}	0.76 ± 0.03^{dC}	0.87 ± 0.06^{cD}
	1	0.39 ± 0.02^{dA}	0.42 ± 0.02^{cA}	0.65 ± 0.05^{cB}	0.78 ± 0.04^{dC}	0.97 ± 0.11^{bcD}
	3	0.59 ± 0.02^{cA}	0.74 ± 0.04^{bB}	0.84 ± 0.03^{bC}	0.93 ± 0.06^{cD}	1.05 ± 0.02^{bE}
	5	0.62 ± 0.02^{bcA}	0.88 ± 0.05^{aB}	1.05 ± 0.03^{aC}	1.16 ± 0.03^{abD}	1.28 ± 0.03^{aE}
	7	0.64 ± 0.03^{bA}	0.71 ± 0.03^{bA}	0.90 ± 0.06^{bB}	1.11 ± 0.07^{bC}	1.34 ± 0.07^{aD}
	9	0.70 ± 0.02^{aA}	0.77 ± 0.03^{bA}	1.02 ± 0.09^{aB}	1.19 ± 0.02^{aC}	1.37 ± 0.05^{aD}
Total sulfhydryl content (mol/10^5g protein)						
	0	8.29 ± 0.12^{aA}	7.63 ± 0.09^{aB}	7.35 ± 0.11^{aC}	6.93 ± 0.19^{aD}	5.76 ± 0.20^{aE}
	1	7.90 ± 0.15^{abA}	7.51 ± 0.36^{aB}	6.52 ± 0.12^{bC}	6.72 ± 0.11^{aC}	5.45 ± 0.03^{abD}
	3	7.62 ± 0.21^{bcA}	7.12 ± 0.14^{bB}	6.31 ± 0.07^{bcC}	6.08 ± 0.06^{bC}	5.34 ± 0.18^{bD}
	5	7.69 ± 0.32^{bcA}	6.44 ± 0.12^{cB}	6.57 ± 0.24^{bB}	5.75 ± 0.11^{bC}	5.19 ± 0.17^{bD}
	7	7.52 ± 0.30^{bcA}	6.79 ± 0.23^{bB}	5.63 ± 0.21^{dC}	5.09 ± 0.31^{cD}	4.32 ± 0.17^{cE}
	9	7.38 ± 0.14^{cA}	5.85 ± 0.09^{dB}	6.09 ± 0.17^{cB}	4.36 ± 0.28^{dC}	3.73 ± 0.33^{dD}

The results are expressed as the mean ± standard deviation (n = 3).
Different letters in the same row (A, B, C, D, E) represent statistical differences ($P < 0.05$).
Different letters in the same column (a, b, c, d) represent statistical differences ($P < 0.05$).

Zhang et al. (2020) also found that pork treated by gamma-ray irradiation exhibited a significant increase in carbonyl levels during the chilled storage from 0 to 3 days. In addition, another consistent finding revealed that carbonyl contents of *Priacanthus tayenus* protein increased with increasing doses of gamma-ray irradiation (Riebroy et al., 2007). As a major oxidative modification in aquatic products, the elevated level of carbonylation could be attributed to several factors. First of all, the generation of free radicals ($OH^{\bullet}$) is conducive to the modification of protein amino groups containing NH– or NH_2–, eventually converting them into carbonyl derivatives through an oxidative deamination reaction (Gautam & Venugopal, 2021; Li et al., 2021). Then, the side chains of some particular amino acids (lysine, arginine, histidine, and proline) participated in the carbonylation process through metal-catalyzed oxidation (Zhang et al., 2022). Furthermore, some proteins, such as alpha skeletal actin, creatine kinase, and glycogen phosphorylase, may be the source of carbonyl compounds. These proteins have been confirmed to undergo carbonylation modification in chilled mackerel muscle (Pazos et al., 2013). Finally, oxidation-induced cleavage of peptides may also trigger the formation of the carbonyl group (Zhang et al., 2020). The formation of carbonyl affects muscle quality. Bu et al. (2022) and Xu et al. (2022) investigated the relationship between protein biochemical characteristics and quality indicators in refrigerated *Thunnus Maccoyii* and *Penaeus chinensis*, respectively. Their findings revealed a significant ($P < 0.05$) negative correlation between the carbonyl group and textural profiles including hardness, springiness, and chewiness.

