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Effect of electron beam irradiation on microbial inactivation, nutritional and quality properties of semi-moist pet foods

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ABSTRACT

The objective of this study was to evaluate the effect of electron beam irradiation (E-beam) on semi-moist pet foods at different absorbed doses. The samples were exposed to e-beam at dose levels of 0, 2.5, 5, and 10 kGy. Then, their effect on microbiological, nutritional, and physicochemical properties was analyzed during 60 days of storage. A dose-dependent effect was observed in bacterial pathogens, especially, 10 kGy sterilized them without further growth throughout the storage period. The nutrient analysis confirmed that 10 kGy of e-beam irradiation did not significantly influence the nutritional components of semi-moist pet foods, ensuring they are nutritionally safe for pets. Irradiated samples showed less changes in quality over time, with lower microbial growth and stable attributes like water activity, pH, and redness (a^*). While samples treated with 10 kGy initially showed more lipid oxidation and protein degradation, these differences were not significant by day 60, suggesting that higher doses did not affect physicochemical properties during storage. In contrast, non-irradiated samples experienced microbial and physicochemical changes over the extended storage. Therefore, we conclude that e-beam irradiation up to 10 kGy can secure microbial safety in semi-moist pet foods, preserving their nutritional and quality attributes for pets' consumption.

1. Introduction

The global pet food market and demand are progressing with an emphasis on safety, nutritional profiles, and quality considerations for pets (Costa et al., 2022). Dry and wet pet foods have dominated the market. However, semi-moist pet foods are also preferred with certain advantages in sensorial quality. Semi-moist pet food consists approximately 25–35 % moisture content on a dry basis (AAFCO, 2003; Adeniyi, 2019; Carrión and Thompson, 2013), which contributes to a desirable soft and chewy texture, enhancing palatability for pets (Niamnuy and Devahastin, 2010; Pibarot et al., 2017). However, it is crucial to acknowledge that semi-moist pet food promotes microbial growth and prolonged storage diminishes its soft texture and shelf life (Deliephan et al., 2023; Pibarot et al., 2017). Further, the appearance and palatability of semi-moist pet foods are enhanced by spraying liquified poultry fat and flavoring to the surface (Lambertini et al., 2016). Nevertheless, it is noteworthy that these ingredients may introduce bacterial pathogens to the manufacturing facility, potentially posing biological hazards (Behravesh et al., 2010). In addition, there is a risk of cross-contamination during the processing and packaging of pet foods (Leiva et al., 2019). Thus, implementing an effective preservation technique for the finished product is essential to ensure its safety without compromising critical quality changes.

Non-thermal technologies, such as high-pressure processing, aseptic packaging, ionizing radiation [electron beam (E-beam), gamma ray, and X-ray irradiation], cold plasma sterilization, and hurdle technology have garnered significant interest in the realm of food preservation (Amit et al., 2017; Tumuluru, 2023). Among them, e-beam irradiation has proven to be a better approach for minimizing the risk of foodborne illnesses, serving as a viable alternative to thermal treatment (Arshad et al., 2020). Also, it does not alter the temperature of processed food, ensuring negligible food quality degradation due to heat (Tahergorabi

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et al., 2012). This process involves accelerating electrons directed at the product (Lewis et al., 2002; Miller, 2006). Notably, high-energy electrons (up to 10 MeV) play a vital role in microbial sterilization (Pillai and Shayanfar, 2018; Tahergorabi et al., 2012), capable of penetrating approximately 8–10 cm in typical food products, leading to the inactivation of foodborne pathogens (Jaczynski and Park, 2003). However, it is vital to note that high-dose irradiation treatment could potentially damage the nutritional profile and physicochemical properties of food (Lung et al., 2015).

In the last decades, e-beam irradiation has been applied to a wide range of semi-moist foods, including beef jerky (Kim et al., 2010); pork jerky (Kang et al., 2012; Kim et al., 2013), and fermented sausages (Lim and Lee, 2007). While many studies have confirmed the effects of e-beam irradiation on semi-moist foods, most of these studies focus on human food. However, there are no studies have reported for semi-moist pet foods. Furthermore, semi-moist pet foods have unique moisture content, composition, and contamination level, affecting microbial survival differently from dry and wet foods (Niamnuy and Devahastin, 2010; Pibarot et al., 2017). Understanding the impact of ionizing radiation on pathogens in these foods is crucial for effective preservation. Considering the effect of e-beam irradiation on similar products, we propose its efficient bactericidal impact, advantageous attributes, and ability to maintain physicochemical integrity in semi-moist pet food applications. Therefore, our objective is to investigate the effect of e-beam irradiation on the decontamination of major pathogenic bacteria (*Salmonella* Typhimurium and *Escherichia coli* O157:H7), as well as the changes in nutritional and physicochemical properties in semi-moist pet foods.

2. Materials and methods

2.1. Sample preparation

Commercial semi-moist pet food was obtained from AT Bio Co. Ltd, Pocheon, Republic of Korea. The samples consisted of flour (40 %), chicken meat (35 %), fish meat (14 %), propylene glycol (5 %), glycerin (5 %), sugar (0.8 %), potassium sorbate (0.15 %), tricalcium phosphate (0.04 %), and food colorant (0.01 %). These samples were cut into uniformly sized slices (approximately 4 × 1.5 × 0.5 cm; 5.00 ± 0.05 g). Samples were placed in sterilized oxygen-impermeable nylon polyethylene/polypropylene bags (2 mL $O_2 \cdot (m^2)^{-1} \cdot 24\ h^{-1}$ at 0 °C, 0.09 mm thickness; Sunkyung Co., Ltd., Seoul, Korea). Both sets of samples were then vacuum packaged and transported to the irradiation center, maintaining at storage condition of 4 °C.

2.2. E-beam irradiation

E-beam irradiation was conducted using an e-beam accelerator (EBILU-10-10, Biomedical Manufacturing Technology Center, Youngcheon, Republic of Korea) with a beam energy of 5 MeV. The beam power and beam current were maintained at 50 kW and 0–7 mA, respectively.

