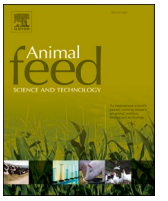
Animal Feed Science and Technology 325 (2025) 116344

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci



Potential of X-ray irradiation for pathogen inactivation in semi-moist pet food and changes in nutritional and physicochemical qualities

Anand Kumar Sethukali [a,b], Hyun Jung Lee [a], Dongbin Park [a], Hyun-Jun Kim [a], Azfar Ismail [a], Jae-Kyung Kim [c], Cheorun Jo [a,d,*]

[a] Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea
[b] Department of Animal Science, Faculty of Agriculture, University of Jaffna, Kilinochchi 44000, Sri Lanka
[c] Advanced Radiation Technology Center, Korea Atomic Energy Research Institute, Jeongup 56212, Republic of Korea
[d] Institute of Green Bio Science and Technology, Seoul National University, Pyeongchang 25354, Republic of Korea

ARTICLE INFO

Keywords:
X-ray irradiation
Semi-moist pet foods
Bacterial pathogen
Nutritional properties
Physicochemical properties

ABSTRACT

The aim of the study was to investigate the effect of X-ray irradiation at different dose levels on semi-moist pet foods. Samples were subjected to X-ray irradiation at 0, 2.5, 5, and 10 kGy, and their microbial, nutritional and physicochemical properties were evaluated for 60 days at 20-day intervals. Among these, samples irradiated at 10 kGy completely sterilized bacterial pathogens and inhibited their growth throughout the storage period. Following this, a dose of 5 kGy showed a better bacterial pathogen reduction. Above 5 kGy irradiated samples exhibited a significant effect on moisture and protein contents. Samples treated with above 5 kGy tended to exhibit a significant decline in water activity, pH, and a^* values during the storage period while continuing to exert microbial stability and quality attributes. However, lipid oxidation and protein degradation were observed in samples irradiated with above 5 kGy of X-ray during storage. Considering all results, we conclude that about 5 kGy X-ray irradiation could prevent microbial activity while maintaining the maximum losses of nutritional and physicochemical properties of semi-moist pet foods.

1. Introduction

Recent interest in pets has extended pet food production (Di Cerbo et al., 2017). Generally, pet foods are commercialized in three major forms, which are dry, semi-moist, and wet pet foods (Carrión and Thompson, 2013). Among them, semi-moist pet foods represent a smaller but significant portion of the manufactured pet food market (Zicker, 2008). They exhibit an attractive nutritional profile, comprising both animal and plant-origin ingredients with moisture content ranging from 25 % to 30 % (AAFCO, 2003; Adeniyi, 2019). However, semi-moist pet foods can harbor mold and harmful bacteria like *Salmonella* and *Escherichia coli* due to its water activity (between 0.60 and 0.80) (Deliephan et al., 2023; Soon et al., 2013; Taylor et al., 2019). In addition, pet foods are susceptible to

* Corresponding author at: Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea.
E-mail address: cheorun@snu.ac.kr (C. Jo).

https://doi.org/10.1016/j.anifeedsci.2025.116344
Received 11 March 2024; Received in revised form 4 March 2025; Accepted 3 April 2025
Available online 8 April 2025

cross-contamination during its storage since they are opened and stored under refrigerator conditions. Consequently, prolonged storage of semi-moist pet foods may reduce shelf life due to microbial activity and alterations in quality parameters.

Ionizing radiation, a non-thermal method, can effectively sterilize food with minimal nutritional and quality changes (Song et al., 2022). Heretofore, electron beam and gamma rays have been primarily used for microbial reduction in food irradiation (Ahn and Lee, 2006; Moosekian et al., 2012). Recently, X-ray irradiation has shown promise for microbial control in human foods and ingredients, surpassing electron beam and gamma ray irradiation (Moosekian et al., 2014; Zehi et al., 2020). X-rays can penetrate thicker and denser materials as they have high energy and short wavelengths (Moosekian et al., 2014). Consequently, this technology enables uniform microbial sterilization without damaging the food's surface, making it suitable for treating already packaged foods and avoiding recontamination of the product (Gautam and Venugopal, 2021; Ricciardi et al., 2019). Notably, X-ray irradiation offers the capability to supply precise dosage, higher kinetic energy, and dose rate than gamma ray, thereby minimizing overexposure and causing less impact on the quality attributes of foods (Pillai and Shayanfar, 2017). Moreover, it has been successfully employed in various foods such as meat and meat products (Ham et al., 2017; Kim et al., 2018; Shin et al., 2014), rice (Begum et al., 2020), red pepper powder (Jung et al., 2015), and potato starch (Lei et al., 2023) for microbial intervention while preserving their physical and chemical characteristics. These findings express that X-ray technology has the potential for application in foods with varying moisture content levels, possibly including semi-moist pet foods.

Despite its proven efficacy, the legal status of irradiation in pet food varies across the globe, influencing its adoption. For example, the European Union enforces strict limitations, allowing irradiation only for specific feed types, while some member states ban it completely (European commission, 2021). In contrast, the US FDA permits irradiation for specific feed ingredients, subject to strict safety assessment and dose restrictions (Jung, 2007). Additionally, the Radura symbol and the term "treated with irradiation" are viewed negatively by consumers due to a lack of understanding, and thinking irradiation is often associated with radioactivity and potential toxicity, affecting their perception of irradiated foods (Tatum, 2016).