Total sulfhydryl (SH) content of MP

Changes in total SH contents of irradiated shrimps at different storage times were shown in Table 1. On 0 d, the control group showed the highest total SH content (8.29 mol/10^5g pro), which was notably greater than that observed in the irradiated shrimps with doses ≥ 3 kGy ($P < 0.05$). Then, the total SH contents in each treatment all declined with prolonged refrigeration. Up to 7th day of preservation, the decreased ratios of total SH contents for 0, 1, 3, 5, 7, and 9 kGy irradiated groups were 30.44 %, 31.03 %, 29.95 %, 32.46 %, 42.53 %, and 49.54 %, respectively. This indicated that the high-dose irradiation seemed to show a promoting effect toward SH oxidation. Riebroy et al. (2007) measured the level of SH in *Priacanthus tayenus* protein and found a decreased trend as the gamma irradiation dose increased. A previous study by Lv et al. (2018) also demonstrated a reduced total SH level of MP from *Tegillarca granosa* upon exposure to high doses of irradiation. Additionally, Xu et al. (2022) also found a declining trend in SH content in shrimp (*Penaeus chinensis*) with prolonged refrigeration.

Irradiation induces a change in the conformation of protein molecules (Lv et al., 2018), causing reactive SH groups to be exposed, whereas their existence seemed to be transitory due to their susceptibility to oxidation or disulfide interchanges (Ekezie et al., 2019). Oxidation of cysteine involving the conversion of SH regions to disulfide bonds and the depletion of SH regions in myosin all contributed to the decline of SH contents. Some substances containing sulfenic acid, sulfinic acid, disulfide cross-links, and other complexes could be formed through oxidation reactions of the thiol group (Lund et al., 2011; Zhang et al., 2022). In addition, the SH groups in proteins are very sensitive to ROS. Reactive free radicals, such as hydroxyl radicals, induced a concentration-dependent decline in the SH level of MP from fish and shrimp (Li et al., 2021; Li et al., 2020; Lund et al., 2011). Thus, irradiation and prolonged storage could be favorable for the oxidation of SH groups due to forming free radicals. In addition, the disulfide bonds formed by moderate oxidation of sulfhydryl groups could improve the protein gel properties by stabilizing the three-dimensional network of protein molecules (Srinivasan & Hultin, 1997; Xiong & Guo, 2020).

Ca^{2+}-ATPase activity of MP

Changes in the Ca^{2+}-ATPase activity of MP from irradiated shrimps at different storage times were revealed in Fig. 2A. Irradiation treatment (≥5 kGy) could significantly decrease ($P < 0.05$) Ca^{2+}-ATPase activity in a dose-dependent way at 0 d refrigeration. Up to 7th day of preservation, a marked decrease ($P < 0.05$) was displayed in Ca^{2+}-ATPase activity in comparison with the samples at the initial stage of storage, the decrease ratios for 0, 1, 3, 5, 7, and 9 kGy treated groups were 61.54 %, 62.57 %, 69.49 %, 67.08 %, 75.66 %, and 70.14 %, respectively.

The globular head region of myosin contains the active site of Ca^{2+}-ATPase. Ca^{2+}-ATPase activity is considered to indicate the structural integrity of myosin in the MP. Shi et al. (2021) discovered an obvious reduction in Ca^{2+}-ATPase activity of MP in grass carp with increasing irradiation dose. A similar result also be found by Lv et al. (2018) from irradiated *Tegillarca granosa* meat. These differences revealed that a high EBI intensity would disrupt the active structure of myosin. Conformational alterations and molecular aggregation occurring within the myosin globular head probably trigger the reduction of Ca^{2+}-ATPase activity. About the impact of storage time, Zhou et al. (2023) explored the changes of Ca^{2+}-ATPase activity in sword prawns during frozen storage, and their result indicated that the decline in Ca^{2+}-ATPase activity associated with the damage/alteration of prawn MP caused by endogenous enzymes-mediated hydrolysis during storage. Additionally, myosin has a particularly high sulfhydryl content, which is the most oxidizable among myofibrillar proteins (Bao et al., 2018). The variation trends of Ca^{2+}-ATPase activity in this research were consistent with the total sulfhydryl content, which further confirmed the occurrence of oxidative modifications induced by irradiation. Additionally, it has been proved that the decreased Ca^{2+}-ATPase activity results in the weakening of muscle fiber contraction, thereby affecting shrimp hardness and springiness. Xu et al (2022) found a significantly ($P < 0.05$) positive correlation between Ca^{2+}-ATPase activity and textural characteristics (hardness, chewiness, and stickiness) of *Penaeus chinensis*.

