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Understanding key volatiles in semi-moist pet foods treated with electron beam and X-ray irradiation

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ABSTRACT

In this study, semi-moist pet food samples were irradiated with electron beam and X-rays at 0, 2.5, 5, and 10 kGy. Samples were stored under refrigeration, and volatile compounds were analyzed on days 0 and 60. X-ray irradiation produced more volatile compounds than electron beam, likely due to differences in free radical formation and radiation-matter interaction characteristics. A dose of 10 kGy promoted oxidative reactions, increasing aldehydes, ketones, and carboxylic compounds, while 5 kGy resulted in more alkanes, indicating hydrocarbon breakdown and distinct flavor development. Notably, 1,3-di-*tert*-butylbenzene increased consistently at 10 kGy compared to controls, suggesting formation through packaging antioxidant degradation and migration into the fat matrix, supporting its potential role as an irradiation- and packaging-related marker in semi-moist pet foods. Storage conditions significantly influenced volatile compound changes, emphasizing the need for further investigation. These results highlight the importance of optimizing irradiation dose and storage conditions to ensure the safety and quality of semi-moist pet foods, with volatile compound profiles used as chemical indicators of potential sensory changes relevant to product quality.

1. Introduction

Semi-moist pet foods are becoming popular worldwide due to their fresh-like sensory attributes (Kiprotich et al., 2023). They appeal to many pet owners for being ready to eat, highly nutritious, microbiologically safe, and having a prolonged shelf life (Qiu et al., 2019; Severini et al., 2008). Nevertheless, proper preservation techniques are essential to maintain their sensory and quality characteristics over time. Traditional methods such as thermal treatments and chemical preservatives are typically used in manufacturing of semi-moist pet foods. However, they have limitations, such as nutrient loss and changes in sensory properties (Niamnuy and Devahastin, 2010).

In recent years, non-thermal preservation techniques, particularly irradiation, have gained attention for their ability to eliminate pathogens and extend shelf life without compromising product quality (Kundu et al., 2014; Li et al., 2015). Irradiation with ionizing energy is commonly used to enhance the physical, chemical, and biological properties of materials and commercial products (Marshall, 2012). It is a safe and effective method for food preservation and is also recognized as a sustainable technology to protect packaged foods from environmental recontamination (Kong et al., 2017; Trindade et al., 2010). Among them, electron beam and X-ray irradiation have emerged as promising options for food preservation compared to gamma ray irradiation (Arshad et al., 2020; Moosekian et al., 2014). They offer the advantage of easy switching between electron and X-ray modes and can be turned off without producing nuclear waste (Pillai and Shayanfar, 2017; Van Calenberg et al., 1999). In addition, both technologies have been extensively used in the human food industry for sterilization, preservation, and nutritional studies (Begum et al., 2020; Ham et al., 2017; Jung et al., 2015; Kang et al., 2012; Kim et al., 2010, 2013; Lei et al., 2023). For instance, electron beam irradiation has been investigated for its effects on protein structure and functionality (Liu et al., 2023; Pi et al., 2021). The selected irradiation dose range (0–10 kGy) in this study is consistent with international safety evaluations by the FAO/IAEA/WHO Joint Expert Committee, which concluded that food and feed products irradiated at average doses up to 10 kGy are toxicologically safe and

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nutritionally adequate for microbial pathogen control (FAO/IAEA/WHO, 1981; IAEA, 2002).

However, there has been little research focused on their application in pet food, particularly semi-moist pet foods. This is a notable gap in the field of food irradiation research, given the growing importance of pet food safety and quality. Despite the growing use of irradiation in various food industries, research on its application in semi-moist pet foods remains limited. Addressing this gap is critical for improving preservation techniques not only for pet foods but also for high-moisture food systems in general. Therefore, understanding the effects of these irradiation methods on semi-moist pet foods is crucial for ensuring their product quality.

Ionizing radiations can impact the flavor volatiles in irradiated food stuffs as it can change the functional groups of volatile compounds (Jo et al., 2018). When food components are exposed to irradiation, various chemical reactions occur, including oxidation, degradation, or formation of volatile compounds, resulting in alterations in product aroma and flavor profiles (Brewer, 2009). Thus, analyzing volatile compounds offers valuable insights into irradiation-induced changes in semi-moist pet foods, aiding in the search for optimized preservation strategies. These changes depend on absorbed dose, facility type, and presence or absence of oxygen and temperature as well as sample type (Panseri et al., 2022). However, the specific effects of irradiation on volatile compounds in semi-moist pet foods have been relatively understudied. Addressing this research gap is essential for enhancing the safety and quality of semi-moist pet foods. Also, in a parallel study, we applied electron beam and X-ray irradiation to semi-moist pet foods and found that both types of irradiation induced lipid oxidation and protein degradation. These changes likely contributed to alterations in the volatile compounds of the semi-moist pet foods (data not shown). Therefore, the research aims to investigate and compare the effects of different doses of electron beam and X-ray irradiation on changes in volatile compounds in semi-moist pet foods during storage.

2. Materials and methods

2.1. Sample preparation

Commercial semi-moist pet food was purchased 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%). The semi-moist pet food matrix used in this study was characterized by an overall moisture content of 19.34 ± 0.32% (wet basis) at day 0 across all samples. The corresponding water activity averaged 0.73 ± 0.01, reflecting the intermediate-moisture nature of this product category. In this context, the term "semi-moist pet food" refers to a commercial classification based on typical moisture content and water activity ranges rather than a treatment dependent parameter.

