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Contents lists available at ScienceDirect

Food Bioscience

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# Effects of E-beam irradiation on the physicochemical properties of Atlantic cod (*Gadus morhua*)

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## ARTICLE INFO

### Keywords:

E-beam irradiation  
Atlantic cod  
SARS-CoV-2  
Physicochemical properties

## ABSTRACT

Electron beam (E-beam) irradiation can effectively inactivate severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in cold-chain seafood. This study evaluated the effects of E-beam irradiation at doses killing SARS-CoV-2 on quality indicators of Atlantic cod. The cod samples were exposed to 0, 2, 4, 7, and 10 kGy E-beam irradiation, and nutrition, texture, color, and sensory attributes were investigated. The results showed that E-beam irradiation significantly increased thiobarbituric acid (TBA) value and decreased hardness, chewiness, and  $\alpha^*$  value of Atlantic cod ( $P < 0.05$ ). E-beam irradiation with 10 kGy significantly lowered total volatile base nitrogen (TVB-N) and reducing sugar content while increasing moisture and ash content ( $P < 0.05$ ). A significant color change was observed after irradiation with 2 kGy–7 kGy E-beam ( $P < 0.05$ ). E-beam irradiation had no effects on sensory attributes ( $P > 0.05$ ). A dose of 4 kGy was recommended considering the keeping quality in Atlantic cod.

## 1. Introduction

Atlantic cod is the most popular groundfish worldwide, accounting for 16% of the global total groundfish supply (around 1142000 MT in 2020) (White, 2020). It is a good

source of omega-3 fatty acids, vitamins, and protein. As cod is a lean protein with almost no carbohydrates, it is lower in calories than oily fish, chicken, and red meat. It has become the most suitable meat choice for diabetes, low-carb, paleo, pescatarian, and gluten-free diets. Cod is considered a low or moderate mercury fish, serving as a healthy food recommended by The U.S. Food & Drug Administration (FDA) for pregnant and lactating people (FDA, 2021). The Atlantic cod is one of the most widely consumed fish by humans all over the globe.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive-sense single-stranded RNA virus that is contagious in humans (Chan et al., 2020). It is known

to cause COVID-19, which World Health Organization (WHO) declared as pandemics

in 2020. Since the outbreak of COVID-19 in 2019, it had spread in more than 222 countries in the six continents, leading to more than 200 million infections, almost 4 million deaths, and the lockdown of one-third of the world's population (Kaplan, Frias, & McFall-Johnsen, 2020; Lu et al., 2021). The number of infected and dead people is still

rising. SARS-CoV-2 is primarily transmitted through respiratory droplets and close contact with infected people and contaminated objects. However, the spread of SARS-CoV-2 through the frozen seafood chain deserves special attention. The Chinese Center

for Disease Control and Prevention had detected and isolated SARS-CoV-2 from the outer packaging of manually transported frozen cod in Qingdao city, Shandong province, China, in September 2020 for the first time, and 12 people were indirectly infected by two infected cases via contaminated frozen cod (Chi, Zheng, Liu, & Wang, 2021). SARS-CoV-2 showed more stability on plastics and the artificially contaminated virus can be detected within 72 h after being applied to these surfaces (Van Doremalen et al., 2020). Results from Feng et al. (2021) also showed that SARS-CoV-2 was more persistent in frozen ( $-20\text{ }^{\circ}\text{C}$ ) than cold storage ( $4\text{ }^{\circ}\text{C}$ ) conditions in contaminated seafood. The infectivity of coronavirus was found to remain up to 2 years during frozen storage and transport at  $-20\text{ }^{\circ}\text{C}$  (Chin et al., 2020). Thus, it has been shown that SARS-CoV-2 can survive for a long time on cold chain cod and its packaging surface once the cod is contaminated.

Further, contaminated cod was processed, packaged, loaded and retailed, resulting into significant risk of transmission of coronavirus to human beings. In July 2020, a person was infected after processing SARS-CoV-2 contaminated imported cold chain seafood, and 79 people were infected one after another in Dalian, China, which indicated the

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<https://doi.org/10.1016/j.fbio.2022.101803>

Received 11 February 2022; Received in revised form 21 May 2022; Accepted 21 May 2022

Available online 6 June 2022

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infections caused by contaminated cold chain products to people indeed deserves worldwide attention (Chi et al., 2021). It is essential to alert the spread of COVID-19 caused by frozen cod via cold chain transportation. On the other hand, the widespread SARS-CoV-2 has caused major disruption in the global cod supply cold chain. Food and Agriculture Organization of the United Nations (FAO) has reported a pronounced decline of fresh whole cod from Norway, falling by 22% (260 tons), and Norwegian export to China and the United Kingdom dropped by 20% and 18.5% (FAO, 2020). Russian frozen Alaska pollock prices fell by as much as 28% in the spring of 2020 as a result of the COVID-19 pandemic (FAO, 2020). To prevent the spread of SARS-CoV-2 and reduce the economic loss of cod industry, it is of great necessity to implement strict measures during the cod cold chain primarily to ensure the safety of frozen cod and its outer packages.

Nonthermal methods, including disinfectant and ultraviolet light (UV-C, the shortest wavelength in the range of 100 nm–280 nm), were commonly used in SARS-CoV-2 inactivation to keep cod safety and quality in the cold chain industry (Anelich, Lues, Farber, & Parreira, 2020). Chemical disinfectants like chlorines, peroxides, silicon nitride, quaternary amines, sodium hypochlorite, and alcohols effectively killed coronavirus (García-Ávila et al., 2020; Kampf, Todt, Pfaender, & Steinmann, 2020; Pezzotti et al., 2020; Yin, Ling, Hong, & Yan, 2020). It is said that 0.05% sodium hypochlorite could reduce more than 5 log cycles of SARS-CoV-2 after 5 min treatment (Yin et al., 2020). Despite the promising results from sanitizers, they are often associated with drawbacks such as high concentration requirements for 100% viral inhibition, limited effectiveness over time, harmful residues behind on food contact surfaces, and possible risks to public health and the environment (Talebian, Wallace, Schroeder, Stellacci, & Conde, 2020). UV-C light is another effective tool for inactivating coronavirus. More than 99.9% reduction of infectious titers was achieved after irradiating with UV-C light for 15 min (Crisuolo et al., 2021). However, UV irradiation is time-consuming and high cost is required for SARS-CoV-2 inactivation, and the sterilization effects of frozen seafood surface and spaces were unknown. Hence, it is more than necessary to employ a method that effectively kills the coronavirus without adverse effects on cod quality and human health. Electron beam (E-beam) is a promising sterilization technology that uses ionizing irradiation to kill microorganisms, characterizing with high reliability, low cost, short process cycle times, and environmental friendliness. It is of high safety and the E-beam sterilized products are not radioactive, have no sterilant residues, and have a demonstrable sterility assurance (E-BEAM Services, 2014). At doses of 10 kGy and below, irradiation was declared safe for food by FAO, WHO, and the International Atomic Energy Agency (IAEA). Ye et al. (2021) found that E-beam irradiation (at least 2 kGy) can inactivate 99.9% SARS-CoV-2 of the cold chain food packaging surface, food surface and spaces. This provides a foundation for the application of E-beam irradiation in the cold chain food industry to protect frozen cod from SARS-CoV-2 contamination.