Zeta potential of MP

Changes in the ζ-potential of MP from irradiated shrimps at different storage times were revealed in Fig. 2B. The ζ-potential of all groups presented a net negative charge. On 0 d, the absolute ζ-potential value displayed a progressive rise as the irradiation dose increased. The highest absolute ζ-potential value (-10.67 mV) was observed in the 7 kGy treatment, which is notably higher ($P < 0.05$) than in control samples (-8.44 mV). Likewise, Li et al. (2018) also discovered a rise in different degrees of net negative charge in irradiated pork MP. When the storage time increased to 7 d, the absolute ζ-potential values for all groups reduced in comparison with the shrimps at the initial stage (0 d), especially for the samples treated with ≥ 3 kGy irradiation, showing a notably reduced trend ($P < 0.05$). This result indicated that weak electrostatic repulsion was observed in the irradiation groups (≥3 kGy), and the protein was more prone to aggregation.

The level of ζ-potential can be used to reveal the filament charges of MP and evaluate the electrostatic interaction between charged particles (Li et al., 2021). Muscle proteins contain positively or negatively charged groups including nonpolar hydrophobic residues, ionic groups, and hydrophilic polar groups. The balance of these groups impacts the final surface charge (Li et al., 2018). The rise in the absolute ζ-potential values of irradiated shrimp MP might be ascribed to several influencing factors. First, the carbonylation of positively charged lysine, arginine, and histidine residues can induce the loss of their positive charges on myofilaments (Bao & Ertbjerg, 2019). Then, oxidation was deemed to influence the isoelectric point (pI) of protein. A shift toward a lower pI value of oxidized MP was observed, accompanied by the ascending level of negative charge (Bao et al., 2018). Last, oxidation promotes the migration of hydrophobic amino acids to the MP surface, which carry negative charges in a neutral environment, endowing the MP with more net negative charges (Liu et al., 2020). While the decrease in net negative charge could be related to partial protein denaturation and aggregation (Li et al., 2021). The present study indicated that oxidative modification induced by EBI treatment and postmortem storage could lead to changes in the electronic arrangement of shrimp myofilaments. And previous studies have demonstrated that the number of surface charges on the protein could affect the protein solubility and muscle water holding capacity. The decrease of net negative charges weakened the electrostatic repulsion between protein molecules, and the protein was prone to aggregate and precipitate, thereby reducing the protein solubility and water holding capacity (Bao & Ertbjerg, 2019; Shi et al., 2021).

Solubility of MP

Changes in solubility of MP from irradiated shrimps at different storage times were revealed in Fig. 3A. On 0 d, compared to the control group, no noticeable distinctions ($P > 0.05$) in protein solubility when the irradiation dose was no more than 5 kGy, while irradiation with ≥ 7 kGy notably decreased the MP solubility ($P < 0.05$). During storage, a fluctuating change in MP solubility was observed in each treatment group. Then, upon contrasting the alteration of MP solubility between 0 and 7 d under the consistent EBI dose, a marked decline trend ($P < 0.05$) was obtained. We also found that the decreased ratios of protein solubility in samples treated with ≥ 7 kGy irradiation were higher than others.