These samples were cut into uniformly sized slices (approximately 4 × 1.5 × 0.5 cm; 5.00 ± 0.05 g). The pet food samples were divided into two sets for two irradiation sources. Samples designated for volatile compound analysis 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. Electron beam and X-ray irradiation

Electron beam irradiation was conducted using an electron beam accelerator (EBILU-10-10, Biomedical Manufacturing Technology Center, Youngcheon, Republic of Korea). The beam energy was set at 5 MeV, with the beam power and current were maintained at 50 kW and 0-7 mA, respectively. Samples were individually irradiated with the absorbed doses of 0, 2.5, 5, and 10 kGy at 0 °C. The absorbed dose was controlled by adjusting the conveyor speed, with conveyor velocities of 15, 8, and 4 m/min. All target doses were delivered in a single pass through the irradiation zone by adjusting the conveyor speed. X-ray irradiation was performed using an electron accelerator-based X-ray irradiation system (linear electron accelerator; Mevex, Canada; Model MBLO-30/3000). X-rays were generated using a tantalum (Ta) target as the X-ray convertor. During irradiation, the beam energy and average beam current were set to 7 MeV and 2 mA, respectively. Samples were transported through the irradiation zone using a conveyor system operated at a speed of 1 m/min under ambient temperature conditions. All irradiations were conducted under identical geometric conditions.

For both irradiation modalities, samples were arranged in a single layer with a thickness of 0.5 cm and a density of approximately 1.67 g/cm3. Dose uniformity across the sample thickness was verified using alanine pellet dosimeters placed on both the top and bottom surfaces of the samples. Dosimetry analysis confirmed a negligible depth dose gradient (<5%), indicating uniform dose delivery throughout the sample. Absorbed dose levels were further verified using alanine pellet dosimeters placed at representative positions on the sample packs and analyzed by 104 Electron Paramagnetic Resonance Unit (EMS-104; Bruker Instruments Inc., USA). The dosimetry system was calibrated against international standards (IAEA, 2002), and the measured values closely matched the intended doses, with uncertainties within ±0.04 kGy (2.5 kGy), ±0.12 kGy (5 kGy), and ±0.29 kGy (10 kGy). This ensured reliable and standardized dose delivery for both irradiation modalities across all treatments in the present study.

Following the successful completion of irradiation, the samples were immediately transported to the laboratory in an icebox, maintaining a temperature of about 4 °C. A standardized small incision was intentionally introduced into the package to simulate post-processing package opening and/or handling-related package compromise during consumer use. The incision was applied uniformly across all treatments and storage times to ensure consistent oxygen exposure. This approach was used to evaluate the oxidative stability of irradiated semi-moist pet foods under controlled post-irradiation air exposure conditions, rather than under fully sealed storage. Subsequently, both irradiated and non-irradiated samples were stored at 4 °C under refrigeration condition until further use. Analysis was conducted on days 0 and 60.

2.3. Volatile compounds analysis

The analysis of volatile compounds in irradiated and non-irradiated pet food samples was performed by solid-phase microextraction and gas chromatography-mass spectrometry (SPME-GC-MS). Five grams of irradiated and non-irradiated pet food samples were placed into a 20-mL headspace vial and sealed with a PTFE-faced silicone septum. The samples were incubated at 40 °C for 5 min, and then, a 65 μm thick polydimethylsiloxane/divinylbenzene fiber (Supelco Inc., Bellefonte, PA, USA) was exposed to the headspace of the vial for 60 min. The volatile compounds were desorbed in the injection port of the GC (Trace 1310, Thermo Fisher Scientific, Waltham, MA, USA) at 270 °C in splitless mode. Helium was used as the carrier gas at a flow rate of 2 mL/min, and volatile compounds were separated using a fused silica capillary column (DB-Wax, 60 m × 0.25 mm i. d., and 0.50 μm film thickness; Agilent Technologies Inc., Santa Clara, CA, USA). The GC oven was programmed as follows: initial temperature of 40 °C, subsequently increased to 180 °C at a rate of 5 °C/min, then increased to 200 °C at a rate of 2 °C/min and held for 5 min, and then increased to a final temperature of 240 °C at a speed of 10 °C/min and held for 10 min. The column was directly coupled to a triple quadrupole mass spectrometer (TSQ 8000, Thermo Fisher Scientific, USA) operating in the electron ionization mode at 70 eV and 250 °C. Mass spectra were obtained with a scan ranging from 35 to 550 m/z at intervals of 0.2 s. The identification of volatile compounds was performed by comparing their mass spectra with those of the National Institute of Standards and Technology (NIST) mass spectral library.

2.4. Statistical analyses

All samples of e-beam and X-ray irradiated semi-moist pet foods for the determination of the volatile compound analysis were performed. The data were evaluated using SAS software (Version 9.4, SAS Institute, Inc., Cary, NC, USA). A three-way ANOVA model was fitted with fixed effects of irradiation type (EB and XR), absorbed dose (0, 2.5, 5, and 10 kGy), storage period (0 and 60 days), and their interactions. Normality and homogeneity were checked using residual diagnostics. Volatile compound data were obtained using a semi-quantitative approach, statistical analyses were applied to evaluate relative differences among treatments under identical analytical conditions. When significant effects were detected ($p < 0.05$), mean comparisons were performed using Tukey's post-hoc test. Results are reported as mean ± standard deviation (SD). Significant interactions are described in the text and illustrated in Figures.

3. Results and discussion

3.1. Volatile compounds

In our preliminary study, semi-moist pet foods were subjected to e-beam and X-ray irradiation at different doses and evaluated over 60 days at 20-day intervals for bacterial pathogen reduction. Results showed that increasing the dose significantly improved pathogen reduction. Notably, samples irradiated at a 10 kGy dose with both e-beam and X-ray exhibited a significant reduction in *Salmonella* Typhimurium and *Escherichia coli* O157:H7 in semi-moist pet foods throughout the 60-day storage period (Data not shown). A number of 44 volatile compounds were identified in all samples (Supplementary Table 1). The compounds were classified into 12 chemical categories including 4 aldehydes, 7 alcohol, 14 alkenes, 2 ketones, 5 carboxylic acid, 1 ether, 1 organofluoride, 1 furans, 1 aromatic compounds, 1 para cresols, and 7 other compounds.