However, it is of great importance to assess whether E-beam irradiation at doses killing coronavirus would pose adverse effects on cod physicochemical properties before applying E-beam irradiation to the cold chain cod industry. Currently, there is no study reporting on the effects of E-beam irradiation on the nutrition, texture, and sensory qualities of Atlantic cod. Therefore, in this work, we studied the physicochemical parameters and sensory attributes of Atlantic cod after irradiated with E-beam. The study aimed to evaluate the impacts of E-beam irradiation on the cod quality and provide data support for further application of E-beam in the cold chain food industry.

## 2. Materials and methods

### 2.1. Cod sample preparation

The whole raw cod was purchased from the grocery store (Wholesale Market of Aquatic.

Products, Hangzhou, China). Meat near the cod head was cut into approximately cylindrical with a radius of 10 cm and a height of 4 cm, and about 500 g cod fillet was vacuum-packaged in a polyethylene bag. Samples were transported to the Electronic Accelerator Platform in Zhejiang University through the entire cold chain (−20 °C). The temperature of the cod was monitored by vertically inserting temperature loggers (ZDR-20Pro, Zeda Instruments Co., Ltd., Hangzhou, China) into the center of cod fillets.

### 2.2. E-beam irradiation

Irradiation treatment was performed at the linear accelerator belt (ESS-010-03, Japan) in the Electronic Accelerator Platform in Zhejiang University. Five target dose levels (0 kGy, 2 kGy, 4 kGy, 7 kGy, and 10 kGy) were chosen according to the previously reported SARS-CoV-2 killing effects (Ye et al., 2021). The cod samples underwent 0, 2, 4, 7, 10 kGy E-beam irradiation at room temperature, respectively. Following irradiation, samples were immediately placed in the freezing incubator and transported to the laboratory. Irradiated cod fillets were stored at −20 °C until subsequent analysis for a maximum of one week.

### 2.3. Nutritional composition analysis

#### 2.3.1. Moisture content

Moisture content in cod fillets was assessed by directly drying the samples to constant weight in the light of the Chinese standard of GB 5009.3–2016. Two gram of cod samples (irradiated and unirradiated meat, representing test and control sample, respectively) were used for determination. Samples were dried, cooled and weighed. The operations are repeated until the mass difference between two measurements was ≤ 2 mg. The constant weight of the weighing bottle ( $m_3$ ) and the mass of the weighing bottle and the cod sample before and after drying ( $m_1$  represents the mass of the weighing bottle and the cod sample before drying,  $m_2$  represents the mass of the weighing bottle and the cod sample after drying) were recorded. Moisture content was calculated according to the following formula:

$$\text{Moisture content (g/100 g)} = [(m_1 - m_2) \times 100] / (m_1 - m_3) \quad (1)$$

#### 2.3.2. Ash content

Ash content was determined as described by the Chinese standard of GB 5009.4–2016. Five grams of cod samples (irradiated and unirradiated meat, representing test and control sample, respectively) were used for determination. Wetting agent magnesium acetate was added to the cod samples. After carbonized, the cod samples were burned and ashed in Muffle Furnace SX2-10-12 (Shanghai Jinwen Instrument Equipment Co., Ltd., China) to constant weight. Ash content was obtained using the following equation:

$$\text{Ash content (g/100 g)} = [(m_1 - m_2 - m_0) \times 100] / (m_3 - m_2) \quad (2)$$

where  $m_0$  is the weight of magnesium oxide,  $m_1$  is the weight of crucible and cod ash,  $m_2$  is the weight of crucible,  $m_3$  is the weight of crucible and the cod samples.

#### 2.3.3. Vitamin A and vitamin E content

The content of vitamin A and vitamin E in cod was estimated employing Reversed-Phase High-Performance Liquid Chromatography (HPLC) according to the Chinese standard of GB 5009.82–2016. Cod fillets (irradiated and unirradiated meat, representing test and control sample, respectively) were first smashed and homogenized. Two grams of homogenized cod sample in each group were saponified under 80 °C water bath for 30 min. The saponification solution was extracted with petroleum ether-diethyl ether mixture and the ether layer was washed with water until it is neutral. The washed ether layer was then condensed, dried and dissolved in MeOH. The prepared solution was filtrated by 0.22- $\mu$ m diameter filter for HPLC determination. The

analytical column was a C<sub>30</sub> column (250 mm × 4.6 mm, 5 μm) (Phenomenex, Aschaffenburg, Germany) and the temperature was held at 20 °C ± 2 °C. Mobile phase (flow rate: 0.8 mL/min) consisted of solvent A (H<sub>2</sub>O) and solvent B (MeOH) by using the following gradient: 0 min (4:96), 13 min (4:96), 20 min (0:100), 24 min (0:100), 24.5 min (4:96) and 30 min (4:96). Detection wavelength of vitamin A was 325 nm and vitamin E was 294 nm. Vitamin A and vitamin E content were calculated via the following equation:

$$X = (\rho \times V \times f \times 100) / m \quad (3)$$

where X is the content of vitamin A/vitamin E (the concentration unit of vitamin A is μg/100 g and vitamin E is mg/100 g), ρ is the concentration of vitamin A/vitamin E based on the standard curve, V is the constant volume (10 mL in the experiment), f is the conversion factor (f = 1 of vitamin A, and f = 0.001 of vitamin E), m is the cod sample weight (2 g in the experiment).