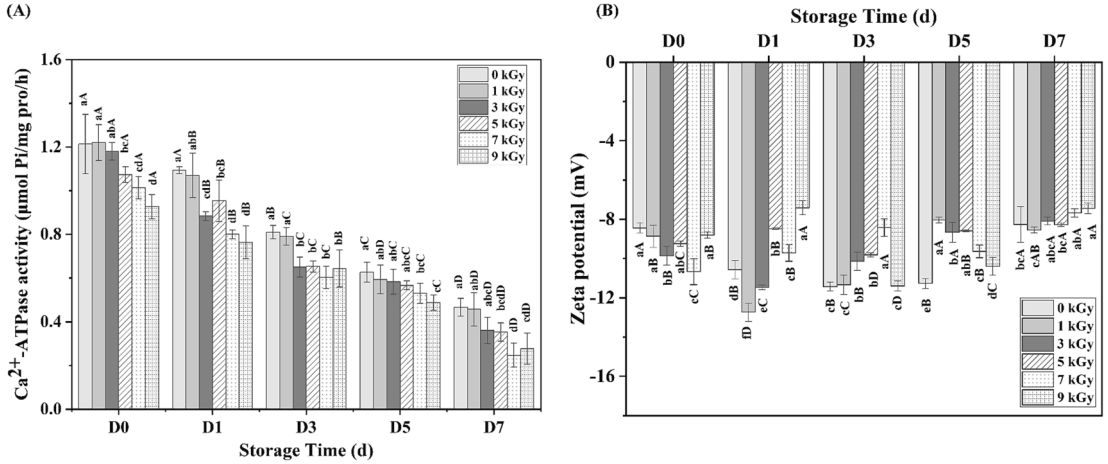

Fig. 2. Ca^{2+}-ATPase activity (A), and zeta-potential (B) of MP from *Litopenaeus vannamei* muscle irradiated with different doses at different storage times.

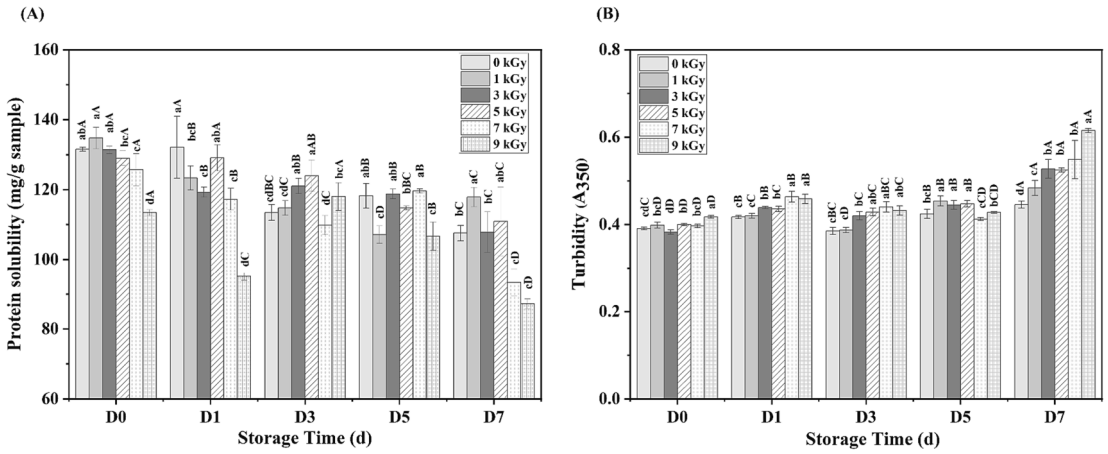

Fig. 3. Changes in solubility (A), and turbidity (B) of MP from *Litopenaeus vannamei* muscle irradiated with different doses at different storage times.

Shi et al. (2021) observed that EBI induced a dose-dependent decrease of solubility in MP from grass carp. The decline in MP solubility was closely associated with the presence of excessive oxidation induced by high-dose irradiation and prolonged storage, leading to an increased extent of protein cross-linking. In addition, protein molecules also tend to form insoluble aggregates through non-covalent interactions, which involve hydrophobic interactions caused by exposed hydrophobic groups. Meanwhile, water might be blocked from entering the internal region of the protein molecule when its structure changes (Kuan et al., 2013; Zhang et al., 2022). It is noteworthy that excessive oxidation can lead to the formation of insoluble aggregates and decrease protein solubility, which is not conducive to protein digestibility from a nutritional point of view (Srinivasan & Hultin, 1997).