Different moisture levels in foods may influence the efficacy of X-ray irradiation in microbial inactivation and physicochemical properties. Further, it was reported that the maximum limit of 10 kGy dose of irradiation causes less or no impact on nutritional and quality attributes of foods (Sahoo et al., 2023). However, higher X-ray intensity in food treatment can lead to increased oxidation in foods due to the powerful oxidative effects of radiolytic products. This suggests that the impact of X-ray treatment becomes more pronounced as food absorbs higher doses (Bliznyuk et al., 2022). Therefore, the use of appropriate dosage can help to prevent undesired changes in the physicochemical and nutritional attributes of foods (Lacivita et al., 2019). Additionally, there is a lack of information on the application of X-ray irradiation as a preservation technique to maintain nutritional and physicochemical characteristics in semi-moist pet foods. Therefore, our objective was to investigate the effect of X-ray irradiation on pathogen inactivation and their changes in nutritional and physicochemical qualities of semi-moist pet foods under refrigeration storage conditions.

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 were composed of flour (40 %), chicken meat (35 %), fish meat (14 %), propylene glycol (5 %), glycerin (5 %), sugar (0.8 %), tricalcium phosphate (0.04 %), and food colour (0.01 %). These samples were then cut into equal sizes of pieces (approximately 4 × 1.5 × 0.5 cm; 5.00 ± 0.05 g). A total of each 180 g of pet food 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) and vacuum-sealed. This packaging method was employed to assess the influence of X-ray irradiation on the nutritional and physicochemical changes of semi-moist pet foods. Subsequently, all packaged samples were stored at 4°C and transported to the irradiation center.

2.2. X-ray irradiation

X-ray irradiation was conducted using an electron beam accelerator (EBUIL-10–10, Biomedical Manufacturing Technology Center, Youngcheon, Korea) equipped with an X-ray converter. The beam energy employed was 5 MeV, and the dose rate was 2.95 kGy/h. Samples were subjected to X-ray irradiation with absorbed doses of 0, 2.5, 5, and 10 kGy at 0°C. Following irradiation, the samples were immediately transported to the laboratory, and the vacuum packs were opened before being stored under refrigeration condition (4°C) for subsequent analysis. The study was carried out for 60 days at 20-day intervals.

2.3. Inoculation test

2.3.1. Bacterial strains and culture preparation

S. Typhimurium (ATCC 13311) and *E. coli* O157:H7 (NCCP 15739) were cultivated in nutrient broth (Difco, Becton Dickinson Co., Sparks, MD, USA) and tryptic soy broth (Difco, Becton Dickinson Co.) respectively. The broths were centrifuged at 4001 × g at 4°C for 10 min (Combi 514 R, Hanil, Incheon, Korea). Subsequently, the supernatant was discarded, and the cells were washed twice with sterile 0.85 % saline solution. Finally, the pellets were 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 and X-ray irradiation

The two selected pathogens were individually inoculated into semi-moist pet foods. For this, each 5 g sample was spot inoculated with 100 μL of the pathogen and air-dried for 1 h inside a biosafety cabinet (biolus® CLASS II Type A2, CHCLAB, Daejeon, Korea). Subsequently, 30 g (6 pieces) of sample for each treatment were prepared and sealed in vacuum packs. After that, the bacterial culture-inoculated samples were stored at 4°C and then transported for the irradiation process. These samples were subjected to X-ray irradiation under the previously described conditions. The applied doses were 0, 2.5, 5, and 10 kGy. Post-irradiation, the samples were stored at 4°C for microbial analysis. The experiment was carried out for 60 days at intervals of 0, 20, 40, and 60 days.

2.3.3. Microbial analysis

A 5 g of each sample was aseptically homogenized using a BagMixer® 400 P (Interscience Co., St Nom, France) for 2 min in a sterile stomacher bag containing 45 mL of sterile saline solution to determine the sterilization effect against *S.* Typhimurium and *E. coli*. Subsequently, the samples were serially diluted in sterile saline (0.85 %) solution, and each diluent (0.1 mL) was plated onto 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, the plates were incubated at 37°C for 48 h, and the microbial count was expressed as colony-forming units per gram (CFU/g).

2.4. 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).

2.5. Nutritional components analysis

The major nutritional components of amino acids, simple sugars, and carboxylic compounds were analyzed using nuclear magnetic resonance (NMR) technique. 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 sec (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 1 s) 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 base-line 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)

2.6. Water activity

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

2.7. 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 512 R, Hanil Co.). The supernatant was then 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).

2.8. Instrumental color

The instrumental color of both control and X-ray 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), b^* (yellowness), were recorded in triplicates.

2.9. 2-Thiobar-bituric acid reactive substance (TBARS)

The TBARS values of the samples were measured following the method outlined by Lee and Lee (2014) with slight modifications. Initially, 5 g of each sample was homogenized at 9600 rpm for 30 s using a homogenizer (T25 basic, Ika Co.) with the addition of 15 mL of distilled water and 50 μL of a 7.2 % butylated hydroxytoluene solution. 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.). Finally, the absorbance of the supernatant was measured at a 532 nm wavelength using a UV/Vis spectrophotometer (SpectroMax M2e, Molecular Devices M2e, Sunnyvale, CA, USA). The TBARS value was calculated as mg malondialdehyde (MDA)/kg of pet food using a standard curve.

2.10. Volatile basic nitrogen (VBN)

The VBN was estimated following the method of Kim et al. (2020). Initially, three grams of ground semi-moist pet food samples were taken to centrifuge tubes and added with 27 mL of deionized distilled water, then homogenized (T25 basic, Ika Co.) at 9600 rpm for 1 min. The homogenate was subsequently filtered using filter paper (No. 1, Whatman International Ltd). After that, 1 mL of filtrate and saturated potassium carbonate were added to the outer section of the Conway unit (Sibata Ltd., Sitama, Japan). Subsequently, 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 then incubated at 37°C for 1 h, followed by titration with 0.01 N hydrochloric acid, and the VBN value of each sample was calculated.

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

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.