#### 2.3.4. Fat and fatty acid content

Soxhlet extraction was applied to fat determination according to GB 5009.6–2016. Briefly, 2 g cod samples (irradiated and unirradiated meat, representing test and control sample, respectively) were fully grounded, and anhydrous sodium sulfate (2 g) was added to the extraction thimble. The extraction process lasted for 6 h with petroleum benzene in a Soxhlet apparatus. After recovering petroleum benzene, lipid extracts were

evaporated, dried, and cooled to constant weight. The fat content in the cod sample was calculated with the following equation:

$$\text{Fat content in the cod sample (g/100 g)} = \text{weight of lipid extracts / sample weight} \times 100 \quad (4)$$

Determination of SFA, MUFA, and PUFA was achieved by the standard internal method

based on the Chinese standard of GB 5009.168–2016. The procedure described briefly: Cod samples (irradiated and unirradiated meat, representing test and control sample, respectively) were first smashed and homogenized. Homogenized cod sample (0.7 g) in each group were hydrolyzed with 10 mL 8.3 mol/L hydrochloric acid under 70 °C–80 °C water bath for 40 min. The hydrolyzed sample was mixed with 10 mL 95% ethanol and the cod fat was extracted using 50 mL diethyl ether-petroleum ether mixture (50/50, v/v). The extracted fat was then saponified and methylated. A fused silica capillary column (100 m × 0.25 mm, 0.2 μm) on an Agilent 7820A gas chromatograph was used to separate and quantify the fatty acid methyl esters. Fatty acid methyl esters were identified by comparison with retention times of standard. The fatty acid content of Atlantic cod was calculated as concentration (g/100 g) = content of fatty acid methyl esters (g/100 g) × conversion factors (factors of conversion of fatty acid methyl esters to fatty acids). The detailed values are given in Supplementary Materials.

#### 2.3.5. Protein and amino acid content

Crude protein was estimated by the Kjeldahl Method. A gram of cod sample (irradiated and unirradiated meat, representing test and control sample, respectively) was digested with copper sulfate (0.4 g), potassium sulfate (6 g) and sulfuric acid (20 mL, 0.0500 mol/L). The digestion continued for 1 h after the temperature of furnace reached 420 °C. After the liquid in tubes turned transparently green, it was cooled down and mixed with 50 mL water. Total nitrogen was determined by Automatic Kjeldahl nitrogen analyzer NKY 6100 (Shanghai Yihong Analytical Instrument Co., Ltd., China). Crude protein content equals the total nitrogen multiplied by 6.5. Concerning the free amino acid determination, 0.1 g cod samples (irradiated and unirradiated meat, representing test and control sample, respectively) were first hydrolyzed by 15 mL 6 mol/L hydrochloric acid (Sinopharm Chemical Reagent Co., Ltd., China) in the hydrolysis tubes. Four drops of phenol were added to the tubes. The tubes were then frozen for 3 min–5 min, vacuumized, filled with

nitrogen, sealed and hydrolyzed for 22 h. The obtained hydrolysate was filtrated, dried and evaporated. Sodium citrate buffer solution (1 mL, pH 2.2) was added to the dried tubes. The filtrated mixture was prepared for amino acid determination by the amino acid automatic analyzer (Biochorm, the UK) based on the Chinese standard of GB 5009.124–2016.

## 2.4. Biochemical properties

#### 2.4.1. Thiobarbituric acid reactive substances (TBARS)

TBARS analysis was performed based on the previous method (Rode & Hovda, 2016). The procedure described briefly: 10 g thawed cod samples (irradiated and unirradiated meat, representing test and control sample, respectively) were homogenized with 30 mL of 7.5% trichloroacetic acid. TBARS was extracted by homogenisation in trichloroacetic acid, filtrated, and added 0.02 M thiobarbituric acid (TBA). Steam distillation was performed in a water bath at 100 °C for 40 min. After cooling to room temperature, the absorbance was measured at 532 nm, and the TBARS value was calculated. A calibration curve was constructed from a dilution series of 0.002 M TEP (1,1,3,3-tetraethoxypropane) stock solution. TBARS values were presented as mg malondialdehyde (MDA)/kg of the cod samples.

#### 2.4.2. The total volatile basic nitrogen (TVB-N)

The method of Eliasson et al. (Eliasson, Arason, Margeirsson, Bergsson, & Pálsson, 2019) was deployed to measure TVB-N by steam distillation and titration. One hundred grams of cod muscle (irradiated and unirradiated meat, representing test and control sample, respectively) were homogenized in 200 mL of 7.5% aqueous trichloroacetic acid solution. The homogenate was centrifuged at 400 × g for 5 min. The supernatant liquid was filtered and distilled with 10% NaOH in the distillation tube using a Kjeldahl-type distillator. Distillation was continued until 40 mL of distillate was obtained in the beaker containing 10 mL of a 4% aqueous boric acid and 0.04 mL of methyl red and bromocresol green indicator for titration of ammonia. Distilled TVB-N was then titrated with the 0.1 N sulfuric acid solution and the neutralization completed when the color turned pink on the addition of a further drop of sulfuric acid. The quantity of TVB-N in mg was determined from the volume of sulfuric acid (n mL) added as follows:

$$\text{TVB-N} = n \times 16.8 \text{ mg of nitrogen/100 g (5)}$$

## 2.5. Texture

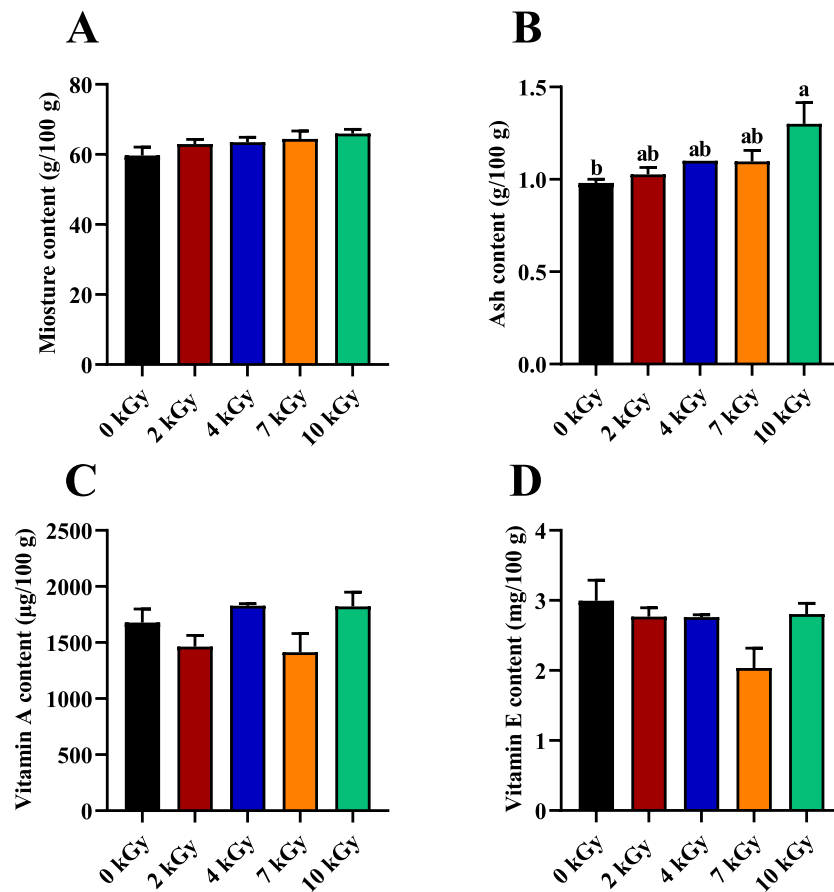
The cod sample with the length of 8 cm, the width of 8 cm, the thickness of 1 cm was prepared to analyze the texture using TA-XT2i Texture Analyzer (Stable Micro Systems, Ltd., Godalming, UK) equipped with a P/5 flat-bottom cylinder probe. Each cod sample was pressed to 50% of its original thickness at a cross-head speed of 1 mm/s, along the direction perpendicular to the muscle fibers. The trigger force was 5 g, and the measuring time of each sample was 5 s. The parameters (hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience) were calculated by Expression PC V.2.1 software.