An increased solubility that occurred during refrigeration might be associated with the changes in the net charges of proteins. From the results of ζ-potential in the present study, a fluctuating change of net negative charges was shown during storage. A rising level of net negative charges promoted the electrostatic repulsion between protein particles and the hydration of charged residues, which were beneficial to increase solubility (Bao & Ertbjerg, 2019; Li et al., 2021). Additionally, protein degradation is a process that occurs simultaneously with protein aggregation during storage. MP was dissociated to form smaller molecules, which provided more contact area for water molecules, thereby obtaining a higher solubility (Li et al., 2021).

Turbidity of MP

The turbidity of MP from irradiated shrimps at various storage times was revealed in Fig. 3B. At 0 days of storage, the control sample showed relatively low turbidity (0.391). While the turbidity in the 9 kGy group was markedly ($P < 0.05$) higher in comparison with others. With increased storage time to 7 d, the turbidity of samples treated with ≥ 1 kGy irradiation raised notably ($P < 0.05$) compared to 0 kGy treatment. In addition, a noticeable variation of turbidity in all groups was exhibited when compared with the turbidity at 0 d, as evidenced by 1.14, 1.21, 1.37, 1.31, 1.38, and 1.47-fold increases ($P < 0.05$) with the enhanced irradiation dose, respectively.

Turbidity is commonly used to reveal the dispersion or aggregation of protein, which can be detected according to the light scattering on the surface of protein particles (Li et al., 2021). It has been found that large protein aggregates were formed with continuous protein oxidation through covalent bonds (disulfide, carbonyl–amine reaction, and dityrosine) or non-covalent interactions, resulting in an increase in turbidity (Feng et al., 2015). In this study, increased turbidity was observed when samples were subjected to high-dose irradiation and protracted chilled storage, which reflected an increased level of insoluble aggregates and the formation of a network structure. The turbidity of MP is negatively correlated with the water holding capacity of muscle. Cao et al. (2022) found that with the increase in beef protein turbidity, its water holding capacity showed a decreasing trend.

Textural properties

Changes in the texture of shrimps treated with EBI at different storage times were revealed in Fig. 4. On 0 d, with a rising irradiation dose, the hardness indicated a gradual increase to the maximum of 20.81 N at 5 kGy, and then a declining trend occurred in samples irradiated by 7 and 9 kGy. Similar variations occurred in springiness and chewiness. Compared to control group, no significant changes in the hardness of shrimps ($P > 0.05$) were observed in all irradiated treatments. But a marked increment ($P < 0.05$) in springiness and chewiness was shown when subjected to ≥ 3 kGy irradiation. Then, textural profiles of all samples descended when the refrigerated duration was extended to 7 d. The highest proportion of hardness and springiness reduction was in the control group, while the minimum occurred in the 5 kGy and 3 kGy treated groups, respectively. Simultaneously, the decreased ratios in chewiness of 1, 7, and 9 kGy treated groups were higher than others. These results illustrated that EBI contributes to inhibiting the textural softening of shrimp during postmortem storage, especially by applying 5 kGy irradiation to shrimps.

Texture is one of the important sensory profiles of shrimp, and textural softening will decrease the consumer acceptability and the economic value of shrimp (Xu et al., 2022). Lv et al. (2018) explored EBI-induced textural changes in *Tegillarca granosa* and obtained a rising level of chewiness in irradiated samples. Yu et al. (2022) revealed a dose-dependent increase in the springiness of shrimp. Rowe et al. (2004) also found that 6.4 kGy irradiation was beneficial in reducing the degree of beef steak tenderization at postmortem storage when compared to steak without irradiation. Protein oxidation has been considered to be one of the explanations for the increase in texture. Oxidative modification could change the spatial conformation of proteins by inducing protein cross-linking and forming protein aggregation (Bao & Ertbjerg, 2019). But, Li et al. (2021) thought moderate oxidation would be useful for enhancing mechanical properties by inducing disulfide cross-linkage of myofibrillar protein to form a dense network microstructure. Nevertheless, excessive oxidation might hinder the orderly interactions of key active groups and decline the level of protein association, which is unfavorable to maintaining mechanical strength. In addition, the textural softening that occurred during chilled storage could be related to the degradation of some structural proteins, such as myofibrillar and connective tissue proteins, which play critical roles in maintaining good texture characteristics (Xu et al., 2022).