2.11. Statistical analyses

All experiments were carried out in triplicate individually. The data were evaluated 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. Inactivation of S. Typhimurium and E. coli O157:H7

The bactericidal effects of X-ray irradiation against *S.* Typhimurium and *E. coli* O157:H7 are illustrated in Fig. 1. Before X-ray irradiation, the initial pathogenic loads of *S.* Typhimurium and *E. coli* O157:H7 were 7.01 and 7.18 log CFU/g in semi-moist pet foods, respectively. Increasing doses of X-ray irradiation significantly reduced both pathogens in a dose-dependent manner. Higher doses of X-ray irradiation express their sterilization effect in food by generating a substantial number of free radicals with enough energy from radiolysis of water (Zehi et al., 2020). These include electrons (e^-_{aq}), hydroxyl radicals ($OH^{\cdot}$), and hydrogen peroxide (H_2O_2), which are very reactive and interfere with the bonds between nucleic acids, causing damage to microbial DNA in a dose-dependent manner

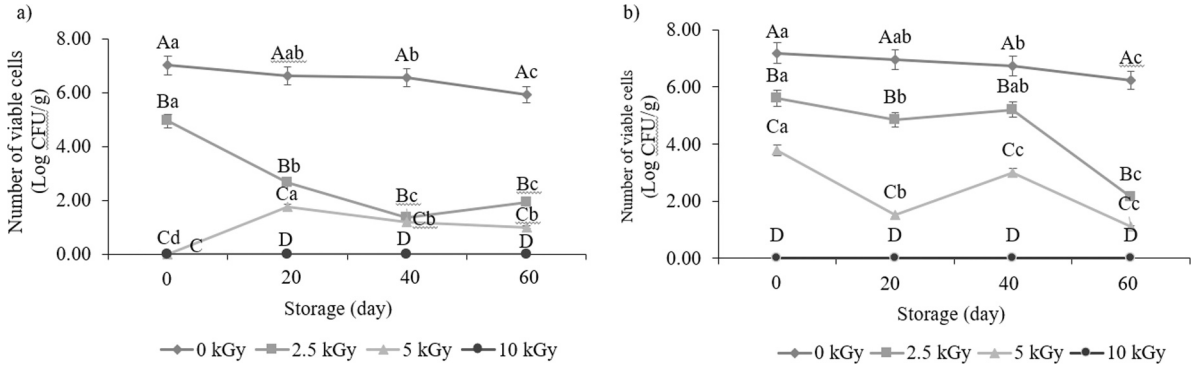


Fig. 1. Effect of X-ray irradiation on the inactivation of *Salmonella* Typhimurium (a) and *Escherichia coli* O175:H7 (b) in pet foods stored at refrigerator condition. $^{A\text{-}D}$Different letters indicate significant differences ($P < 0.05$) among different irradiation doses. $^{a\text{-}d}$Different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

(Begum et al., 2020; Mahmoud et al., 2016). However, different effect can be observed for different microorganisms (Nasab et al., 2023). In this study, samples irradiated with 10 kGy were completely eliminated both *S.* Typhimurium and *E. coli* O157:H7 in semi-moist pet foods (Fig. 1). Subsequently, a dose of 5 kGy significantly reduced bacterial counts of *S.* Typhimurium by 7.01 log CFU/g and *E. coli* O157:H7 by 3.39 log CFU/g. Following this, the number of *S.* Typhimurium and *E. coli* O157:H7 were significantly reduced by 2.07 and 1.58 log CFU/g, respectively (Fig. 1; $P < 0.05$). It seems that *E. coli* O157:H7 was less effectively reduced by X-ray compared to *S.* Typhimurium in semi-moist pet foods. Although ionizing radiation targets microbial DNA and induces changes at the cellular level, these changes can vary with the microorganism for different reasons (Tahergorabi et al., 2012). Even though *S.* Typhimurium and *E. coli* O157:H7 belong to the gram-negative bacterial group, differences in the thickness of their peptidoglycan layer in the cell wall, DNA repair mechanisms, and tolerance to harsh conditions may influence their responses to different doses of X-ray irradiation (Calado et al., 2014; Nasab et al., 2023; Song et al., 2014). Similarly, higher X-ray had better impact on *S.* Typhimurium in semi-moist pet foods than that on *E. coli* O157:H7.

On the other hand, X-ray irradiation at 10 kGy resulted in no detectable growth of both pathogens in pet food samples until 60 days of refrigerated storage. Interestingly, doses of 2.5 kGy and 5 kGy tended to show a significant bacteria reduction over the storage period mainly due to the injured bacterial cells by X-ray irradiation (Fig. 1). It was reported that application of X-ray irradiation could prevent repairing DNA material of bacteria (Park and Ha, 2019). Additionally, non-irradiated samples also showed a slight downward trend in microbial growth over the storage period. It may be attributed to the inclusion of potassium sorbate, which acts as an antimicrobial agent (Deliephan et al., 2023). However, higher pathogenic load of non-irradiated samples on day 60 implying that the efficacy of potassium sorbate was not sufficient to control pathogens in semi-moist pet foods. Considering the microbial results, a dose of 10 kGy X-ray irradiation were effective in reducing both *S.* Typhimurium and *E. coli* O157:H7 in semi-moist pet foods. In addition, X-ray had better efficient than electron beam irradiation when the same dose was treated (Data not shown). This clearly indicates that X-ray irradiation at 5 kGy could be efficient sterilization for reducing both *S.* Typhimurium and *E. coli* O157:H7 in semi-moist pet foods.