Samples were e-beam irradiated with the target doses of 0, 2.5, 5, and 10 kGy at a conveyor velocities of 15, 8, and 4 m/min, respectively. The samples, with a thickness of 0.5 cm and a density of 1.67 g/cm^3, were arranged in a single layer to optimize irradiation efficiency. This thickness was well within the 5 MeV e-beam's penetration depth (approximately 3 cm in water equivalent for 1 g/cm^3 material), ensuring effective irradiation. The negligible depth dose gradient (<5 %) eliminated the need for internal dose measurements. Alanine pellet dosimeters (0.28 cm thick) were placed on the top and bottom of the samples to verify the delivered dose. Dosimetry measurements were taken using a 104 Electron Paramagnetic Resonance unit (EMS-104; Bruker Instruments Inc., USA). The uncertainty of absorbed dose for each dose was 2.5 ± 0.04 kGy, 5 ± 0.12 kGy, and 10 ± 0.29 kGy, respectively.

Following the successful completion of e-beam irradiation, the samples were immediately transported to the laboratory in an iced box. Prior to storage, an approximately 3 cm incision was made in all sample packs, aligning with the customary storage practice of pet owners. Subsequently, both irradiated and non-irradiated samples were stored at 4 °C under refrigeration condition until further use. The experiment was designed for 60 days at 20-day intervals.

2.3. Bactericidal effect

2.3.1. Bacterial strains and culture preparation

For the pathogens' sterilization test, *S.* Typhimurium (ATCC 13311) and *E. coli* O157:H7 (NCCP 15739) were cultured in nutrient broth (Difco, Becton Dickinson Co., Sparks, MD, USA) and tryptic soy broth (Difco, Becton Dickinson Co.), respectively. Then, each broth was centrifuged at 4001×*g* at 4 °C for 10 min (Combi 514R, Hanil, Incheon, Korea). The supernatant was discarded, and the pellets were washed with a 0.85 % saline solution, repeating this process twice. The pellets were then re-suspended in sterile 0.85 % saline solution at a final concentration of 10^7 to 10^8 colony-forming units (CFU)/mL by measuring the optical density at 600 nm ($OD_{600} = 0.2$).

2.3.2. Inoculation of pathogens

Prior to inoculating the target pathogens, all samples, including controls, were exposed to ultraviolet light for 30 min using a 40 W UV-C lamp with a wavelength of 253.7 nm. This step was performed to sterilize and eliminate endogenous microorganisms, ensuring that microbial counts post-inoculation could be attributed solely to the introduced pathogens. Each pathogenic solution (0.1 mL) of *S.* Typhimurium and *E. coli* O157:H7 was spot inoculated on each slice of semi-moist pet food and air-dried for 1 h to ensure the fixation of microorganisms. Subsequently, the samples were vacuum-packaged in a single layer to ensure complete penetration of e-beam irradiation as described above.

2.3.3. Microbial analysis

Each (5 g) of irradiated and non-irradiated samples were transferred to a sterile stomacher bag containing 45 mL of sterile 0.85 % saline solution. They were then aseptically homogenized for 2 min in a stomacher (Bag Mixer® 400P, Interscience Co, St. Nom la Bretèche, France). Subsequently, the samples were serially diluted in sterile saline (0.85 %) solution, and 0.1 mL of each diluent was added to the xylose lysine deoxycholate agar (Difco, Becton Dickinson Co.), and eosin methylene blue agar (Difco, Becton Dickinson Co.) medium to determine the inactivated counts of *S.* Typhimurium and *E. coli*, respectively. Finally, plates were incubated at 37 °C for 48 h. The number of microorganisms was expressed as colony-forming units per gram (CFU/g) based on the homogenization of the entire 5 g sample, showing the total microbial load of the sample post-inoculation.

2.4. Nutrient analysis

2.4.1. Proximate composition

The moisture, crude protein, crude fat, crude fiber, and ash contents of the non-irradiated and e-beam irradiated semi-moist pet food samples were determined using the official methods of the AOAC International (Shin et al., 2020).

2.4.2. Nutritional profile analysis

The nuclear magnetic resonance (NMR) technique was applied to analyze nutritional compounds of free amino acids, simple sugars, and carboxylic compounds. Polar metabolites were extracted as described by Kwon et al. (2022). Each 5 g of samples were thawed at 4 °C for 24 h. Followed by melting, the samples were homogenized with 0.6 M perchloric acid at 1720×*g* for 30 s (T25 basic, Ika Co., Staufen, Germany). The homogenate was centrifuged at 3000×*g* for 15 min (Continent 512 R, Hanil Co., Incheon, Korea). The supernatant was titrated to 7.0 using potassium hydroxide. Then extract was filtered using filter

paper (No. 1, Whatman International Ltd., Kent, UK) and lyophilized (Freezer dryer 18, Labco Corp., Kansas City, MO, USA). The lyophilized extracts were reconstituted using 1 mL of 20 mM phosphate buffer (pH 7.4) with deuterium oxide containing 1 mM 3-(trimethylsilyl) propionic-2,2,3,3-d4 acid. Subsequently, it was placed in a water bath at 35 °C for 10 min, and centrifuged at 3000×*g* for 15 min. The supernatants were transferred to a microcentrifuge tube and centrifuged at 17,000×*g* for 10 min. Finally, the supernatant was transferred to an NMR tube (5 mm) before NMR analysis.