In general, total amount of volatile compounds was increased with both e-beam and X-ray treatment (Fig. 1). It has been evident that Ahn et al. (2000) reported irradiation enhanced the volatile compounds production in foods compared to non-irradiated samples. Increasing doses in irradiation can generate highly reactive species that accelerate oxidative processes in the proteins and lipids, producing many secondary compounds and other volatile compounds (Bliznyuk et al., 2022; Zianni et al., 2023). The total amount of volatile compounds generated by X-ray irradiation was higher compared to electron beam irradiated samples. This difference may be attributed to differences in irradiation modality and radiation-matter interaction characteristics, which influence free radical generation and subsequent chemical reactions in the food matrix. Further, the rise in volatile compounds during storage indicates oxidative reactions triggered by oxygen exposure over time (Zianni et al., 2023). These outcomes suggested that X-ray irradiation at higher dosage levels and extended storage contribute to more volatile compounds production in semi-moist pet foods. These differences between electron beam and X-ray may reflect distinct radiation-matter interaction characteristics associated with the two irradiation modalities (Pillai and Shayanfar, 2017). This enhances radical formation in the food matrix, promoting lipid and protein oxidation during storage (Bliznyuk et al., 2022). Such physical distinctions are consistent with the higher levels of oxidation-derived volatiles observed under X-ray at 10 kGy. These outcomes suggest that higher-dose X-ray irradiation, while effective in microbial reduction, also increases oxidative by-products, affecting volatile compound profiles. This underscores the importance of tailored irradiation conditions for semi-moist pet foods and potentially other high-moisture food products. It was known that the effect of irradiation could be varied depending on the specific class of volatile compounds (Jo et al., 2018). Each result will be discussed in the following sections.

3.1.1. Aldehydes and ketones

The presence of aldehydes and ketones are used as indicators of lipid and protein oxidation induced by irradiation which affects volatile compounds in foods (Zheng et al., 2022). Both aldehydes (hexanal and benzaldehyde) and ketones (1-hydroxy-2-propanone) production were significantly pronounced in X-ray irradiated samples than in electron beam irradiated samples (Table 1). A dose-dependent effect was observed for hexanal, nonanal, benzaldehyde, and 1-hydroxy-2-propanone. Samples irradiated at 10 kGy exhibited the highest concentrations of aldehydes and 1-hydroxy-2-propanone compared to other treatments. Further, the production of aldehydes was gradually increased during the storage period, except for octanal (Table 1). Ionizing radiation induces fatty acids break down into alcohols, which, in turn, break down into aldehydes and ketones (Bliznyuk et al., 2022; Zianni et al., 2023). Hexanal and nonanal are formed through the ω-oxidation process from linoleic and oleic acids, respectively (Ayseli and Coskun, 2022). Moreover, saturated aldehydes (hexanal, nonanal) and benzaldehyde are considered as marker for lipid oxidation by irradiation (Ahn et al., 1998, 2000; Feng and Ahn, 2016). Additionally, Strecker degradation is involved in aldehydes and ketones formation by the breakdown of specific amino acid (Feng and Ahn, 2016). The presence of these compounds is often associated with off-flavor and off-aroma development in foods which can negatively influence aroma and sensory quality (Samant et al., 2021). The odor threshold levels of hexanal and benzaldehyde in food are 0.0045 ppm and 0.35 ppm, respectively (Belitz et al., 2004). Irradiation can lead to the formation of compounds such as aldehydes and ketones, which are commonly associated with changes in aroma characteristics and chemical stability in food systems (Hu, 2016). In the study, variations in the relative abundance of these compounds were observed among irradiation treatments, reflecting differences in irradiation conditions and subsequent storage-related oxidative processes. These findings highlight potential

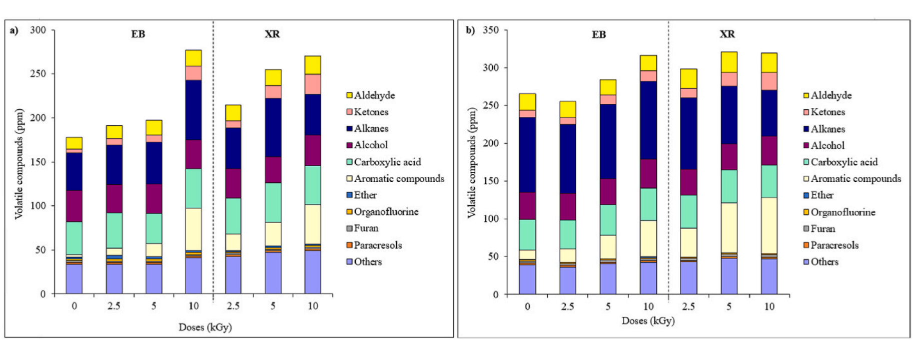

Fig. 1. Stacked bar chart of volatile compounds in semi-moist pet foods irradiated by electron beam and X-ray at different doses during the storage period of day 0 (a) and day 60 (b). EB, electron beam; XR, x-ray.

Table 1
Concentration of aldehydes and ketones in electron beam and X-ray irradiated semi-moist pet foods during storage periods of 0 and 60 days (in ppm).