3.2. Proximate composition

The primary purpose of proximate composition analysis in pet food is to measure the quantity of available nutrients and to ensure that the nutrient contents meet the nutritional requirements for pets (Dodd et al., 2021). There were no significant changes observed in proximate composition in irradiated semi-moist pet foods, except for moisture and crude protein contents (Table 1). Samples treated with 5 and 10 kGy of X-ray irradiation had significantly lower moisture and higher crude protein contents compared to non-irradiated samples ($P < 0.05$). The radiolysis of water reduced moisture content at higher doses irradiated samples (Calado et al., 2014). Samples were oven dried to evaporate moisture before measuring crude protein indicating that high temperature affect crude protein content. Protein content is essential for pets to obtain energy for growth and development and to provide essential amino acids which cannot be synthesized by them (Ahmed et al., 2021). However, the difference in protein content between higher doses and non-irradiated samples was minimal, ranged from 13.20 % to 13.55 %. It may indicate that X-ray irradiation influenced protein levels while still maintaining their required quantity. This shows that X-ray irradiation did not negatively impact the nutritional value of semi-moist pet foods. Samples irradiated at 10 kGy had the highest fat content, while the control group exhibited the lowest fat content. However, no significant differences were observed between X-ray irradiated samples and the control. Increasing irradiation doses reduced water content and changed the food matrix, leading to fat concentration and a higher detected percentage (Soladoye et al., 2015). Fiber content was lowest at 2.5 kGy, whereas while 5 and 10 kGy showed values like control, indicating minimal effects on fiber stability at higher doses. Low dose irradiation at 2.5 kGy may subtly alter fiber structure and degrade integrity, thereby reducing measure fiber content (Le Moigne et al., 2017). However, low doses are less likely to significantly affect other nutrients or the overall food matrix. A gradual decline in ash content with increasing doses, suggesting that higher doses cause mineral denaturation in semi-moist pet foods (Stefanova et al., 2010). Despite changes in fat, fiber, and ash content were not statistically significantly, these trends show that X-ray irradiation selectively affects the nutritional composition of semi-moist pet foods. Therefore, based on the current result, about 5 kGy X-ray irradiation underwent slight nutritional changes in terms of moisture and protein contents, but it did not affect the overall nutritional composition in semi-moist pet foods.

Table 1
Proximate composition of X-ray irradiated semi-moist pet foods on day 0.

Items	Irradiation dose (kGy)				SEM[1)]	P value
	0	2.5	5	10		
Moisture	20.63^{A}	19.61^{A}	16.22^{B}	17.19^{B}	0.291	*
Crude protein	13.20^{C}	13.35^{BC}	13.55^{A}	13.50^{AB}	0.042	*
Crude fat	7.50	9.50	9.22	10.14	0.949	NS
Crude fiber	0.32	0.19	0.33	0.26	0.062	NS
Ash	1.31	1.31	1.16	1.04	0.062	NS

[1)]Standard error of the means (n = 12).
[A-C]Different letters indicate significant differences ($P < 0.05$) among different irradiation doses.
*: $P < 0.05$; NS: Not significant.

3.3. Nutritional profile

A number of 17 compounds (nine amino acids, three monosaccharides, two disaccharides, and three carboxylic compounds) were identified (Table 2). Such compounds have different roles in growth and development, reproduction, and metabolic processes of pet (Case et al., 2010). In this study, the results showed no significant difference in nutritional compounds between X-ray irradiated and non-irradiated samples. Specifically, the nine essential amino acids detected (methionine, lysine, glutamate, glycine, histidine, aspartate, valine, isoleucine, and alanine) play crucial roles in the nutritional value of pet foods and the physiological processes of pets (Hendriks et al., 2015; Rooijen et al., 2014). Although X-ray irradiation significantly affected the protein content, it does not create significant influence on amino acids (Matloubi et al., 2004). Amino acids are protected within complex protein structures and additional energy is needed to break down amino acids' bonds (Matloubi et al., 2004). Additionally, among monosaccharides, ribose exhibited a significant difference regarding irradiation dose, implying that 10 kGy of X-ray irradiation may depolymerize complex carbohydrates to generate simple sugars while maintaining overall nutritive value in semi-moist pet foods (Methacanon et al., 2011). Regarding storage days, a significant reduction in amino acids (methionine, lysine, glycine, valine, isoleucine, and alanine), mono and disaccharides (galactose and sucrose), and carboxylic compounds (fumarate and lactate) were observed ($P < 0.05$; Table 2). This could be attributed due to oxidation, enzymatic activity, and the exposure to light and air during storage period (Niamnuy and Devahastin, 2010). Interestingly, the interaction of irradiation dose and storage days was not found, implying that only storage conditions might have an influence on nutritional components.

3.4. Water activity

Significant effect was observed in water activity in semi-moist pet foods when treated by X-ray irradiation (Fig. 2; $P < 0.05$). When semi-moist pet foods exposed to 10 kGy of X-ray irradiation, a significant increase in water activity was found on day 0. This result suggests that more free radicals induced by irradiation break down complex biomolecules and release more free water molecules as by-products, leading to higher water activity (Pan et al., 2020). During storage, all treatments tended to show a significant decrease in water activity within the range of 0.65–0.69. This implies that substances such as glycerin and propylene glycol might absorb water, reducing water activity during storage (Deliephan et al., 2023). Meanwhile, we explained the decrease in pathogenic bacteria as the effect of X-ray irradiation as well as glycerin and propylene glycol. Furthermore, water activity is crucial factor for microbial growth and survival, and maintaining an appropriate level is essential for upholding the quality of semi-moist pet foods (Niamnuy and Devahastin, 2010). Therefore, the reduction in water activity might contribute to pathogenic reduction in semi-moist pet foods during

Table 2
Nutritional compounds of X-ray irradiated semi-moist pet foods at different along with storage periods of 0 and 60 days using nuclear magnetic resonance (NMR) technique.