## 2.6. Color analysis

The surface color of raw cod fillets was measured by Minolta Chroma Meter CR400 (Minolta, Osaka, Japan). The cod color was characterized by CIELab coordinates, where L\* indicated the lightness within the scale range of 0–100 points from black to white, a\* represented the position between red (+) and green (–), and the parameter of b\* implied the position between yellow (+) and blue (–) with the scale range of 127 (–127) points. The whiteness and ΔE of cod fillets were calculated as the following equation:

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2} \quad (6)$$

$$\Delta E = \sqrt{[(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2]} \quad (7)$$



**Fig. 1.** Effect of E-beam irradiation doses on (A) Moisture content, (B) Ash content, (C) Vitamin A content, and (D) Vitamin E content in Atlantic cod. Each data column represents the mean of three replications. Vertical bars represent the standard error of means. a-b Different letters differ significantly ( $P < 0.05$ ). Atlantic cod were irradiated with doses of 0 kGy, 2 kGy, 4 kGy, 7 kGy, 10 kGy, respectively.

## 2.7. Sensory assessment

The sensory panel consisted of 10 males and 10 females who had been pre-trained according to international standards (ISO, 1993). The evaluation was carried out in a sensory evaluation laboratory of Zhejiang University (25 °C, 60% RH, 250 lx, < 35 dB). Cod fillets were trimmed to get the uniform size (8 × 4 × 1 cm) and equal weight and were boiled for 5 min in water (water: cod fillets, 2:1, m/m). Sensory attributes included odor, flavor, and texture properties, and evaluation was conducted with a 10-line scale (from 0 = immensely dislike to 10 = immensely like). The total score was calculated according to the scores of each index.

## 2.8. Statistical analysis

All experiments were conducted in triplicate for each group. The results were analyzed using GraphPad Prism 8.3 (GraphPad Software Inc., New York, USA) and expressed as mean ± SEM. Data were subjected to one-way analysis of variance (ANOVA) with Tukey's multiple comparison posttests. Differences at  $P < 0.05$  were considered significant.