One-dimensional ^{1}H NMR spectra were recorded in deuterium oxide at 298 K using a Bruker 850 MHz cryo-NMR spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany). The one-dimensional ^{1}H NMR was analyzed using a zg30 (recycle delay of 1s) pulse sequence default in Topspin 3.6.2 (Bruker Biospin GmbH). The pulse sequence was performed using 128 scans, 64 K data points, and a sweep width of 17,007.803 Hz. Chemical shifts (δ) were referenced to the TSP resonance, and the baseline corrections were performed manually. Metabolite peaks (little or no overlap) were identified using the biological magnetic resonance bank (BMRB; bmrb.wisc.edu), the human metabolome database (HMDB; hmdb.ca), standard compounds, and Chenomx NMR suite 7.1 (Chenomx. Edmonton, AB, Canada). The quantification and one-dimensional ^{1}H NMR spectra were developed and processed as described by the method of Kwon et al. (2022). Each metabolite was calculated using 1 mM TSP as the internal standard. The concentrations of the metabolites were quantified using the following equation:

Metabolite concentration = (Numbers of proton (internal standard))/(Numbers of proton (metabolite)) × (Intensity of peak (metabolite))/(Intensity of peak (internal standard)) × Concentration (internal standard)

2.5. Quality attributes

2.5.1. Water activity

The water activity of irradiated and non-irradiated pet food samples was measured using a water activity meter (HygroPalm HP23-AW-A, Rotronic AG, Bassersdorf, Switzerland).

2.5.2. pH

Before analysis, the pH meter was calibrated with standardized buffer solutions (pH 4.01, 7.0, and 9.21) at room temperature. Ground pet food samples (1 g) were homogenized (T25 basic, Ika Co.) with 9 mL of deionized distilled water and centrifuged at 2265×*g* for 10 min at 4 °C (Continent 512R; Hanil Co.). The supernatant was filtered using filter paper (No. 4, Whatman International Ltd.). The pH of the homogenates was measured using a pH meter (Seven2Go S2, Mettler-Toledo International Inc., Schwerzenbach, Switzerland).

2.5.3. Instrumental color

The instrumental color of both the non-irradiated and e-beam irradiated samples was obtained using a colorimeter (CM-5, Konica Minolta Co., Osaka, Japan) with a 13 mm diameter aperture, a D65 light source, and a 2° standard observer throughout the experiment. Prior to analysis, the instrument was calibrated using standard black and white plates. The CIE L^* (lightness), a^* (redness), and b^* (yellowness) were recorded.

2.5.4. 2-Thiobar-bituric acid reactive substance (TBARS)

The TBARS values of the samples were determined following the method described by Lee and Lee (2014) with slight modifications. Initially, 5 g of each sample with 15 mL of distilled water and 50 μL of 7.2 % butylated hydroxytoluene solution was homogenized at 9600 rpm for 30 s using a homogenizer (T25 basic, Ika Co.). Subsequently, 1 mL of the homogenate was mixed with 2 mL of 20 mM 2-thiobarbituric acid in 15 % trichloroacetic acid. The mixture was then placed in a water bath at 90 °C for 15 min, cooled, and centrifuged at 2000×*g* for 10 min (Continent 512 R; Hanil Co). The absorbance of the supernatant was measured at a 532 nm wavelength using a spectrophotometer (SpectroMax M2e, Molecular Devices, Sunnyvale, CA, USA). The TBARS value was calculated as mg malondialdehyde/kg of pet food using a standard curve.

2.5.5. Volatile basic nitrogen (VBN)

The method described by Kim et al. (2020) was adapted to evaluate VBN. Three grams of ground semi-moist pet food samples were mixed with 27 mL of deionized distilled water (1:9) and homogenized at 9600 rpm for 1 min (T25 basic, Ika Co.). The homogenate was then filtered using filter paper (No. 1, Whatman International Ltd.). Subsequently, 1 mL of filtrate and saturated potassium carbonate were added to the outer section of the Conway unit (Sibata Ltd., Sitama, Japan). Following this, 1 mL of 0.01 N boric acid was added to the inner space along with Conway's indicator (0.066 % methyl red: 0.066 % bromocresol green, 1:1; v/v), and the Conway unit was immediately sealed with grease. The samples were placed in an incubator at 37 °C for 1 h, followed by titration with 0.01 N hydrochloric acid, and the VBN value of each sample was calculated.

$$\text{VBN (mg/100 g sample)} = 0.14\ (a - b) \times 5 \times 100$$

Where a is the titration volume of 0.01 N HCl (mL) in the sample and b is the titration volume of 0.01 N HCl (mL) in the blank.

2.6. Statistical analyses

All experiments were carried out in triplicate individually. The data were analyzed using SAS software (Version 9.4, SAS Institute, Inc., Cary, NC, USA) and subjected to one-way analysis of variance with Tukey's test to determine the differences between means at a confidence level of $P < 0.05$.

3. Results and discussion

3.1. Bactericidal effect

In general, the bactericidal effect of ionizing radiation is predominantly linked to the damage inflicted on bacterial DNA by radiation-induced free radicals, and the magnitude of this damage is directly proportional to the irradiation dose (Ahn et al., 2013). Their effect can be varied with different food ingredients (Zheng et al., 2022). The initial microbial contamination of non-treated samples for both *S.* Typhimurium and *E. coli* O157 were undetected level. The samples were then UV-treated for sterilization, however we did not measure the microbial count afterward. In this study, e-beam had a dose-dependent bactericidal effect on both *S.* Typhimurium and *E. coli* O157:H7 inoculated on semi-moist pet foods (Fig. 1a and b; $P < 0.05$). When e-beam was not applied, the initial loads of *S.* Typhimurium and *E. coli* O157:H7 were 6.87 and 7.81 log CFU/g, respectively. However, no microbial growth for both pathogens was observed, when samples were subjected to 10 kGy of e-beam irradiation. Followed by a dose of 5 kGy reduced the viable count of *S.* Typhimurium and *E. coli* O157:H7 by 2.97 and 2.01 log CFU/g, respectively, while the weakest bactericidal effect was found at 2.5 kGy (*S.* Typhimurium: 0.67 log CFU/g; *E. coli* O157:H7: 1.07 log CFU/g). We found similar tendencies when e-beam irradiation was treated on pork jerky, which has similar water content (about 20–28 %) and water activity (<0.60) of semi-moist pet foods (Kang et al., 2012). This dose-dependent relationship of e-beam irradiation has been previously observed that higher doses lead to increased DNA damage due to the augmented generation of free radicals by e-beam (Kim et al., 2014). During the 60-day storage period, the growth of both pathogens was not detected in 10 kGy irradiated samples (Fig. 1a and b). The 2.5 and 5 kGy irradiated samples showed a significant reduction in the detection count of *S.* Typhimurium over the storage period. For *E. coli*, 2.5 and 5 kGy irradiated samples exhibited a significant decline until 40 days and remained constant. The lower growth of both pathogens could also be