Compounds	Irradiation dose (kGy)				SEM[1)]	Storage (day)		SEM[2)]	P value		
	0	2.5	5	10		0	60		Dose	Day	Dose*Day
Amino acids											
Methionine	0.15	0.13	0.14	0.13	0.010	0.15^{A}	0.12^{B}	0.007	NS	*	NS
Lysine	0.29	0.28	0.28	0.25	0.019	0.30^{A}	0.26^{B}	0.013	NS	*	NS
Glutamate	0.18	0.17	0.17	0.16	0.012	0.18	0.16	0.009	NS	NS	NS
Glycine	14.92	13.78	13.60	13.15	1.024	15.36^{A}	12.38^{B}	0.724	NS	*	NS
Histidine	0.02	0.02	0.03	0.02	0.005	0.02	0.02	0.003	NS	NS	NS
Aspartate	0.05	0.05	0.06	0.05	0.004	0.05	0.05	0.003	NS	NS	NS
Valine	0.09	0.08	0.08	0.08	0.006	0.09^{A}	0.07^{B}	0.004	NS	*	NS
Isoleucine	0.23	0.22	0.21	0.20	0.014	0.23^{A}	0.20^{B}	0.010	NS	*	NS
Alanine	0.30	0.29	0.28	0.25	0.019	0.31^{A}	0.25^{B}	0.014	NS	*	NS
Monosaccharides											
Ribose	3.60^{B}	4.54^{AB}	5.99^{A}	5.55^{AB}	0.519	4.93	4.91	0.367	*	NS	NS
Galactose	120.60	111.39	112.34	109.58	7.967	124.48^{A}	102.47^{B}	5.633	NS	*	NS
Fucose	1.50	1.62	1.76	1.76	0.165	1.79	1.53	0.117	NS	NS	NS
Disaccharides											
Maltose	0.87	0.87	0.85	0.82	0.113	0.96	0.74	0.079	NS	NS	NS
Sucrose	3.86	3.94	3.89	3.56	0.387	4.40^{A}	3.22^{B}	0.274	NS	**	NS
Carboxylic acids											
Fumarate	0.05	0.05	0.04	0.04	0.005	0.05^{A}	0.03^{B}	0.004	NS	**	NS
Malate	0.13	0.12	0.12	0.11	0.010	0.13	0.11	0.007	NS	NS	NS
Lactate	1.30	1.18	1.14	1.08	0.075	1.30^{A}	1.05^{B}	0.053	NS	**	NS

[1)]Standard error of the mean ($n = 24$), [2)]$n = 24$.
[A-B]Different letters indicate significant differences ($P < 0.05$) among different irradiation doses.
*: $P < 0.05$; **: $P < 0.01$; NS: Not significant.

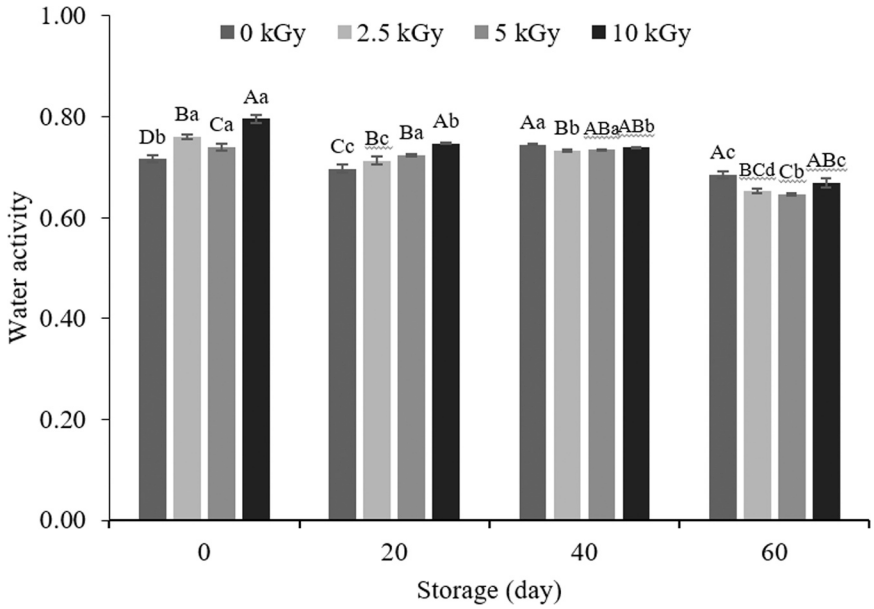


Fig. 2. Changes in the water activity of X-ray irradiated pet foods at different doses during cold storage. [A-D]Different letters indicate significant differences ($P < 0.05$) among different irradiation doses. [a-d]Different letters indicate significant differences ($P < 0.05$) among different storage days within the same treatment.

storage period (Fig. 1). In general, bacteria prefer a water activity of 0.85 or higher for growth and multiplication (Tapia et al., 2020). So semi-moist pet foods with 0.65–0.69 of water activity may not be susceptible to additional bacterial growth during storage. Additionally, no significant found between samples irradiated with 10 kGy and non-irradiated samples for water activity after 40 days of storage (Fig. 2). It suggests that higher doses of X-ray irradiation did not affect structural changes that lead to water loss. Thus, X-ray irradiation at dosage levels above 5 kGy exhibited preservative effect by reducing water activity in semi-moist pet foods while holding the required water activity levels for maintaining quality attributes during the storage period.