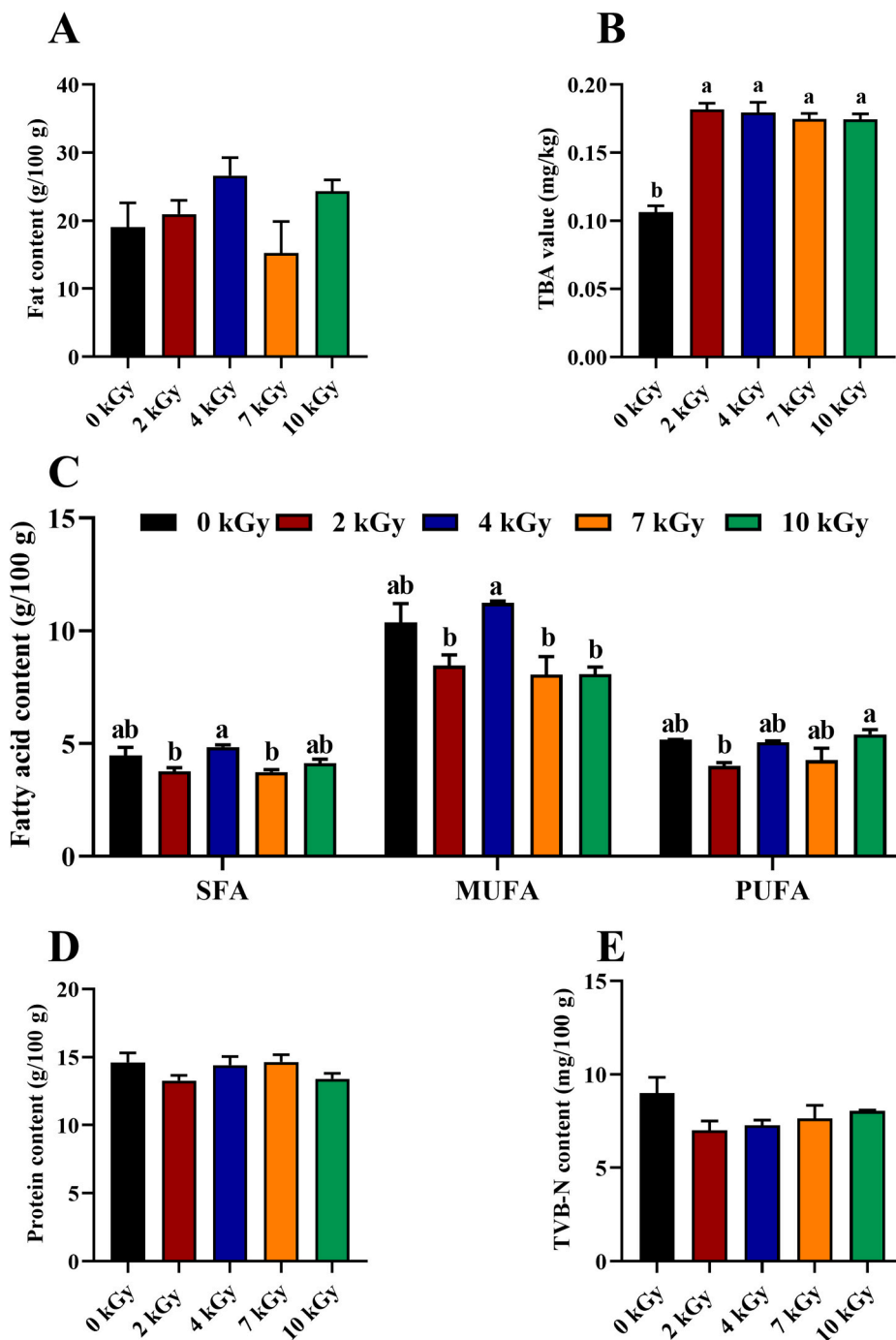
## 3. Results and discussion

### 3.1. Changes of moisture, ash, vitamin A, and vitamin E content

Moisture and ash content in Atlantic cod after 0 kGy–10 kGy E-beam irradiation are shown in Fig. 1. Per hundred grams of Atlantic cod had a moisture content of 59.67 g ± 2.45 g at 0 kGy E-beam irradiation, and it increased to 62.93 g ± 1.38 g, 63.50 g ± 1.40 g, 64.43 g ± 2.28 g, 65.97 g ± 1.19 g after 2, 4, 7, 10 kGy E-beam irradiation (Fig. 1A,  $P > 0.05$ ). Immobilized water was reported as the primary water in hake (*Merluccius merluccius*, L.) muscle, accounting for 90%–92%, which is higher than *Micropterus salmoides* (MS) meat and grass carp (Zu et al., 2021). It has been confirmed that the reduced moisture was accompanied by the lower immobilized water and enhanced binding force of the MS meat to the residual water after irradiation (Zu et al., 2021). Contrarily, considering the slightly increased moisture after E-beam irradiation, we speculated that E-beam irradiation might weaken the binding force between the Atlantic cod muscles and the residual water.

Ash refers to inorganic materials in food, including essential minerals, which play a critical role in maintaining several bodily functions. The ash content of cod was slightly increased under 0 kGy–7 kGy E-beam irradiation (0.98 g ± 0.02 g at 0 kGy, 1.03 g ± 0.04 g at 2 kGy, 1.10 g ± 0.00 g at 4 kGy, 1.10 g ± 0.06 g at 7 kGy) and notably increased to 1.30 g ± 0.12 g at 10 kGy E-beam irradiation (Fig. 1B,  $P < 0.05$ ). The significant increase of ash content in cod fillets might attribute to the chelating reaction induced by 10 kGy E-beam irradiation, according to Chumwaengwapee et al. (2013).





**Fig. 2.** Effect of E-beam irradiation doses on (A) Fat content, (B) TBA value, (C) Fatty acid content including saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA), (D) Protein content, and (E) TVB-N content in Atlantic cod. Each data column represents the mean of three replications. Vertical bars represent the standard error of means. a-c Different letters differ significantly ( $P < 0.05$ ). Atlantic cod were irradiated with doses of 0 kGy, 2 kGy, 4 kGy, 7 kGy, 10 kGy, respectively.

Vitamin A and vitamin E are recognized as radiation-sensitive vitamins in foods. However, the vitamin A and vitamin E content of Atlantic cod irradiated with various E-beam doses showed no difference with the control group ( $P > 0.05$ ) (Fig. 1C–D). Vitamin A content was unaffected in fresh dogfish after 0.3 Mrad irradiation but halved by 3 Mrad, indicating irradiation has an impact on vitamin A content (Mameesh, Boge, & Brækkan, 1964). The difference might attribute to the fish species difference, leading to the difference in vitamin A sensitivity to irradiation. Previous studies reported that irradiation caused vitamin E loss in Spanish mackerel and Australian marine fish (AL-KAHTANI et al., 1996; Armstrong, Wyllie, & Leach, 1994). Similarly, vitamin E content of Atlantic cod decreased after E-beam irradiation in this study ( $P > 0.05$ ). However, losses could not be correlated with treatment dosage. Our work proved the stability of vitamin A and vitamin E in Atlantic cod

under 2 kGy–10 kGy E-beam irradiation.

### 3.2. Changes of fat, fatty acid content, and TBA value

The meat's fat and fatty acid content are primary determinants of its shelf-life and storage stability, affecting its flavor, texture, and aromatic taste profile. Fig. 2A and Fig. 2C showed the changes of fat and fatty acid content of Atlantic cod after E-beam irradiation. No significant changes in fat content in Atlantic cod were observed after irradiation ( $P > 0.05$ ) (Fig. 2A). Irradiation with doses of 2 kGy–10 kGy did not significantly influence the saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) content ( $P > 0.05$ ) (Fig. 2C). Previous studies have reported the increase of SFA and the decrease of unsaturated fatty acid (USFA) in grass carp surimi and other

**Table 1**  
The amino acid content of cod under different E-beam irradiation doses.

Amino acid (g/ 100 g)	E-beam irradiation doses (kGy)				
	0	2	4	7	10
Aspartic acid	1.36 ± 0.04 <sup>a</sup>	1.26 ± 0.03 <sup>a</sup>	1.33 ± 0.05 <sup>a</sup>	1.32 ± 0.04 <sup>a</sup>	1.32 ± 0.03 <sup>a</sup>
Threonine	0.62 ± 0.02 <sup>a</sup>	0.60 ± 0.01 <sup>a</sup>	0.63 ± 0.02 <sup>a</sup>	0.62 ± 0.01 <sup>a</sup>	0.62 ± 0.02 <sup>a</sup>
Serine	0.60 ± 0.02 <sup>a</sup>	0.58 ± 0.01 <sup>a</sup>	0.62 ± 0.02 <sup>a</sup>	0.60 ± 0.02 <sup>a</sup>	0.59 ± 0.03 <sup>a</sup>
Glutamic acid	2.01 ± 0.07 <sup>a</sup>	1.99 ± 0.02 <sup>a</sup>	2.08 ± 0.07 <sup>a</sup>	1.98 ± 0.07 <sup>a</sup>	2.05 ± 0.06 <sup>a</sup>
Proline	0.47 ± 0.02 <sup>a</sup>	0.45 ± 0.01 <sup>a</sup>	0.50 ± 0.04 <sup>a</sup>	0.47 ± 0.04 <sup>a</sup>	0.44 ± 0.04 <sup>a</sup>
Glycine	0.61 ± 0.06 <sup>a</sup>	0.61 ± 0.03 <sup>a</sup>	0.68 ± 0.12 <sup>a</sup>	0.63 ± 0.11 <sup>a</sup>	0.60 ± 0.07 <sup>a</sup>
Alanine	0.79 ± 0.02 <sup>a</sup>	0.77 ± 0.01 <sup>a</sup>	0.81 ± 0.02 <sup>a</sup>	0.77 ± 0.02 <sup>a</sup>	0.79 ± 0.03 <sup>a</sup>
Valine	0.64 ± 0.01 <sup>b</sup>	0.66 ± 0.02 <sup>b</sup>	0.71 ± 0.03 <sup>a</sup>	0.70 ± 0.00 <sup>a</sup>	0.71 ± 0.00 <sup>a</sup>
Methionine	0.41 ± 0.01 <sup>a</sup>	0.40 ± 0.01 <sup>a</sup>	0.42 ± 0.01 <sup>a</sup>	0.41 ± 0.01 <sup>a</sup>	0.40 ± 0.00 <sup>a</sup>
Isoleucine	0.61 ± 0.01 <sup>a</sup>	0.58 ± 0.01 <sup>a</sup>	0.61 ± 0.02 <sup>a</sup>	0.58 ± 0.02 <sup>a</sup>	0.60 ± 0.02 <sup>a</sup>
Leucine	1.09 ± 0.03 <sup>a</sup>	1.04 ± 0.00 <sup>a</sup>	1.09 ± 0.04 <sup>a</sup>	1.04 ± 0.04 <sup>a</sup>	1.07 ± 0.03 <sup>a</sup>
Tryptophan	0.40 ± 0.02 <sup>a</sup>	0.38 ± 0.00 <sup>a</sup>	0.40 ± 0.02 <sup>a</sup>	0.38 ± 0.01 <sup>a</sup>	0.39 ± 0.01 <sup>a</sup>
Phenylalanine	0.51 ± 0.04 <sup>a</sup>	0.51 ± 0.00 <sup>a</sup>	0.52 ± 0.01 <sup>a</sup>	0.50 ± 0.01 <sup>a</sup>	0.51 ± 0.02 <sup>a</sup>
Histidine	0.31 ± 0.02 <sup>f</sup>	0.32 ± 0.01 <sup>c</sup>	0.36 ± 0.06 <sup>bc</sup>	0.42 ± 0.01 <sup>ab</sup>	0.43 ± 0.01 <sup>a</sup>
Lysine	1.32 ± 0.04 <sup>a</sup>	1.22 ± 0.01 <sup>a</sup>	1.26 ± 0.04 <sup>a</sup>	1.26 ± 0.05 <sup>a</sup>	1.28 ± 0.02 <sup>a</sup>
Arginine	0.79 ± 0.03 <sup>a</sup>	0.75 ± 0.02 <sup>a</sup>	0.81 ± 0.02 <sup>a</sup>	0.75 ± 0.01 <sup>a</sup>	0.76 ± 0.03 <sup>a</sup>
Total Content	12.54	12.12	12.83	12.43	12.56