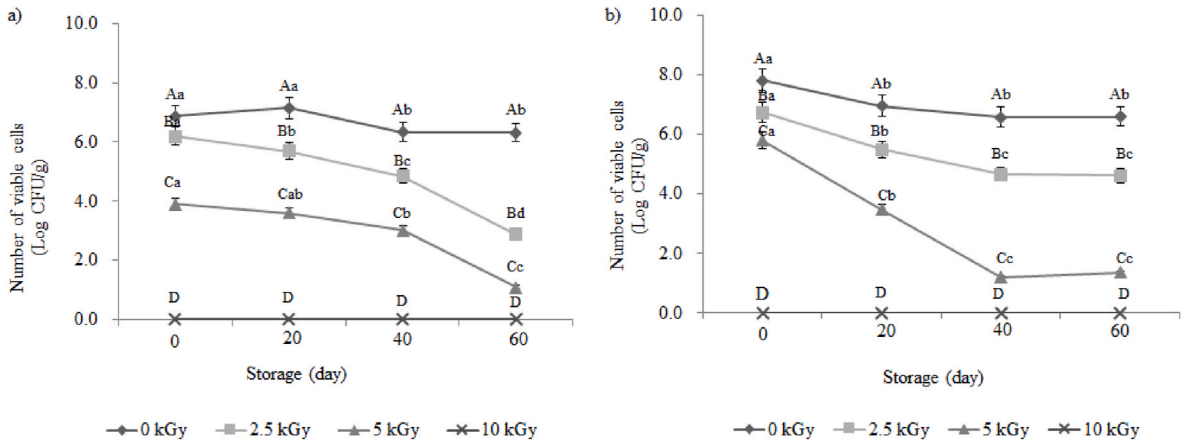

Fig. 1. Effect of electron beam irradiation on the inactivation of *Salmonella* Typhimurium (a) and *Escherichia coli* O175:H7 (b) in pet foods stored at refrigerator condition. $^{A-D}$Different letters indicate significant differences ($P < 0.05$) among different irradiation doses. $^{a-d}$Different letters indicate significant differences ($P < 0.05$) among different storage days within the same treatments.

attributed to the higher e-beam irradiation (Kim et al., 2013). The rapid increase in reactive oxygen species (ROS) can be induced by 10 kGy doses of e-beam irradiation as it can decline the intracellular ATP concentration in bacteria, depolarize the cell membrane, and have a significant effect on intracellular nucleic acid and protein levels, resulting in cell injury and cell death (Chang et al., 2023; Lung et al., 2015). Moreover, non-irradiated samples also exhibited a slightly decreasing tendency for pathogens to survive during storage, possibly by unfavorable growth conditions, such as low water activity, limited nutrients, and the surface topography of the food (Chun et al., 2010). Based on the results, our study suggests that irradiation of up to 10 kGy using e-beam is effective in inhibiting *S.* Typhimurium and *E. coli* O157:H7 below the detection limit in semi-moist pet foods until 60 days.

Meanwhile, the D_{10} values of *S.* Typhimurium and *E. coli* O157:H7 were 1.51 kGy and 1.42 kGy, respectively. When exposed to e-beam irradiation, there was no significant difference observed between pathogens for D_{10} values. The D_{10} value refers to the radiation dose required to inactivate 90 % of a viable microbial population (Smith and Pillai, 2004). The proximity of D_{10} values for both pathogens suggests that they have similar sensitivity under the given experimental conditions ($P > 0.05$). Additionally, radiation sensitivity is another parameter used to define the sensitivity of organisms to the effects of ionizing radiation. Accordingly, *S.* Typhimurium and *E. coli* O157:H7 showed radiation sensitivities of 0.95 and 0.93, respectively, when subjected to e-beam irradiation. Based on the results, it is assumed that the uniformity in their resistance could imply common DNA damage repair processes that affect both pathogens similarly under the influence of e-beam irradiation.

3.2. Nutrient analysis

3.2.1. Proximate composition

Determining the proximate composition of pet foods is vital for selecting a well-balanced diet and ensuring the exact levels of essential nutrients are available (Rolinec et al., 2016). After e-beam was treated onto the samples, no significant difference was found between non-irradiated and irradiated semi-moist pet foods, except for their moisture content (Table 1). These results indicate that up to 10 kGy of e-beam irradiation did not interfere with the nutritional constituents of semi-moist pet foods, as the doses used were below the threshold to cause significant changes in the chemical bonds and molecular structures of the main components (Zhang et al., 2023). Furthermore, it has been evident that irradiation of food with a maximum dose of 10 kGy poses no nutritional problems (Sahoo et al., 2023). Additionally, samples irradiated with doses of 5 kGy and 10 kGy of e-beam exhibited a significant increase in moisture content compared to non-irradiated samples, possibly due to increased reactions between free radicals and other molecules, leading to water production as a by-product (Pan et al., 2020). Considering the results, e-beam irradiation at doses of 5 kGy and 10 kGy did not affect the nutritional profile of the samples, indicating an effective preservation technique for semi-moist pet food.

Table 1
Proximate composition (%) of electron beam irradiated semi-moist pet foods on day 0.