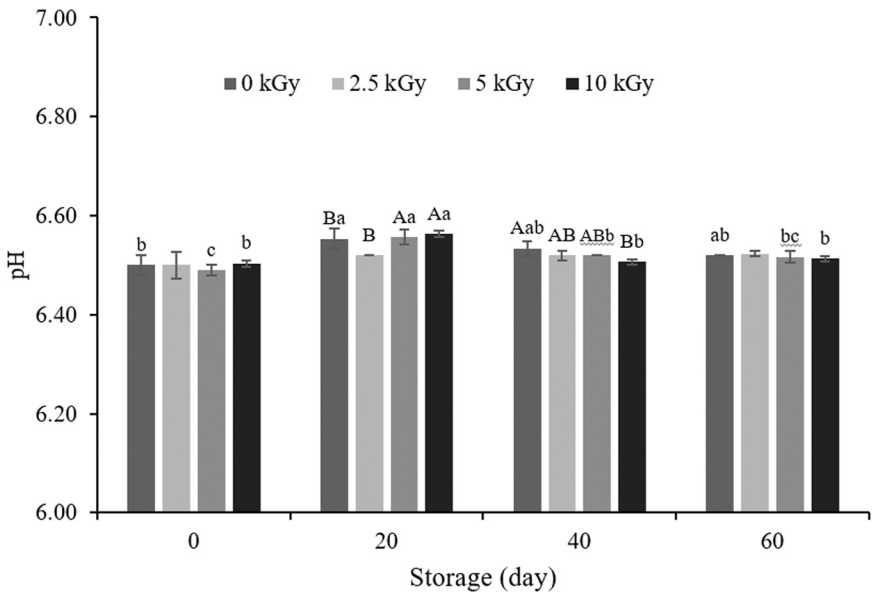


Fig. 3. Changes in the pH of X-ray irradiated pet foods at different doses during cold storage. [A-B]Different letters indicate significant differences ($P < 0.05$) among different irradiation doses. [a-c]Different letters indicate significant differences ($P < 0.05$) among different storage days within the same treatment.

3.5. pH

The typical pH of semi-moist pet foods falls within the range of 5.5–6.5, indicating a slightly acidic nature serves to preserve them from microbial intervention (Adeniyi, 2019). On day 0, there was no significant difference between irradiated and non-irradiated samples (Fig. 3, $P < 0.05$). During the 60 days of storage, 10 kGy of X-ray irradiated samples exhibited no significant reduction in pH. Samples irradiated with 5 kGy showed a slight increase over the storage period but fell within the range. Meanwhile there were no significant changes in pH observed in samples irradiated with 2.5 kGy throughout the storage period. This is attributed that both lower and higher doses of X-ray irradiation led to less microbial growth, thereby maintaining pH stability in semi-moist pet foods over the extended storage period (Chen et al., 2023). Additionally, a significant increase was observed only in non-irradiated samples during the storage period, implying that the different products produced by microbial population led to pH changes in semi-moist pet foods (Deliephan et al., 2023). Based on the results, samples treated with X-rays at above 5 kGy maintained slightly acidic conditions throughout the storage period, indicating a preservative effect against microbial actions.

3.6. Instrumental color

It is crucial in maintaining consistency in color for semi-moist pet food manufacturers (Watson et al., 2023). The results revealed that higher irradiation doses of 5 kGy and 10 kGy exhibited significantly higher L^*, a^*, and b^* values compared to those in non-irradiated samples on day 0 (Table 3). Samples irradiated with 2.5 kGy exhibited significantly higher L^* values than non-irradiated samples, with no significant differences found for a^* and b^* values on day 0. Additionally, significantly lower a^* and b^* values were observed in samples treated with 2.5 kGy compared to those treated with 5 and 10 kGy. This clearly shows that about 5 kGy doses influenced the color in semi-moist pet foods compared to 2.5 kGy irradiated samples regardless of a^* and b^* values. Considering at day 60, both treatments showed higher b^* values, while L^* and a^* values declined compared to other treatments (Table 3). This result indicates that b^* values strongly correlate with water activity and moderately correlate with thermal treatments during feed processing and storage (Rajkumar et al., 2022). Hence, the color changes in samples treated with 5 and 10 kGy of X-ray irradiation doses were likely caused by significantly reduced water content or changes in protein structure due to heating in manufacturing process. The results indicate that higher doses of X-ray irradiation led to significant color changes in semi-moist pet foods during storage. This may affect the purchasing behavior of pet owners, however since these color changes are difficult for both humans and pets to distinguish, it's not a significant concern.

3.7. 2- Thiobarbituric acid reactive substances (TBARS)

TBARS values are indicative of lipid oxidation and are linked to the sensory quality of food products (Ahn et al., 2000a, 2000b; Zhao et al., 2017). The TBARS values were significantly increased with higher doses of X-ray irradiation in a dose-dependent manner (Fig. 4). This indicates that X-ray irradiation induces lipid oxidation by generating free radicals, thereby resulting in higher TBARS Values (Trindade et al., 2010). Samples treated with 10 kGy had significantly higher TBARS values than other treatments. This shows that higher irradiation doses produce more reactive oxygen species, causing excessive oxidative changes in the food (Tian et al., 2013). Additionally, 5 kGy had significantly lower TBARS valus than 10 kGy. However, no significant difference was found in TBARS levels between samples irradiated with 2.5 and 5 kGy. This implies that 5 kGy exhibited comparatively less lipid oxidation than 10 kGy in semi-moist pet foods. During storage, all irradiated samples exhibited a significant increase in TBARS values due to the accumulation of

Table 3
Changes in the colour of X-ray irradiated pet foods at different doses during cold storage.

Colour attributes	Storage (day)	Irradiation dose (kGy)				SEM[2)]
		0	2.5	5	10	
L^*	0	46.19^{Bb}	50.83^{Aa}	50.17^{Aa}	49.96^{Aa}	0.432
	20	43.16^{Ac}	40.63^{Bc}	43.29^{Ad}	45.23^{Ac}	0.487
	40	47.81^{Aa}	47.47^{Bb}	49.85^{Bc}	47.65^{AB}	0.048
	60	45.18^{Cb}	46.54^{Bb}	48.27^{Ab}	45.61^{Cc}	0.091
	SEM[1)]	0.310	0.402	0.150	0.391	
a^*	0	4.42^{Ca}	4.23^{Ca}	5.01^{Ba}	5.39^{Aa}	0.055
	20	3.20^{Ad}	2.57^{Cc}	2.74^{Bc}	3.30^{Ad}	0.034
	40	3.92^{Bb}	3.52^{Db}	3.83^{Cb}	4.04^{Ab}	0.029
	60	3.73^{Bc}	3.47^{Db}	3.67^{Cb}	3.82^{Ac}	0.010
	SEM[1)]	0.034	0.024	0.040	0.025	
b^*	0	15.6^{Bc}	16.32^{Ba}	16.30^{Ad}	16.3^{Ab}	0.142
	20	17.33^{Ab}	13.60^{Bb}	17.08^{Ac}	17.66^{Aa}	0.393
	40	18.5^{Aa}	16.89^{Ba}	18.73^{Aa}	17.22^{Aab}	0.078
	60	16.53^{Cbc}	17.24^{Ba}	17.91^{Ab}	17.37^{Aa}	0.041
	SEM[1)]	0.250	0.232	0.143	0.210	

[1)]Standard error of the means (n = 12), [2)]n = 12.
[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.