<sup>a-c</sup> different superscript letters indicate significant difference ( $P < 0.05$ ).

fishes after E-beam irradiation (Wenjiao, Yuanlong, & Shuo, 2008; Yang, Zhang, Wang, Zhang, Wang, & Ye, 2016). The different results observed in our study might attribute to the irradiated seafood difference.

TBA value represents a measure of malonaldehyde formed through hydroperoxides. It is an indicator of lipid oxidation and is responsible for the rancid odor and tastes developing during food storage. TBA value of 1 mg MA kg<sup>-1</sup>-2 mg MA kg<sup>-1</sup> is considered the limit beyond acceptable odor and taste in fish flesh, and its value below 3 mg MA kg<sup>-1</sup> suggested the fish with perfect quality (Motalebi, Hoseini, & Javan, 2011). In our work, a significant increase of TBA value was obtained in E-beam irradiated Atlantic cod, from 0.11 mg/kg ± 0.00 mg/kg of 0 kGy irradiated group to 0.18 mg/kg ± 0.00 mg/kg, 0.18 mg/kg ± 0.01 mg/kg, 0.17 mg/kg ± 0.00 mg/kg, 0.17 mg/kg ± 0.00 mg/kg of 2 kGy, 4 kGy, 7 kGy and 10 kGy irradiated groups, respectively ( $P < 0.05$ ) (Fig. 2B). The highest TBA value occurred in the 2 kGy E-beam irradiation group of 0.18 mg/kg ± 0.00 mg/kg, which revealed that irradiated and unirradiated Atlantic cod had no abnormal odor and taste. We observed that the initial TBA value of the control samples was 0.11 mg/kg ± 0.00 mg/kg, and it increased to approximately 0.17 mg/kg-0.18 mg/kg after E-beam irradiation. Zhang, Wang, Zhang, Wang, and Ye (2016) found a similar increase of TBA value in vacuum-packaged grass carp surimi under 0 kGy-7 kGy (0 kGy, 1 kGy, 3 kGy, 5 kGy, 7 kGy) E-beam irradiation from the initial 0.24 mg/100 g ± 0.02 mg/100 g to 0.34 mg/100 g ± 0.01 mg/100 g, 0.39 mg/100 g ± 0.04 mg/100 g, 0.36 mg/100 g ± 0.05 mg/100 g and 0.38 mg/100 g ± 0.02 mg/100 g, respectively. Yang et al. (2014) also reported that the TBA value of vacuum-packaged Atlantic salmon increased from 0.11 mg/kg ± 0.03 mg/kg to about 0.15 mg/kg-0.3 mg/kg after E-beam irradiation. Consistent with the previous results, TBA values in the irradiated group were always higher than unirradiated fish samples, and there are no obvious dose-effects between TBA value and E-beam irradiation doses in Atlantic cod.

However, Fan, Chi, and Zhang (2008) showed an almost tenfold increase of TBA value to 3.09 mg/kg compared with the control group of 0.37 mg/kg in silver carp. The difference might ascribe that vacuum packaging plays an important role in blocking oxygen and preventing the increase of TBA value. On the other hand, it was confirmed that oxygen exerts a positive effect on lipid oxidation, and it might be necessary to reduce O<sub>2</sub> content in contact with irradiated cod considering the long-term preservation and storage of Atlantic cod.

### 3.3. Changes of protein, amino acid, and TVB-N content

Fish protein consists of sarcoplasmic proteins, myofibrillar proteins, and stroma proteins. Amino acid, the building material of proteins, determines proteins' conformational structure, chemical, and biological properties based on its type and rank order. In Atlantic cod, no significant difference ( $P > 0.05$ ) of protein content was observed after irradiation with 0 kGy-10 kGy E-beam doses (Fig. 2D). The content of 14 amino acids remained unchanged compared with the 0 kGy irradiated group ( $P > 0.05$ ) (Table 1.). Valine and histidine content both increased after E-beam irradiation. (Table 1.). Al-Kahtani et al. (1996) proposed similar findings that irradiation led to minimal changes of protein and increased some amino acids level in tilapia and Spanish mackerel. Proteins in fish muscles play a critical role in binding with water (Gokoglu & Yerlikaya, 2015). Considering the significantly increased moisture content after 10 kGy E-beam ( $P < 0.05$ ) (Fig. 1A), it was speculated that E-beam irradiation destructed protein structure of Atlantic cod, stroking cleavage of peptide bonds, and making easier extraction of amino acids.