Compounds	Type of irradiation		Absorbed dose levels (kGy)				Storage period (days)	
	EB	XR	0	2.5	5	10	0	60
Aldehyde								
Hexanal	9.64 ± 0.01^{b}	11.09 ± 0.02^{a}	9.24 ± 0.03^{c}	10.36 ± 0.09^{b}	10.71 ± 0.18^{ab}	11.15 ± 0.03^{a}	7.31 ± 0.77^{b}	13.42 ± 0.05^{a}
Octanal	1.56 ± 0.17	1.58 ± 0.04	1.65 ± 0.01^{a}	1.53 ± 0.06^{b}	1.52 ± 0.14^{b}	1.60 ± 0.07^{a}	1.67 ± 0.08^{a}	1.47 ± 0.01^{b}
Nonanal	1.21 ± 0.05	1.23 ± 0.06	0.84 ± 0.12^{d}	1.16 ± 0.04^{c}	1.34 ± 0.03^{b}	1.55 ± 0.08^{a}	1.17 ± 0.05^{b}	1.27 ± 0.06^{a}
Benzaldehyde	5.83 ± 0.12^{b}	7.78 ± 0.09^{a}	5.90 ± 0.14^{c}	6.89 ± 0.17^{b}	7.21 ± 0.12^{ab}	7.24 ± 0.03^{a}	6.60 ± 0.17^{b}	7.02 ± 0.12^{a}
Ketones								
1-Hydroxy-2-propanone	7.99 ± 0.26^{b}	11.49 ± 0.31^{a}	4.52 ± 0.04^{d}	6.68 ± 0.11^{c}	10.41 ± 0.08^{b}	17.35 ± 0.09^{a}	10.14 ± 0.21	9.35 ± 0.05
2-Methyl-3-octanone	2.32 ± 0.16^{b}	2.83 ± 0.15^{a}	2.42 ± 0.08	2.70 ± 0.22	2.72 ± 0.88	2.45 ± 0.03	0.54 ± 0.01^{b}	4.61 ± 0.02^{a}

EB, electron beam; XR, x-ray.
Mean ± Standard deviation (n = 5).
[a-d]Different superscripts letters within a row indicate significant differences among treatments ($p < 0.05$).

implications for sensory quality based on volatile profiles, without implying direct effects on animal acceptance or health. The results clearly indicate that X-ray irradiation at a 10 kGy dose leads to higher lipid and protein oxidation in semi-moist pet foods, resulting in a shorter shelf life during storage. The findings highlight that X-ray irradiation at 10 kGy accelerates oxidative reactions, increasing lipid and protein oxidation markers, which can negatively affect sensory quality and shelf life. However, irradiation doses such as 5 kGy demonstrated a more favorable impact, supporting their potential application in food systems where both safety and sensory quality are critical. This demonstrates that adjusting irradiation dose is key for optimizing semi-moist pet food quality, as moderate doses can effectively reduce pathogens while minimizing off-flavour formation, supporting a balance between microbial safety and chemical indicators of sensory quality.

3.1.2. Alkanes

The highest proportions of volatile compounds observed among classes was alkanes. The alkanes are tested for irradiated lipid-containing foods, both meat-based and non-meat-based products, especially 2-alkylcyclobutanones and aliphatic hydrocarbons (Blanch et al., 2009). Based on results, most of the alkanes were significantly influenced by X-ray irradiation compared to electron beam irradiated semi-moist pet foods. Among alkanes, 3,7-dimethyloctane was not significantly affected by either irradiation sources (Table 2). Comparatively, semi-moist pet foods irradiated at 5 kGy exhibited higher relative alkanes levels than other treatments. All detected alkane compounds showed a significant increase during the storage period. Alkanes are long chain hydrocarbons; irradiation can make different cleavage point of carbon molecules and generate more alkanes. The primary mechanism is that the formation of hydrocarbon free radicals from alkyl chains (Merritt et al., 1967). In the present study, higher alkane levels observed under X-ray irradiation are discussed in terms of irradiation-induced hydrocarbon cleavage rather than penetration-related dose effects. Additionally, it is assumed that 5 kGy was actively involved in breaking of hydrocarbon molecules rather than other chemical reactions (Ahn et al., 2000). Alkanes are typically tasteless and odorless, so they do not contribute to the flavor or aroma of semi-moist pet foods. However, high concentrations of alkanes might mask other volatile compounds, potentially altering overall aroma perception (Adenot and Abdelhakim, 2022). On the positive side, alkanes are inert, meaning they do not easily react with other substances, which can help preserve the stability and shelf life of semi-moist pet foods (Adenot and Abdelhakim, 2022). Based on the results, while X-ray irradiation at 5 kGy increased alkane formation, the observed changes primarily reflect shifts in the volatile profile rather than the generation of compounds commonly associated with off-flavours. Controlled irradiation ensures microbial safety while preserving sensory quality, supporting its role in optimizing semi-moist pet foods.

3.1.3. Alcohols

The alcohols are representative volatile compounds for identifying oxidative changes and microbial preservation (Stefanova et al., 2010). The results showed that X-ray treated samples had higher concentrations of alcohol compounds than electron beam treated semi-moist pet foods, with the exception of ethanol (Table 3). Ethanol and 1-pentanol were higher in non-irradiated samples than irradiated samples. Additionally, 1-tridecanol and 1-octen-3-ol showed an increasing trend in terms of increasing dose levels. During storage, 1-hexanol, 1-octen-3-ol, and

Table 2
Concentration of alkanes in electron beam and X-ray irradiated semi-moist pet foods during storage periods of 0 and 60 days (in ppm).