Items	Irradiation dose (kGy)			
	0	2.5	5	10
Moisture	18.58 ± 0.49^{B}	18.55 ± 0.37^{B}	20.21 ± 0.95^{A}	19.39 ± 0.21^{AB}
Crude protein	13.33 ± 0.14^{A}	13.06 ± 0.28^{A}	13.07 ± 0.14^{A}	13.37 ± 0.03^{A}
Crude fat	6.53 ± 0.89^{A}	7.00 ± 0.40^{A}	6.08 ± 0.66^{A}	8.57 ± 0.10^{A}
Crude fiber	0.33 ± 0.06^{A}	0.40 ± 0.16^{A}	0.20 ± 0.11^{A}	0.14 ± 0.16^{A}
Ash	1.14 ± 0.05^{A}	1.05 ± 0.11^{A}	1.19 ± 0.12^{A}	1.19 ± 0.09^{A}

Mean ± Standard deviation (n = 3).
^{A,B}Different letters indicate significant differences ($P < 0.05$) among different irradiation doses.

3.2.2. Nutritional profile

The NMR technique was conducted to evaluate the nutritional composition changes induced by e-beam irradiation at various doses in semi-moist pet foods. This ensures that irradiated pet foods are nutritious for our pets while also preserving food quality (Panseri et al., 2022). A total of 17 compounds (nine amino acids, three monosaccharides, two disaccharides, and three carboxylic compounds) were characterized from a nutritional perspective (Table 2). As a result, no significant differences were found between the nutritional compounds of non-irradiated and irradiated samples. Interestingly, the nine essential amino acids detected (methionine, lysine, glutamate, glycine, histidine, aspartate, valine, isoleucine, and alanine) are highly required for pets for the efficacy of their metabolic processes (McCusker et al., 2014). Besides ribose and fucose were the only components that showed a significant increase regardless of the dose levels, suggesting that high doses of irradiation might lead to the breakdown of polysaccharides like starch, resulting in the production of monosaccharides without significantly affecting their overall nutritional value (Byun et al., 2007). This clearly shows that doses of 5 kGy and 10 kGy of e-beam irradiation maintain the nutritional components of semi-moist pet foods without significant changes throughout the storage period.

Table 2
Nutritional compounds (μg/g) of electron beam irradiated semi-moist pet foods at different doses along with storage periods of 0 and 60 days using nuclear magnetic resonance (NMR) technique.

Compounds	Irradiation dose (kGy)				Storage (day)		P value		
	0	2.5	5	10	0	60	Dose	Day	Dose*Day
Amino acids									
Methionine	0.10 ± 0.06^{A}	0.12 ± 0.02^{A}	0.13 ± 0.03^{A}	0.14 ± 0.04^{A}	0.14 ± 0.02^{A}	0.11 ± 0.03^{A}	NS	NS	NS
Lysine	0.20 ± 0.08^{A}	0.26 ± 0.03^{A}	0.23 ± 0.06^{A}	0.27 ± 0.08^{A}	0.25 ± 0.05^{A}	0.23 ± 0.07^{A}	NS	NS	NS
Glutamate	0.12 ± 0.06^{A}	0.16 ± 0.02^{A}	0.15 ± 0.03^{A}	0.17 ± 0.04^{A}	0.16 ± 0.03^{A}	0.14 ± 0.03^{A}	NS	NS	NS
Glycine	9.86 ± 0.39^{A}	12.91 ± 0.16^{A}	10.81 ± 0.56^{A}	12.96 ± 0.72^{A}	12.88 ± 0.49^{A}	10.39 ± 0.12^{A}	NS	NS	NS
Histidine	0.02 ± 0.02^{A}	0.03 ± 0.01^{A}	0.02 ± 0.01^{A}	0.02 ± 0.01^{A}	0.02 ± 0.01^{A}	0.02 ± 0.01^{A}	NS	NS	NS
Aspartate	0.03 ± 0.03^{A}	0.05 ± 0.01^{A}	0.05 ± 0.01^{A}	0.06 ± 0.02^{A}	0.05 ± 0.01^{A}	0.05 ± 0.02^{A}	NS	NS	NS
Valine	0.06 ± 0.03^{A}	0.08 ± 0.01^{A}	0.07 ± 0.02^{A}	0.08 ± 0.02^{A}	0.08 ± 0.01^{A}	0.06 ± 0.01^{A}	NS	NS	NS
Isoleucine	0.15 ± 0.07^{A}	0.20 ± 0.02^{A}	0.18 ± 0.04^{A}	0.20 ± 0.04^{A}	0.20 ± 0.04^{A}	0.17 ± 0.02^{A}	NS	NS	NS
Alanine	0.20 ± 0.09^{A}	0.26 ± 0.04^{A}	0.24 ± 0.07^{A}	0.26 ± 0.07^{A}	0.26 ± 0.05^{A}	0.21 ± 0.08^{A}	NS	NS	NS
Monosaccharides									
Ribose	2.67 ± 0.14^{B}	4.63 ± 0.44^{AB}	4.76 ± 0.93^{AB}	7.10 ± 0.25^{A}	4.33 ± 0.67^{A}	5.26 ± 0.55^{A}	**	NS	NS
Galactose	79.20 ± 0.06^{A}	106.16 ± 0.54^{A}	96.44 ± 0.46^{A}	111.97 ± 0.54^{A}	107.08 ± 0.31^{A}	89.81 ± 0.06^{A}	NS	NS	NS
Fucose	1.02 ± 0.49^{B}	1.57 ± 0.14^{AB}	1.45 ± 0.36^{AB}	2.02 ± 0.65^{A}	1.60 ± 0.03^{A}	1.43 ± 0.09^{A}	*	NS	NS
Disaccharides									
Maltose	0.46 ± 0.21^{A}	0.65 ± 0.10^{A}	0.66 ± 0.26^{A}	0.89 ± 0.46^{A}	0.74 ± 0.15^{A}	0.59 ± 0.25^{A}	NS	NS	NS
Sucrose	2.46 ± 0.10^{A}	3.39 ± 0.49^{A}	2.93 ± 0.75^{A}	4.01 ± 0.19^{A}	3.62 ± 0.78^{A}	2.78 ± 0.88^{A}	NS	NS	NS
Carboxylic acids									
Fumarate	0.03 ± 0.02^{A}	0.04 ± 0.00^{A}	0.03 ± 0.01^{A}	0.04 ± 0.02^{A}	0.04 ± 0.01^{A}	0.03 ± 0.01^{A}	NS	NS	NS
Malate	0.09 ± 0.03^{A}	0.11 ± 0.02^{A}	0.10 ± 0.02^{A}	0.11 ± 0.06^{A}	0.11 ± 0.02^{A}	0.10 ± 0.04^{A}	NS	NS	NS
Lactate	0.87 ± 0.38^{A}	1.09 ± 0.13^{A}	0.99 ± 0.25^{A}	1.08 ± 0.23^{A}	1.11 ± 0.02^{A}	0.91 ± 0.03^{A}	NS	NS	NS