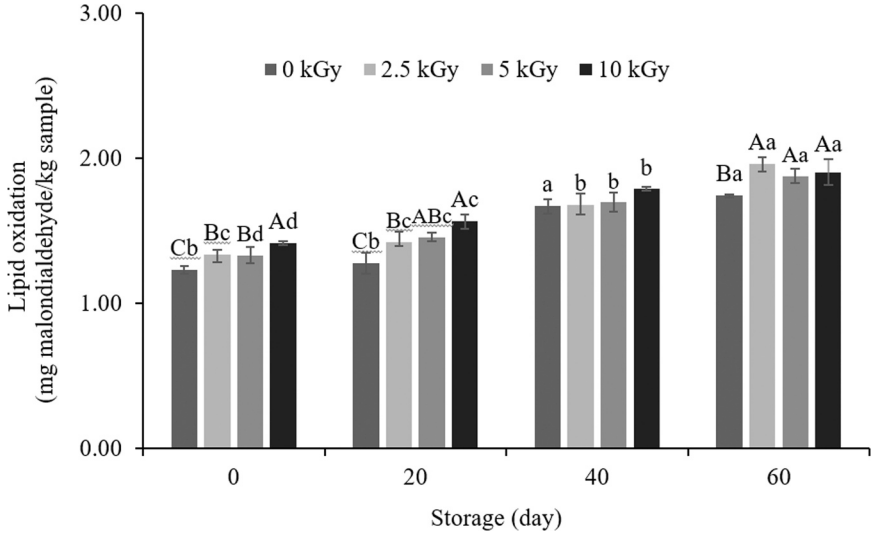


Fig. 4. Changes in TBARS of X-ray irradiated pet foods at different doses (0, 2.5, 5, and 10 kGy) over 60 days of refrigeration storage period. [A-C]Different letters indicate significant differences ($P < 0.05$) among different irradiation doses. [a-d]Different letters indicate significant differences ($P < 0.05$) among different storage days within the same treatment.

secondary lipid oxidation products in semi-moist pet foods over the extended storage period (Bhoir et al., 2019; Hassanzadeh et al., 2017). After 60 days of storage, no significant differences were found in TBARS values in all irradiated samples (Fig. 4; $P > 0.05$), suggesting that lipids in semi-moist pet foods may have already undergone substantial oxidation (Li et al., 2017). Considering lipid oxidation results, below 5 kGy dose of X-ray irradiation resulted in less lipid oxidation without changing the lipid components of semi-moist pet foods over the extended storage period.

3.8. Volatile basic nitrogen (VBN)

VBN is an important parameter used to measure the release of nitrogenous compounds due to microbial action (Kim et al., 2022). Accordingly, the VBN was determined in both X-ray treated and non-treated samples during 60 days of storage (Fig. 5). During storage, the VBN values were significantly increased in non-irradiated samples which were attributed to the microbial actions (Al-Bachir and Zeinou, 2009). In this study, there was no significant differences for VBN in between irradiated and non-irradiated samples until day 40. This outcome exhibits that increasing doses of X-ray irradiation contributed for less microbial actions in semi-moist pet foods (Thakur and Singh, 1994). However, a significant increase in VBN was observed in all irradiated samples compared to non-irradiated samples on day 60. This suggests that free-radicals produced by X-rays had a slow and delayed effect on protein molecules via peptide bond cleavage, which released nitrogen containing amino acids, amines, and ammonia in semi-moist pet foods (Ahn et al., 2016; Thakur and Singh, 1994). This result shows that both lower and higher doses of X-ray irradiation has potential to effectively control microbial activity while causing less protein degradation losses in semi-moist pet foods throughout the storage period.

4. Conclusion

In this investigation, we conducted a pioneering examination of the feasibility of applying X-ray radiation to semi-moist pet foods. Among different treatments, the efficacy of X-ray treatment at a 10 kGy dose was better against both *S.* Typhimurium and *E. coli* O157: H7 compared to other treatments. However, it led to some nutritional and physicochemical losses. We found that a dose of 5 kGy had the second highest bactericidal effect, while causing minimal changes in nutritional and quality attributes in semi-moist pet foods. However, 5 kGy irradiated samples contributed for slight lipid oxidation and protein degradation. Therefore, we can conclude that about 5 kGy of X-ray have the potential to treat semi-moist pet foods effectively to overcome post-processing contamination issues, whilst minimal alterations in the nutritional and physicochemical properties of the products. Further studies will explore the combined use of 5 kGy X-ray radiation and feasible hurdle technology in semi-moist pet foods, which will enable us to strongly recommend their utilization.