Compounds	Type of irradiation		Absorbed dose levels (kGy)				Storage period (days)	
	EB	XR	0	2.5	5	10	0	60
2,2,6-Trimethyloctane	2.12 ± 0.08^{a}	1.88 ± 0.06^{b}	2.14 ± 0.04^{a}	2.08 ± 0.08^{a}	2.08 ± 0.13^{a}	1.72 ± 0.07^{b}	1.43 ± 0.02^{b}	2.58 ± 0.07^{a}
2,2,7,7-Tetramethyloctane	1.06 ± 0.04^{b}	1.30 ± 0.02^{a}	1.12 ± 0.03^{b}	1.11 ± 0.18^{b}	1.29 ± 0.07^{a}	1.19 ± 0.09^{ab}	0.88 ± 0.21^{b}	1.48 ± 0.05^{a}
2,2,4,6,6-Pentamethylheptane	8.20 ± 0.06^{a}	7.98 ± 0.16^{b}	8.40 ± 0.19^{a}	8.21 ± 0.21^{a}	8.24 ± 0.04^{a}	7.51 ± 0.05^{b}	5.27 ± 0.13^{b}	10.91 ± 0.10^{a}
Decane	4.13 ± 0.06^{a}	3.42 ± 0.02^{b}	3.63 ± 0.04^{ab}	3.53 ± 0.02^{b}	4.00 ± 0.03^{a}	3.93 ± 0.06^{a}	3.60 ± 0.01^{b}	3.94 ± 0.08^{a}
3,7-Dimethyloctane	7.43 ± 0.07	7.44 ± 0.03	7.65 ± 0.08^{a}	7.44 ± 0.01^{a}	7.63 ± 0.07^{a}	7.02 ± 0.16^{b}	4.83 ± 0.04^{b}	10.04 ± 0.10^{a}
2,6,10-Trimethyldodecane	1.83 ± 0.02^{b}	2.07 ± 0.06^{a}	1.88 ± 0.04^{b}	1.85 ± 0.10^{b}	2.02 ± 0.02^{b}	2.06 ± 0.10^{a}	1.20 ± 0.07^{b}	2.70 ± 0.16^{a}
2,6,8-Trimethyldecane	3.66 ± 0.03^{b}	3.86 ± 0.08^{a}	3.72 ± 0.16^{b}	3.67 ± 0.15^{b}	3.91 ± 0.06^{a}	3.75 ± 0.01^{b}	2.44 ± 0.09^{b}	5.08 ± 0.11^{a}
Hexadecane	1.29 ± 0.11^{a}	1.22 ± 0.04^{b}	1.20 ± 0.09^{c}	1.12 ± 0.04^{d}	1.45 ± 0.18^{a}	1.26 ± 0.03^{b}	0.38 ± 0.10^{b}	2.14 ± 0.03^{a}
2-Methyl decane	12.15 ± 0.15^{b}	12.70 ± 0.15^{a}	12.42 ± 0.04	12.33 ± 0.02	12.70 ± 0.01	12.25 ± 0.04	8.03 ± 0.21^{b}	16.82 ± 0.35^{a}
2,3,5-Trimethyldecane	3.66 ± 0.11^{b}	3.86 ± 0.05^{a}	3.72 ± 0.09^{b}	3.67 ± 0.09^{b}	3.91 ± 0.01^{a}	3.75 ± 0.35^{b}	2.44 ± 0.09^{b}	5.08 ± 0.03^{a}
Tetradecane	3.78 ± 0.05^{b}	4.02 ± 0.03^{a}	3.84 ± 0.01^{b}	3.84 ± 0.35^{b}	4.02 ± 0.02^{a}	3.90 ± 0.06^{ab}	2.57 ± 0.81^{b}	5.23 ± 0.15^{a}
2,3,6,7-Tetramethyloctane	1.06 ± 0.01^{b}	1.30 ± 0.05^{a}	1.12 ± 0.35^{b}	1.11 ± 0.11^{b}	1.29 ± 0.03^{a}	1.19 ± 0.08^{ab}	0.88 ± 0.03^{b}	1.48 ± 0.06^{a}
5-Ethyl-2,2,3-trimethylheptane	8.20 ± 0.24^{b}	9.83 ± 0.01^{a}	8.89 ± 0.15^{b}	8.98 ± 0.18^{b}	9.47 ± 0.09^{a}	8.74 ± 0.16^{b}	5.99 ± 0.02^{b}	12.05 ± 0.01^{a}
Dodecane	3.38 ± 0.35^{a}	2.07 ± 0.33^{b}	2.49 ± 0.08^{c}	2.61 ± 0.07^{bc}	2.93 ± 0.54^{a}	2.88 ± 0.14^{ab}	2.48 ± 0.08^{b}	2.98 ± 0.16^{a}

EB, electron beam; XR, x-ray.
Mean ± Standard deviation (n = 5).
[a-d]Different superscripts letters within a row indicate significant differences among treatments ($p < 0.05$).

Table 3
Concentration of alcohols in electron beam and X-ray irradiated semi-moist pet foods during storage periods of 0 and 60 days (in ppm).

Compounds	Type of irradiation		Absorbed dose levels (kGy)				Storage period (days)	
	EB	XR	0	2.5	5	10	0	60
Ethanol	7.68 ± 0.16^{a}	6.44 ± 0.06^{b}	7.56 ± 0.07^{a}	6.84 ± 0.23^{ab}	7.21 ± 0.08^{ab}	6.63 ± 0.23^{b}	6.99 ± 0.17	7.13 ± 0.09
1-(2-Ethoxyproxy)-2-Propanol	1.70 ± 0.05^{b}	1.83 ± 0.18^{a}	1.68 ± 0.29^{b}	1.72 ± 0.12^{b}	1.74 ± 0.18^{b}	1.92 ± 0.21^{a}	1.80 ± 0.02^{a}	1.73 ± 0.43^{b}
1-Pentanol	9.26 ± 0.06	9.28 ± 0.22	10.42 ± 0.09^{a}	9.20 ± 0.04^{b}	7.86 ± 0.33^{c}	9.59 ± 0.17^{b}	9.94 ± 0.03^{a}	8.60 ± 0.44^{b}
1-Hexanol	6.78 ± 0.28	6.82 ± 0.13	7.15 ± 0.25^{a}	6.98 ± 0.34^{b}	6.68 ± 0.21^{c}	6.39 ± 0.08^{d}	6.72 ± 0.12^{b}	6.88 ± 0.02^{a}
1-Tridecanol	0.54 ± 0.18^{b}	0.68 ± 0.24^{a}	0.18 ± 0.11^{d}	0.37 ± 0.54^{c}	0.70 ± 0.19^{b}	1.25 ± 0.34^{a}	0.59 ± 0.27	0.62 ± 0.80
1-Octen-3-ol	6.58 ± 0.11^{b}	7.50 ± 0.04^{a}	6.69 ± 0.30^{c}	6.92 ± 0.57^{bc}	7.06 ± 0.79^{b}	7.50 ± 0.38^{a}	5.41 ± 0.39^{b}	8.67 ± 0.23^{a}
1-Heptanol	2.00 ± 0.09^{b}	2.11 ± 0.08^{a}	2.17 ± 0.22^{a}	2.01 ± 0.13^{b}	1.86 ± 0.14^{c}	2.18 ± 0.17^{a}	1.99 ± 0.06^{b}	2.12 ± 0.01^{a}

EB, electron beam; XR, x-ray.
Mean ± Standard deviation (n = 5).
[a-d]Different superscripts letters within a row indicate significant differences among treatments ($p < 0.05$).