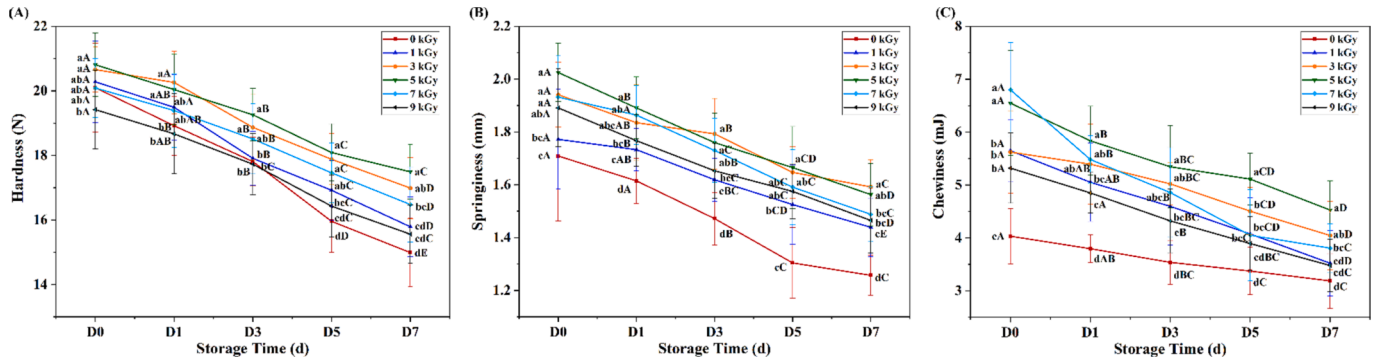

Fig. 4. Changes in textural properties including hardness (A), springiness (B), and chewiness (C) of irradiated *Litopenaeus vannamei* at different storage times.

Conclusions

EBI treatment is beneficial for extending the shelf life of *Litopenaeus vannamei*, but it also affects the muscle protein and texture quality of the shrimp. EBI accelerated protein oxidation of shrimp during refrigerated storage. Compared to the control group, high-dose (≥7 kGy) irradiation treatment significantly induced the production of ROS in the shrimp muscle, promoting the loss of sulfhydryl groups and the generation of carbonyl groups in MP, disrupting the integrity of myosin, triggering a decline in Ca^{2+}-ATPase activity. They also weakened the electrostatic repulsion between protein molecules, making proteins more prone to aggregation. Compared to the high-dose groups, low-dose (≤5 kGy) irradiation treatment increased the protein solubility and reduced the turbidity of shrimps. Additionally, suitable doses of irradiation were able to delay the softening of shrimp texture. Results revealed that irradiation with 3 kGy and 5 kGy retarded the descent of hardness, springiness, and chewiness of shrimp muscle. Therefore, appropriate EBI doses of 3–5 kGy contribute to extending the shelf life of refrigerated shrimp and preserving a good texture quality of the shrimp by inducing moderate protein oxidation, which can serve as a theoretical foundation for the application of EBI in shrimp preservation. In this study, the effects of EBI on protein oxidation and textural properties of shrimp during refrigerated storage were presented, and further analysis should be performed to identify the oxidative modification sites and abundance of muscle proteins in irradiated shrimp (Muhammad et al., 2021; Muhammad et al., 2022; Zhang et al., 2022). This will be useful in revealing the specific protein oxidation mechanisms underlying the changes in the texture quality of irradiated shrimp.

CRediT authorship contribution statement

Haoran Wang: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft. **Ran Suo:** Conceptualization, Methodology, Writing – review & editing. **Yangyang Wang:** Data curation, Validation, Software. **Jianfeng Sun:** Conceptualization. **Yaqiong Liu:** Validation. **Wenxiu Wang:** Writing – review & editing. **Jie Wang:** Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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