Mean ± Standard deviation (n = 3).
^{A,B}Different letters indicate significant differences ($P < 0.05$) among different irradiation doses or storage periods.
*: $P < 0.05$; **: $P < 0.01$; NS: Not significant.

3.3. Quality attributes

3.3.1. Water activity

The water activity is a key factor in preventing microbial growth, often serving as the primary parameter influencing food stability, modulating microbial response, and determining the types of microorganisms present in food (Tapia et al., 2020). When e-beam irradiation was treated on semi-moist pet foods, we found no significant differences in their water activity by e-beam irradiation (Table 3). Meanwhile, during 60 days of storage, their values varied from 0.70 to 0.74 on day 0 and tended to a significant downward trend with values ranging between 0.66 and 0.70. It is predicted that such moisture loss from samples due to evaporation during storage, along with chemical changes such as oxidation and binding of water molecules, might affect the availability of water molecules, reducing water activity (Deliephan et al., 2023).

On the other hand, the low water activity of semi-moist pet foods may support the reduction in pathogen growth during storage (Fig. 1a and b). Pathogenic bacteria are unable to grow and multiply when water activity is 0.85 or lower (Tapia et al., 2020). Başer and Yalcin (2017) reported that it is recommended to follow pasteurization, pH control, or add preservatives to semi-moist pet foods to control microbial activity, even though its water activity ranges from 0.60 to 0.80. Further, the water activity of samples irradiated with doses of 5 kGy and 10 kGy decreased significantly during storage; however, the overall change in water activity was minimal and within the typical range. The slight reduction in water observed in samples irradiated with doses of 5 kGy and 10 kGy might be attributed to a decrease in the water holding capacity of proteinaceous materials in semi-moist pet foods (Samant et al., 2021). However, no significant changes in protein content were observed in semi-moist pet foods (Table 1), ensuring that the physicochemical attributes were not significantly altered by high doses of e-beam irradiation.

Table 3
Changes in the water activity and pH of electron beam irradiated pet foods at different doses during cold storage.

	Storage (day)	Irradiation dose (kGy)			
		0	2.5	5	10
Water activity	0	0.73 ± 0.00^{ABa}	0.70 ± 0.05^{Bb}	0.71 ± 0.04^{Bb}	0.74 ± 0.02^{Aa}
	20	0.75 ± 0.03^{Aa}	0.72 ± 0.02^{Aab}	0.71 ± 0.02^{Ab}	0.71 ± 0.01^{Ab}
	40	0.73 ± 0.00^{Aa}	0.74 ± 0.01^{Aa}	0.74 ± 0.02^{Aa}	0.74 ± 0.01^{Aa}
	60	0.66 ± 0.03^{Bb}	0.69 ± 0.06^{Ab}	0.70 ± 0.08^{Ab}	0.68 ± 0.01^{Ab}
pH	0	6.50 ± 0.01^{Ab}	6.50 ± 0.02^{Aa}	6.50 ± 0.01^{Aa}	6.49 ± 0.02^{Aa}
	20	6.51 ± 0.01^{Ab}	6.51 ± 0.02^{Aa}	6.51 ± 0.01^{Aa}	6.51 ± 0.00^{Aa}
	40	6.53 ± 0.01^{Aa}	6.52 ± 0.00^{BCa}	6.51 ± 0.01^{BCa}	6.50 ± 0.01^{Ca}
	60	6.49 ± 0.01^{Ab}	6.49 ± 0.01^{Ab}	6.49 ± 0.01^{Aa}	6.50 ± 0.02^{Aa}

Mean ± Standard deviation (n = 3).
$^{A-C}$Different letters indicate significant differences ($P < 0.05$) among different irradiation doses.
^{a,b}Different letters indicate significant differences ($P < 0.05$) among different storage days within the same treatment.

3.3.2. pH

The slightly acidic pH of semi-moist pet foods helps maintain microbiological stability, structure, and quality (Niamnuy and Devahastin, 2010). In our study, the pH of non-irradiated semi-moist pet foods significantly decreased over the storage period, while that of samples irradiated with 5 kGy and 10 kGy of e-beam irradiated samples was not significantly changed (Table 3). This result may suggest that e-beam irradiation can minimize pH changes by reducing the production of various microbial byproducts, leading to a lower pH (Chen et al., 2023). However, regardless of e-beam irradiation, all samples had a higher pH close to neutral (ranged 6.49–6.53), suggesting that e-beam irradiation regulates microbial activity and maintains the structural integrity of semi-moist pet foods, ensuring pH stability (Deliephan et al., 2023). Thus, doses of 5 kGy and 10 kGy of e-beam irradiation are a

reliable method for sterilizing semi-moist pet foods without affecting their quality.