Volatile compounds such as trimethylamine, ammonia, and dimethylamine, generated by destructive activities of microorganisms and enzymes on proteins and non-protein nitrogenous materials, are considered TVB-N, which is regarded as one of the most common freshness indexes to monitor the quality and safety of seafood (Moosavi-Nasab, Khoshnoudi-Nia, Azimifar, & Kamyab, 2021). In marine fish, TVB-N content in the range of 5 mg/100 g-20 mg/100 g is indicative of good quality, whereas values higher than 30 mg/100 g are considered as a limit of acceptability (Zhang et al., 2016). In our study, no significant difference was observed between the control and E-beam irradiated groups ( $P > 0.05$ ) (Fig. 1E). The TVB-N content of Atlantic cod was at a level of about 8 mg/100 g under 0 kGy-10 kGy E-beam irradiation (9.01 mg/100 g ± 0.84 mg/100 g, 7.00 mg/100 g ± 0.50 mg/100 g, 7.27 mg/100 g ± 0.28 mg/100 g, 7.64 mg/100 g ± 0.71 mg/100 g, 8.05 mg/100 g ± 0.05 mg/100 g in 0, 2, 4, 7, 10 kGy E-beam irradiated group) (Fig. 1E), which suggested the good quality of both the control and irradiated cod samples. Our results were similar to Zhang et al. (2016), vacuum-packaged grass carp surimi irradiated with 0 kGy-7 kGy (0 kGy, 1 kGy, 3 kGy, 5 kGy, 7 kGy) E-beam had about 15 mg/100 g TVB-N. Similarly, TVB-N content of Atlantic salmon irradiated with E-beam at doses of 0 kGy-3 kGy was reported to be approximately 17 mg/100 g based on the findings from Yang et al. (2014). It was proposed that E-beam irradiation combined with vacuum packaging could inhibit the microbial count and enzyme activity in fish, thus effectively suppressing the decomposition of fish fillets regarding TVB-N content (Yang et al., 2014; Zhang et al., 2016). Several studies have revealed that irradiation could inhibit the increase of TVB-N during storage period (Li et al., 2022; Yang et al., 2014; Zhang et al., 2016). Further studies are needed to investigate whether E-beam irradiation inhibits the increase in TVB-N of Atlantic cod.

### 3.4. Texture properties

Frozen fish texture, a critical attribute of flesh quality, arouses most attention in the seafood industry since freezing and long-term storage lead to marked increases in toughness and dryness of the tissues (Dunajski, 1980). Slaughter procedures, postmortem treatment, and intrinsic biological properties affected the muscle texture (Tang et al., 2012). Texture properties including hardness, adhesiveness, springiness,

**Table 2**  
Texture properties of Atlantic cod irradiated by different doses of E-beam.

Texture properties	E-beam irradiation doses (kGy)				
	0	2	4	7	10
Hardness	836.40 ± 83.15 <sup>a</sup>	273.40 ± 30.17 <sup>b</sup>	435.6 ± 70.75 <sup>b</sup>	347.10 ± 63.06 <sup>b</sup>	345.40 ± 22.08 <sup>b</sup>
Adhesiveness	-9.62 ± 1.88 <sup>a</sup>	-6.42 ± 1.17 <sup>a</sup>	-7.63 ± 3.05 <sup>a</sup>	-7.99 ± 2.72 <sup>a</sup>	-6.16 ± 0.39 <sup>a</sup>
Springiness	0.45 ± 0.04 <sup>c</sup>	0.41 ± 0.03 <sup>c</sup>	0.76 ± 0.03 <sup>a</sup>	0.60 ± 0.03 <sup>b</sup>	0.44 ± 0.02 <sup>c</sup>
Cohesiveness	0.53 ± 0.05 <sup>a</sup>	0.60 ± 0.03 <sup>a</sup>	0.48 ± 0.03 <sup>a</sup>	0.52 ± 0.04 <sup>a</sup>	0.63 ± 0.02 <sup>a</sup>
Gumminess	351.80 ± 54.93 <sup>a</sup>	207.80 ± 46.94 <sup>a</sup>	203.70 ± 28.69 <sup>a</sup>	260.10 ± 28.08 <sup>a</sup>	218.00 ± 12.43 <sup>a</sup>
Chewiness	152.50 ± 16.70 <sup>a</sup>	62.09 ± 10.77 <sup>b</sup>	65.42 ± 12.29 <sup>b</sup>	79.23 ± 7.69 <sup>b</sup>	89.88 ± 4.26 <sup>b</sup>
Resilience	0.24 ± 0.03 <sup>a</sup>	0.25 ± 0.02 <sup>a</sup>	0.19 ± 0.02 <sup>a</sup>	0.20 ± 0.02 <sup>a</sup>	0.28 ± 0.03 <sup>a</sup>

<sup>a-c</sup> different superscript letters indicate significant difference ( $P < 0.05$ ).

**Table 3**  
 $L^*$ ,  $a^*$  and  $b^*$  values of Atlantic cod under different E-beam irradiation doses.

Index	E-beam irradiation doses (kGy)				
	0	2	4	7	10
$L^*$ Values	75.30 ± 0.37 <sup>ab</sup>	71.81 ± 0.38 <sup>b</sup>	72.96 ± 0.65 <sup>b</sup>	78.30 ± 2.59 <sup>a</sup>	75.01 ± 0.56 <sup>ab</sup>
$a^*$ Values	-1.77 ± 0.13 <sup>a</sup>	-2.79 ± 0.19 <sup>b</sup>	-2.68 ± 0.02 <sup>b</sup>	-2.49 ± 0.07 <sup>b</sup>	-2.37 ± 0.06 <sup>b</sup>
$b^*$ Values	4.51 ± 0.65 <sup>a</sup>	2.01 ± 0.90 <sup>b</sup>	2.13 ± 0.22 <sup>b</sup>	2.96 ± 0.27 <sup>ab</sup>	4.97 ± 0.88 <sup>a</sup>
Whiteness	74.79 ± 0.31 <sup>a</sup>	72.03 ± 0.14 <sup>a</sup>	72.75 ± 0.65 <sup>a</sup>	77.95 ± 2.59 <sup>a</sup>	74.38 ± 0.54 <sup>a</sup>
$\Delta E$	0.00 <sup>b</sup>	4.57 ± 0.29 <sup>a</sup>	3.48 ± 0.44 <sup>a</sup>	4.62 ± 1.41 <sup>a</sup>	1.61 ± 0.14 <sup>ab</sup>