1-heptanol exhibited a significant increase. Irradiation generated free radical's breakdown hydrogen peroxides and produce lower molecular compounds including alcohols (Zheng et al., 2022). The breakdown rates are different from irradiation source due to variations in dose rate and energy (Zheng et al., 2022). Microbial fermentation induced the production of alcohols, especially ethanol, in which, it was higher in non-irradiated samples than irradiated samples. The alcohols can be precursors to malondialdehyde, a secondary product of lipid oxidation, indicates extensive lipid peroxidation (Feng and Ahn, 2016), as its levels increase in conjunction with the progression of oxidation, which can be measured by TBARS. In addition, 1-heptanol and 1-tridecanol, byproducts of lipid oxidation in stored pet foods, are commonly associated with rancid or tallowy aroma notes that may negatively influence sensory quality (Fan et al., 2018; Xu et al., 2019). Besides, ethanol and 1-pentanol contribute alcoholic and balsamic aromas to foods, and at high concentrations, such alcohols are known to impart undesirable aroma characteristics in food systems (Belitz et al., 2004; Bonkohara et al., 2016; Dong et al., 2015). The production of these compounds suggests that irradiation and storage promoted chemical changes within the food matrix, which may have implications for oxidative stability and shelf-life characteristics. As per the results, the shelf life of semi-moist pet foods were gradually declining regardless of alcohol productions during extended storage period. Dose-dependent changes in alcohol formation show that optimized irradiation conditions may help microbial safety with chemical stability, while minimizing irradiation-induced alterations to the volatile profile.

3.1.4. *Carboxylic compounds*

The carboxylic compounds contribute to the flavor, antioxidant activity, and chemical stability of irradiated foods (Brewer, 2009; Nawar, 1986). X-ray irradiation contributed to the generation of more carboxylic compounds in semi-moist than electron beam irradiation. Non-irradiated samples exhibited lower concentrations of carboxylic compounds than irradiated samples, with 10 kGy irradiated samples showing higher concentrations than other treatments (Table 4). Butanoic acid recorded the highest concentration in non-irradiated and 2.5 kGy irradiated samples. This compound exhibited a dose-dependent effect and demonstrated a significant reduction during the storage period. In irradiated samples, butanoic acid was formed by decarboxylation and deamination of α-carbon moiety from amino acids through the actions of ozone produced from oxygen by irradiation (Equation (1); Ahn et al., 2016). For control samples, the butanoic acid is a saturated fatty acid resulted by the hydrolysis of animal fat and plant oils. It has a strong, distinctive smell often described as rancid butter (Bonkohara et al., 2016), which are commonly associated with strong rancid aroma at elevated concentrations. However, it also acts as a preservative by inhibiting the growth of certain bacteria and fungi, thus extending the shelf life of pet foods (Dwidar et al., 2012). X-ray irradiation increased carboxylic compounds, but dose effects were limited, indicating preserved flavour development and stability. These findings support irradiation as a practical method to ensure safety while maintaining quality in semi-moist pet foods.

3.1.4.1. *Decarboxylation*

$$RCH(NH_2)COOH + O_3 + O_2 + H_2O \rightarrow RCHO + CO_2 + NH_3 + H_2O_2 + O_2$$

3.1.5. *Aromatic compounds*

Ionizing radiations can more affect the aroma profile of treated foodstuffs with direct interactions. Direct interactions occur with matrix components, i.e., biomolecules and water molecules, breaking covalent bonds such as C–S–H, C–O–H, and C–N–H, with consequent production of free radicals and other species (Zianni et al., 2023). Aromatic compounds were predominantly contributed by X-ray irradiation rather than electron beam irradiation. The predominant aromatic volatile compound was 1,3-di-*tert*-butylbenzene, meanwhile they showed an increasing trend regardless of increasing dose levels (Table 4). Moreover, their concentration significantly increased during the storage

Table 4
Concentration of carboxylic acids and aromatic compounds in electron beam and X-ray irradiated semi-moist pet foods during storage periods of 0 and 60 days (in ppm).

Compounds	Type of irradiation		Absorbed dose levels (kGy)				Storage period (days)	
	EB	XR	0	2.5	5	10	0	60
Carboxylic compounds								
2-Methoxyacetic acid	1.29 ± 0.04^{a}	1.22 ± 0.01^{b}	1.20 ± 0.20^{c}	1.12 ± 0.03^{d}	1.45 ± 0.18^{a}	1.27 ± 0.01^{b}	0.38 ± 0.12^{b}	2.14 ± 0.36^{a}
Propanoic acid	2.95 ± 0.22^{b}	3.22 ± 0.46^{a}	2.84 ± 0.35^{c}	3.03 ± 0.08^{b}	3.11 ± 0.06^{b}	3.37 ± 0.05^{a}	3.11 ± 0.39	3.06 ± 0.14
Butanoic acid	25.10 ± 0.36^{b}	26.70 ± 0.39^{a}	24.67 ± 0.14^{c}	25.77 ± 0.18^{b}	25.78 ± 0.23^{b}	27.42 ± 0.04^{a}	26.23 ± 0.18^{a}	25.59 ± 0.23^{b}
2-Methyl-Hexanoic acid	5.34 ± 0.27^{b}	5.57 ± 0.05^{a}	5.27 ± 0.07^{b}	5.42 ± 0.68^{b}	5.37 ± 0.43^{b}	5.77 ± 0.21^{a}	5.49 ± 0.04	5.42 ± 0.27
4-Methyl_Pentanoic_acid	5.22 ± 0.15^{b}	5.57 ± 0.22^{a}	5.08 ± 0.42^{b}	5.36 ± 0.11^{b}	5.22 ± 0.47^{b}	5.93 ± 0.57^{a}	5.40 ± 0.03	5.40 ± 0.26
Aromatic compounds								
1,3-di-*tert*-butylbenzene	24.62 ± 0.35^{b}	42.31 ± 0.07^{a}	18.95 ± 0.26^{d}	20.79 ± 0.57^{c}	34.79 ± 0.24^{b}	59.34 ± 0.03^{a}	20.88 ± 0.38^{b}	46.05 ± 0.49^{a}