3.3.3. Instrumental color

The importance of measuring color in pet foods primarily relates to the perception and acceptance of the product by both pet owners and pets (Hobbs, 2019; Watson et al., 2023). The non-irradiated sample had a higher L^* value than the irradiated sample on day 0; however, samples treated with 10 kGy of e-beam irradiation had significantly higher L^* values than the non-irradiated and other treatments at the end of the experiment (Table 4). Regarding a^* value, a dose-dependent effect was found at day 0, in contrast, a^* values were comparatively lower in the non-irradiated samples than in irradiated ones. In terms of b^* values, the 10 kGy irradiated samples exhibited the highest value throughout the experiment period. However, although the values of color were significantly varied, the color of both irradiated and non-irradiated samples was not visually changed until the end of the experiment. This resembles the stability of semi-moist pet foods over irradiation and storage conditions, possibly due to their low water activity (Table 3). The minimal changes in water activity did not result in any significant alterations in the optical properties of the semi-moist pet foods (Mathlouthi, 2001). This led to fewer or no quality changes in semi-moist pet food irradiated at high doses. Therefore, it is evident that different doses of e-beam irradiation have a limited effect on the alteration of meat pigmentation and the complex molecular structures of cereal flour in semi-moist pet foods, contributing to the color attributes.

3.3.4. 2-Thiobar-bituric acid reactive substance (TBARS)

Ionizing radiation can promote the formation of free radicals and accelerate lipid oxidation (Zheng et al., 2022). Free radicals breakdown hydroperoxides and yield distinctive flavor compounds, which directly influences the quality and acceptability of irradiated foods (Jin et al., 2012). Therefore, the TBARS method was used to estimate secondary lipid oxidation products, represented as mg MDA/kg of pet food (Non-irradiated: 1.19–1.69; Irradiated: 1.26–2.04 mg MDA/kg). In this study, when semi-moist pet food was treated with e-beam, the highest TBARS value was observed in 10 kGy irradiated sample, followed by 2.5, 5 kGy, and the non-irradiated had the lowest value ($P < 0.05$; Fig. 2). The present results could be attributed to the production of ROS by e-beam irradiation treatment with increasing irradiation doses (Chang et al., 2023). Tian et al. (2013) outlined that high doses are associated with overproduced ROS ($O_2^{\cdot-}$ and H_2O_2) causing lipid peroxidation and leading to the accumulation of MDA, such a trend was observed in our study. Additionally, ionizing radiation can degrade compounds like fats and vitamins, but it can also release bioactive compounds previously bound to other molecules, leading to increased measurable content. The dose used in our study may have caused minor structural changes that enhanced compound release without significant degradation. Meanwhile, after 60 days of storage, there was no significant difference between the irradiated samples (Fig. 2). The increased TBARS levels in both irradiated and non-irradiated samples are influenced by the food matrix and storage conditions (Zheng et al., 2022). The increase is attributed to the reaction of dissolved oxygen in pet foods with lipid compounds, generating more lipid oxidation products and contributing to the enhanced TBARS values over the storage period (Arshad et al., 2019; Chen et al., 2023). The study implies that, regardless of the irradiation dose, e-beam irradiation may influence the dynamics of lipid oxidation in pet food, leading to similar TBARS values during extended storage. Hence, varying e-beam doses do not notably affect alterations in lipid compounds in semi-moist pet foods. This suggests that quality parameters remain unaltered even with doses of 10 kGy of e-beam irradiation in semi-moist pet foods.

3.3.5. Volatile basic nitrogen (VBN)

In general, VBN evaluation aims to experimentally measure protein degradation and the release of ammonia and nitrogenous compounds by microbial action (Karthik et al., 2010). During 60 days of storage, the changes in VBN were assessed for irradiated and non-irradiated semi--moist pet foods (Fig. 3). A significant difference was observed between irradiated and non-irradiated samples for VBN until day 40. The higher VBN in irradiated samples was associated with an increase in protein breakdown by free radicals generated by e-beam irradiation (Kim et al., 2013). However, this distinction disappeared by day 60 and significantly

Table 4
Changes in the color of electron beam irradiated pet foods at different doses during cold storage.

Color attributes	Storage (day)	Irradiation dose (kGy)			
		0	2.5	5	10
L^*	0	48.47 ± 0.53^{Aa}	44.89 ± 0.38^{Ba}	46.21 ± 0.13^{Bab}	44.98 ± 0.98^{Bb}
	20	43.49 ± 0.19^{Bc}	43.35 ± 0.47^{Bb}	46.80 ± 0.52^{Aa}	44.67 ± 0.43^{ABb}
	40	44.56 ± 0.12^{Cb}	45.17 ± 0.09^{Ca}	46.27 ± 0.09^{Bab}	49.08 ± 0.47^{Aa}
	60	43.82 ± 0.13^{Cc}	44.69 ± 0.11^{Ba}	44.76 ± 0.09^{Bb}	48.55 ± 0.11^{Aa}
a^*	0	4.06 ± 0.05^{Ca}	4.13 ± 0.04^{Ca}	4.60 ± 0.05^{Ba}	5.46 ± 0.07^{Aa}
	20	3.34 ± 0.01^{Bc}	2.91 ± 0.03^{Cc}	3.02 ± 0.08^{Cc}	3.51 ± 0.05^{Ad}
	40	3.89 ± 0.03^{BCb}	3.75 ± 0.15^{Cb}	4.05 ± 0.04^{Bb}	4.32 ± 0.03^{Ab}
	60	4.11 ± 0.04^{Aa}	3.73 ± 0.04^{Db}	3.95 ± 0.03^{Bb}	3.82 ± 0.02^{Cc}
b^*	0	15.41 ± 0.15^{Bc}	15.82 ± 0.12^{ABc}	16.23 ± 0.03^{Ab}	16.24 ± 0.43^{Ac}
	20	17.18 ± 0.16^{BCa}	16.72 ± 0.31^{Cb}	18.13 ± 0.59^{ABa}	18.54 ± 0.55^{Ab}
	40	17.07 ± 0.05^{Ca}	17.54 ± 0.06^{Ba}	16.67 ± 0.08^{Db}	19.49 ± 0.06^{Aa}
	60	16.54 ± 0.01^{BCb}	16.47 ± 0.06^{Cb}	16.59 ± 0.02^{Bb}	18.22 ± 0.05^{Ab}

Mean ± Standard deviation (n = 3).
$^{A-D}$Different letters indicate significant differences ($P < 0.05$) among different irradiation doses.
$^{a-d}$Different letters indicate significant differences ($P < 0.05$) among different storage days within the same treatment.