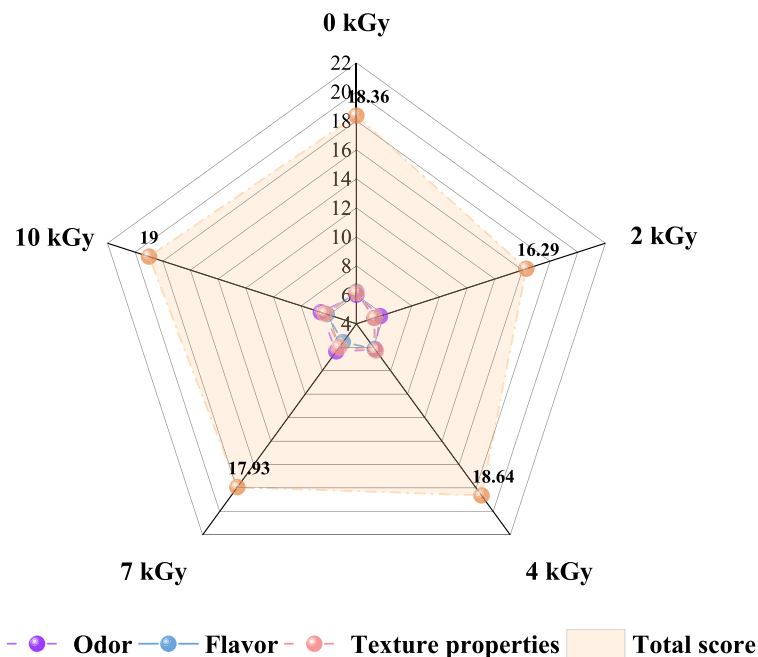
<sup>a-b</sup> different superscript letters indicate significant difference ( $P < 0.05$ ).

cohesiveness, gumminess, chewiness, and resilience of Atlantic cod under 5 doses of E-beam irradiation were given in Table 2. Irradiation treatment caused a significant decrease in hardness and chewiness ( $P <$

0.05). Similar findings were reported by Zhang et al. (2016) that the hardness and chewiness of grass carp surimi were significantly lower in the E-beam irradiated group. The decrease of hardness and chewiness of fish fillets implies a reduction in the firmness of muscle tissue (Xu et al., 2014). Irradiation was reported to induce protein denaturation, favoring proteolysis, and increasing the softness of fish flesh (Rodrigues et al., 2017). Delgado, Pandit, and Zeugolis (2014) have proved collagen degradation in fish fillets after irradiation. Therefore, we speculated that E-beam irradiation caused protein denaturation in Atlantic cod, making the protein more susceptible to pressure effects.

### 3.5. Color

The color attribute is one of the essential parameters evaluating fish freshness and quality. It can be influenced by several factors, such as heme pigments content, oxidation status, ligand formation of heme pigments, and physical characteristics (pH, temperature, processing, storage time) (Qiao, Fletcher, Smith, & Northcutt, 2001). We quantified the color of Atlantic cod fillets by  $L^*$  (lightness),  $a^*$  (redness-greenness), and  $b^*$  (yellowness-blueness) values. Table 3 shows the  $L^*$ ,  $a^*$ ,  $b^*$  values, whiteness, and  $\Delta E$  of Atlantic cod under different E-beam irradiation doses. The results showed the unchanged  $L^*$  values, a significant decrease of  $a^*$  values from  $-1.77 \pm 0.18$  to  $-2.79 \pm 0.32$ ,  $-2.68 \pm 0.03$ ,  $-2.49 \pm 0.12$ ,  $-2.37 \pm 0.10$  after 2–10 kGy E-beam irradiation ( $P < 0.05$ ), and lowered  $b^*$  values under 2 kGy–7 kGy E-beam irradiation (Table 3). Montiel et al. (2013) reported that the decrease in  $a^*$  was attributed to astaxanthin degradation in salmon. Considering the low pigment content and higher TBA value of Atlantic cod after irradiation, we guessed that E-beam led to the browning reaction by oxidizing lipid, which in turn decreased  $a^*$  value in cod muscles (Mahmoud, Nannapaneni, Chang, Wu, & Coker, 2016). Less yellowness in E-beam irradiated groups was also observed by Lee, Ameer, Kim, Chung, and Kwon (2018). The whiteness of the cod samples exhibited an inconspicuous difference between the control and irradiated groups.  $\Delta E$  indicated the significant color changes of Atlantic cod after 2 kGy–7 kGy E-beam irradiation with  $4.57 \pm 0.50$ ,  $3.48 \pm 0.77$ , and  $4.62 \pm 2.43$ , respectively ( $P < 0.05$ ) (Table 3). Although E-beam irradiation brought about the significant color change of Atlantic cod, according to  $\Delta E$ , it is difficult to



**Fig. 3.** Sensory profiles for odor, flavor, and texture properties of Atlantic cod after E-beam irradiation at 0 kGy, 2 kGy, 4 kGy, 7 kGy, and 10 kGy.



discern the color difference by the naked eyes.

### 3.6. Sensory evaluation

Changes in the sensory scores of the odor, flavor, and texture properties of Atlantic cod after E-beam irradiation at 0 kGy, 2 kGy, 4 kGy, 7 kGy, and 10 kGy are shown in Fig. 3. E-beam irradiation did not significantly change the cod samples' odor, flavor, and texture, and the total score of three indexes in each group showed an indistinctive difference ( $P > 0.05$ ). The results indicated that E-beam irradiation did not induce the perception of sensory defects or off-flavors, and it had excellent application potential to keep the safety and quality in Atlantic cod.

## 4. Conclusion

This study demonstrated the effects of 2, 4, 7 10 kGy E-beam irradiation that inactivated SARS-CoV-2 on the composition, texture, color, and sensory attributes of Atlantic cod. It was observed that 10 kGy E-beam irradiation significantly lowered the reducing sugar content, TVB-N content and notably increased moisture and ash content. Although E-beam significantly promoted lipid oxidation of Atlantic cod, TBA values were at an acceptable level. The hardness and chewiness of cod conspicuously reduced after E-beam irradiation, while springiness increased under 4 and 7 kGy doses. The significant color change was shown after 2 kGy–7 kGy E-beam irradiation, manifesting significantly decreased  $a^*$  and  $b^*$  values. It was proved that E-beam irradiation at doses in our work would not affect the sensory evaluation of Atlantic cod. Overall, E-beam irradiation is a promising technology for preventing SARS-CoV-2 contamination and keeping seafood products quality, and 4 kGy E-beam irradiation dose is recommended for further application in the cold-chain seafood industry.

### 4.1. Declaration of interest statement in the conflict of interest

Declarations of interest: none.

### Author statement

**Huilin Yu:** Conceptualization, Methodology, Formal analysis, Writing-original draft. **Junhui Zhang:** Conceptualization, Methodology, Formal analysis.

**Honghao Li:** Methodology.

**Yan Zhao:** Methodology.

**Juan Chen:** Methodology.

**Yang Qiu:** Formal analysis.

**Shengyao Xia:** Formal analysis.

**Jiajin Zhu:** Project administration.

### Acknowledgment

The researchers gratefully acknowledge the grants from Hangzhou Global Scientific and Technological Innovation Center of Zhejiang University (Grant number KC2021ZY0B0003).

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fbio.2022.101803>.

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