EB, electron beam; XR, x-ray.
Mean ± Standard deviation (n = 5).
[a-d]Different superscripts letters within a row indicate significant differences among treatments ($p < 0.05$).

period. This storage-related increase observed in control samples indicates that the formation of 1,3-di-*tert*-butylbenzene is not exclusively irradiation-driven and may also rise from time-dependent packaging additive degradation and migration processes. While this increase during storage limits its use as a definitive irradiation marker, the distinct pattern of increase observed in irradiated samples suggests that it could still be indicative of irradiation, provided that its production due to storage alone is carefully considered. The formation of aromatic compounds results from reactions between free radicals and chemical components in the food matrix. These compounds may serve as markers of irradiation when their specific occurrence or concentration is linked to ionizing radiation exposure (Blanch et al., 2009). In fact, the study of Kim et al. (2005) reported that this compound was identified as a volatile marker to detect irradiated beef extract powder. Mechanistically, irradiation generates primary reactive species (·OH, e_aq^-, and H·) via water hydrolysis (step 1-2). These radicals attack *tert*-butyl-containing antioxidants such as (BHT) embedded in polythene/polypropylene packaging, producing phenoxy radicals (step 3). Subsequent radical-induced demethylation yields 2,4-di-*tert*-butylphenol (step 4), which can undergo deoxygenation and radical arrangements to form 1, 3-di-*tert*-butylbenzene (step 5). Additionally, radical coupling routes involving *tert*-butyl and aryl radicals may also contribute (step 6-7) (Krzymien et al., 2001). Being a small, lipophilic volatile, this compound can readily migrate from the packaging into the fat-rich pet food matrix (Lee et al., 2004). Especially, its concentration was highest in 10 kGy samples and present at substantially lower levels in control samples, supporting its potential utility as an irradiation-associated, packaging-derived indicator, provided that storage related contributions are carefully considered.

3.1.5.1. Initiation (radiolysis)

1) $H_2O \rightarrow \cdot OH + e_aq^- + H\cdot$

2) $RH + \cdot OH \rightarrow R\cdot + H_2O$

3.1.5.2. Antioxidant activation (BHT)

3) 2,6-di-*tert*-butyl-4-methylphenol (BHT) $\cdot OH/\ e_aq^- \rightarrow$ BHT-O· + H_2O /OH^- (phenoxy radical formation)

3.1.5.3. Side chain cleavage (demethylation). 4) BHT-O· → 2,4-di-*tert*-butylphenol + $\cdot CH_3$ (radical-induced demethylation)

3.1.5.4. Deoxygenation/rearrangement to volatile marker

5) 2,4-di-*tert*-butylphenol → 1,3-di-*tert*-butylbenzene + ·OH/ H_2O

6) Ar· + $(CH_3)_3C\cdot \rightarrow$ Ar-tBu

7) Ar-tBu + $(CH_3)_3C\cdot \rightarrow$ 1,3-di-*tert*-butylbenzene + H· (dehydroxylation / rearrangement)

Although, the amount of 1,3-di-*tert*-butylbenzene in irradiated samples was extremely higher, a trace amount of this compound was found in control samples. This is also in agreement with the findings of Caja et al. (2008) in the study of identifying irradiated marker in ground beef concerned on both 1,3-di-*tert*-butylbenzene and 2-dodecylcyclobutanone, and also in Shim et al. (2009) in medicinal herb of *Paeoniae Radix*. X-ray irradiation at 10 kGy produced higher levels of aromatic compounds, likely due to both extensive chemical reactions in the food matrix and radiolytic degradation of packaging antioxidants, with migration and accumulation enhanced during storage. In contrast, moderate doses preserved acceptable aroma in pet foods.

3.1.6. Other compounds

Further, electron beam irradiation significantly increased the levels of ether and para-cresols, while X-ray irradiation led to a significant increase in organofluorine and furan. Regarding irradiation dose, organofluroine showed a dose-dependent effect, while no significance was found for pentyl-furan (Table 5). Ether and organoflurouine gradually decreased during the storage period, while para cresols and furans increased during storage. It is assumed that electron beams directly deposit energy into the material, while X-rays indirectly interact through ionization processes. These differences may result in distinct chemical outcomes. Additionally, post storage condition may also contribute for the formation of different chemical compounds in irradiated and non-irradiated semi-moist pet foods. Pentyl-furan has an undesirable green bean-like aroma formed due to the oxidation of linoleic acid and can cause off-flavors, potentially negatively impacting the sensory quality of semi-moist pet foods (Dong et al., 2015). Its presence indicates the oxidation and degradation of fats and oils, signaling reduced quality, freshness, and shelf life of semi-moist pet foods. Edible fats are incorporated into pet food formulations as an energy source and to enhance textural and sensory attributes; however, during processing and storage, these added fats may undergo oxidative reactions, influencing volatile compound formation and overall product quality (Kiprotich et al., 2023; Lin et al., 1998). Moreover, lipid oxidation is a time-dependent degenerative chemical phenomenon, results in the formation of volatile compounds that alter the sensory characteristics and diminish the nutritional value of pet foods (Hołda and Głogowski, 2016; Morelli et al., 2021). Therefore, proper pet food storage is essential to slow chemical deterioration, and appropriate irradiation doses reduce unwanted compounds while maintaining safety and quality in semi-moist pet foods.