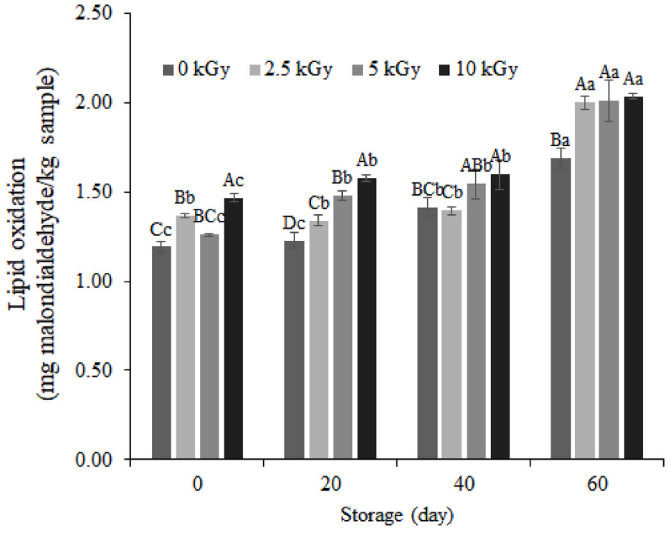

Fig. 2. Changes in TBARS of electron beam irradiated pet foods at different doses (0, 2.5, 5, and 10 kGy) over 60 days of refrigeration storage period. $^{A-D}$Different letters indicate significant differences ($P < 0.05$) among different irradiation doses. $^{a-c}$Different letters indicate significant differences ($P < 0.05$) among different storage days within the same treatment.

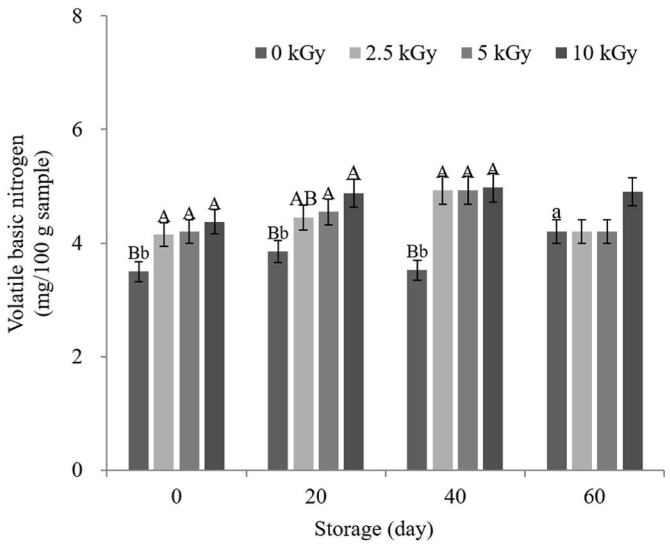

Fig. 3. Changes in VBN of electron beam irradiated pet foods at different doses (0, 2.5, 5, and 10 kGy) over 60 days of refrigeration storage period. $^{A-B}$Different letters indicate significant differences ($P < 0.05$) among different irradiation doses. $^{a-b}$Different letters indicate significant differences ($P < 0.05$) among different storage days within the same treatment.

increased values for VBN during storage were observed exclusively only in non-irradiated samples. In contrast, increasing doses of e-beam irradiation minimized the changes in VBN over the storage period possibly by less microbial activity by e-beam irradiation. It can be reasonably concluded that protein breakdown in irradiated samples did not continue during storage due to less microbial growth, which can be attributed to damage to their nucleic acids by e-beam irradiation (Karthik et al., 2010). This also suggests that e-beam irradiation at both low and high doses effectively curtails microbial spoilage and stabilizes VBN levels in semi-moist pet foods over an extended storage period.

4. Conclusion

The study aimed to assess the effect of e-beam irradiation on the bactericidal effect and nutritional and quality attributes of semi-moist pet foods. No microbial growth observed for both *S.* Typhimurium and *E. coli* O157:H7 throughout the storage period occurred at a dose of 10 kGy, signifying the effectiveness of high doses of e-beam in microbial sterilization of semi-moist pet foods. Nutrient analysis declared that a 10 kGy dose of e-beam irradiation maintains the nutritional components without altering the overall quality of semi-moist pet foods. During storage, samples irradiated at 10 kGy exhibited a significant reduction in water activity, an increase in b^* values, and no significant changes in pH, revealing the microbiological and physicochemical stability of semi-moist pet foods at a dose of 10 kGy of e-beam irradiation exposure. Although TBARS and VBN were significantly higher in the 10 kGy irradiated samples at the beginning, no significant differences were found on day 60, implying that the dose of 10 kGy still did not adversely affect the quality of semi-moist pet foods. Therefore, it is recommended to use e-beam irradiation up to 10 kGy for the microbial reduction and preservation of nutritional and quality attributes in semi-moist pet foods. Further studies investigating the detection of flavor and taste compounds in e-beam-irradiated semi-moist pet foods would be beneficial for assessing their impact on quality and safety.

CRediT authorship contribution statement

Anand Kumar Sethukali: Writing – original draft, Visualization, Methodology, Formal analysis. **Hyun Jung Lee:** Writing – review & editing, Validation, Methodology, Conceptualization. **Dongbin Park:** Writing – review & editing, Methodology. **Hyun Jun Kim:** Writing – review & editing, Validation. **Hag Ju Lee:** Writing – review & editing, Methodology. **Cheorun Jo:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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.

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Data availability

Data will be made available on request.

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