4. Conclusion

Irradiation conducted a complex role in the flavor volatiles of semi-moist pet foods. While 10 kGy increased off-flavor compounds like hexanal and benzaldehyde, 5 kGy induced a more favorable balance. Alkanes, which are inert compounds, may not contribute to off-flavors, even helping preserve flavor stability. In addition, certain aldehydes and ketones at controlled levels contribute to aroma complexity, with descriptors such as nutty or toasted notes reported in food systems. Aromatic compounds such as 1,3-di-*tert*-butylbenzene reflected irradiation- and packaging related chemical changes, with implications for volatile profiles rather than direct sensory outcomes.

Taken together, moderate-dose irradiation at 5 kGy effectively balances microbial safety with the sensory quality of semi-moist pet foods, while higher doses may introduce oxidative changes that compromise sensory attributes. Palatability was not directly evaluated in this study, and sensory implications were inferred from volatile compound profiles. These findings underscore the potential of irradiation technologies in preserving not only pet foods but also other high-moisture food systems. Optimizing irradiation conditions in combination with controlled storage is essential for ensuring quality and extending shelf life. By clarifying the role of irradiation-induced oxidative mechanisms, this study supports the development of improved preservation strategies and provides practical guidance for industry: moderate irradiation doses optimize safety and sensory quality, ensuring product integrity and quality, thereby supporting industry confidence and consumer trust.

CRediT authorship contribution statement

Anand Kumar Sethukali: Writing – original draft, Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Dongbin Park:** Methodology, Investigation, Formal analysis. **Cheorun Jo:** Writing – original draft, Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Hyun Jung Lee:** Writing – original draft, Writing – review & editing, Validation, Data curation,

Table 5
Concentration of other volatile compounds detected in electron beam and X-ray irradiated semi-moist pet foods during storage periods of 0 and 60 days (in ppm).

Compounds	Type of irradiation		Absorbed dose levels (kGy)				Storage period (days)	
	EB	XR	0	2.5	5	10	0	60
Ether								
2,5,8,11,14-Pentaoxapentadecane	1.72 ± 0.23[a]	0.69 ± 0.14[b]	1.35 ± 0.39[a]	1.44 ± 0.23[a]	1.17 ± 0.16[ab]	0.86 ± 0.09[b]	2.22 ± 0.12[a]	0.19 ± 0.07[b]
Organofluorine								
1-Benzyloxy-2,4-Difluorbenzene	2.00 ± 0.18[a]	1.76 ± 0.06[b]	1.61 ± 0.15[c]	1.80 ± 0.11[bc]	1.98 ± 0.15[ab]	2.12 ± 0.03[a]	2.10 ± 0.11[a]	1.66 ± 0.44[b]
Furan								
2-Pentylfuran	2.06 ± 0.39[b]	2.38 ± 0.23[a]	2.18 ± 0.18	2.21 ± 0.03	2.21 ± 0.36	2.28 ± 0.42	1.76 ± 0.96[b]	2.69 ± 0.12[a]
Para cresols								
2,4,6-Trimethylphenol	2.05 ± 0.16[a]	1.98 ± 0.06[b]	2.03 ± 0.61[b]	2.03 ± 0.07[b]	2.09 ± 0.04[a]	1.91 ± 0.31[c]	1.95 ± 0.10[b]	2.08 ± 0.16[a]
Others								
Trimethylamine	16.49 ± 0.17[b]	17.95 ± 0.08[a]	14.74 ± 0.52[c]	16.13 ± 0.08[c]	18.20 ± 0.11[b]	19.80 ± 0.27[a]	17.21 ± 0.29	17.23 ± 0.26
Decycltegclycol	0.82 ± 0.12	0.84 ± 0.06	0.36 ± 0.31[d]	0.69 ± 0.29[c]	0.97 ± 0.01[b]	1.30 ± 0.02[a]	0.92 ± 0.01[a]	0.74 ± 0.00[b]
1,2,4-Triazole,4-(N-(2-hydroxyethyl))	1.43 ± 0.15[a]	0.34 ± 0.30[b]	1.17 ± 0.01[a]	1.09 ± 0.04[a]	0.88 ± 0.03[b]	0.42 ± 0.03[c]	1.69 ± 0.71[a]	0.09 ± 0.01[b]
3-Methyl-1-butenyl	13.40 ± 0.29	13.51 ± 0.31	13.73 ± 0.01[a]	13.17 ± 0.03[b]	13.15 ± 0.05[b]	13.76 ± 0.07[a]	13.67 ± 0.04[a]	13.24 ± 0.06[b]
3-Trifluroacetoxytetradecane	0.32 ± 0.42[b]	0.45 ± 0.04[a]	0.07 ± 0.00[c]	0.07 ± 0.01[c]	0.51 ± 0.02[b]	0.90 ± 0.30[a]	0.38 ± 0.05	0.39 ± 0.06
2-Ethoxy-ethanol	5.83 ± 0.08[b]	7.78 ± 0.15[a]	5.90 ± 0.02[c]	6.89 ± 0.42[b]	7.21 ± 0.04[ab]	7.23 ± 0.02[a]	6.60 ± 0.02[b]	7.02 ± 0.15[a]
Ethanol, 2-nitro propionate ether	2.95 ± 0.26[b]	3.22 ± 0.03[a]	2.84 ± 0.24[c]	3.03 ± 0.26[b]	3.11 ± 0.66[b]	3.37 ± 0.21[a]	3.11 ± 0.10	3.06 ± 0.27

EB, electron beam; XR, x-ray.
Mean ± Standard deviation (n = 5).
[a-d]Different superscripts letters within a row indicate significant differences among treatments ($p < 0.05$).

Conceptualization.

Declaration of competing interest

The authors have declared no conflict of interest.-

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Appendix A. supplementary data

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Data availability

Data will be made available on request.

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