CRediT authorship contribution statement

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

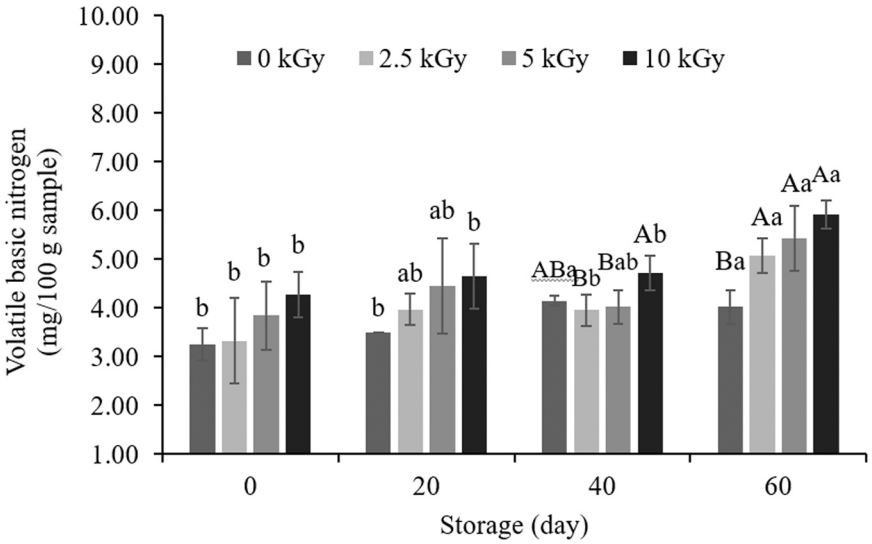


Fig. 5. Changes in VBN of X-ray irradiated pet foods at different doses (0, 2.5, 5, and 10 kGy) over 60 days of refrigeration storage period. [A-B]Different letters indicate significant differences ($P < 0.05$) among different irradiation doses. [a-b]Different letters indicate significant differences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

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.

Acknowledgements

This work was supported by a project of Development of Functional Pet Food and Safety Assurance Technology with Korean Livestock Products (No. PJ016891).

References

Adeniyi, A.E., 2019. Evaluation of Salmonella and Shiga Toxin-producing Escherichia coli Isolated from Pet Food and Antimicrobial Susceptibility Patterns (Doctoral dissertation).

Ahmed, I., Fatma, İ.N.A.L., Roshan, R.İ.A.Z., 2021. Insects usage in pets food. Vet. Hekim. Der. Derg. 93 (1), 87–98. https://doi.org/10.33188/vetheder.909963.

Ahn, D.U., Jo, C., Du, M., Olson, D.G., Nam, K.C., 2000. Quality characteristics of pork patties irradiated and stored in different packaging and storage conditions. Meat Sci. 56 (2), 203–209. https://doi.org/10.1016/S0309-1740(00)00044-9.

Ahn, D.U., Jo, C., Olson, D.G., 2000. Analysis of volatile components and sensory characteristics of irradiated raw pork. Iowa State Univ. Anim. Ind. Rep. 1 (1).

Ahn, D.U., Lee, E.J., 2006. Mechanisms and prevention of quality changes in meat by irradiation. Food Irradiat. Res. Technol. 58, 127–142.

Ahn, D.U., Lee, E.J., Feng, X., Zhang, W., Lee, J.H., Jo, C., Nam, K., 2016. Mechanisms of volatile production from non-sulfur amino acids by irradiation. Radiat. Phys. Chem. 119, 64–73. https://doi.org/10.1016/j.radphyschem.2015.09.008.

Al-Bachir, M., Zeinou, R., 2009. Effect of gamma irradiation on microbial load and quality characteristics of minced camel meat. Meat Sci. 82 (1), 119–124. https://doi.org/10.1016/j.meatsci.2008.12.012.

Association of American Feed Control Officials (AAFCO), 2003. Official Publication. AAFCO, Oxford, IN. ⟨https://www.aafco.org/resources/official-publication/⟩ (Accessed 10 January 2024).

Begum, T., Follett, P.A., Hossain, F., Christopher, L., Salmieri, S., Lacroix, M., 2020. Microbicidal effectiveness of irradiation from Gamma and X-ray sources at different dose rates against the foodborne illness pathogens *Escherichia coli, Salmonella* Typhimurium and *Listeria monocytogenes* in rice. LWT 132, 109841. https://doi.org/10.1016/j.lwt.2020.109841.

Bhoir, S.A., Jhaveri, M., Chawla, S.P., 2019. Evaluation and predictive modeling of the effect of chitosan and gamma irradiation on quality of stored chilled chicken meat. J. Food Process Eng., e13254 https://doi.org/10.1111/jfpe.13254.

Bliznyuk, U., Avdyukhina, V., Borshchegovskaya, P., Bolotnik, T., Ipatova, V., Nikitina, Z., Nikitchenko, A., Rodin, I., Studenikin, F., Chernyaev, A., Yurov, D., 2022. Effect of electron and X-ray irradiation on microbiological and chemical parameters of chilled turkey. Sci. Rep. 12 (1), 750. https://doi.org/10.1038/s41598-021-04733-3.

Calado, T., Venâncio, A., Abrunhosa, L., 2014. Irradiation for mold and mycotoxin control: a review. Compr. Rev. Food Sci. Food Saf. 13 (5), 1049–1061. https://doi.org/10.1111/1541-4337.12095.

Carrión, P.A., Thompson, L.J., 2013. Pet Food. In: Motarjeni, Y., Lelieveld, H. (Eds.), Food Safety Management. Elsevier Inc., Massachusetts.

Case, L.P., Daristotle, L., Hayek, M.G., Raasch, M.F., 2010. Canine and feline nutrition: a resource for companion animal professionals. Elsevier Health Sciences.

Chen, W., Yang, J., Huang, N., Zhang, Q., Zhong, Y., Yang, H., Liu, W., Yue, Y., 2023. Effect of combined treatments of electron beam irradiation with antioxidants on the microbial quality, physicochemical characteristics and volatiles of vacuum-packed fresh pork during refrigerated storage. Food Control 145, 109480. https://doi.org/10.1016/j.foodcont.2022.109480.

Deliephan, A., Dhakal, J., Subramanyam, B., Aldrich, C.G., 2023. Effects of liquid smoke preparations on shelf life and growth of wild type mold and *Aspergillus flavus* in a model semi moist pet food. Front. Microbiol. 14, 1154765. https://doi.org/10.3389/fmicb.2023.1154765.

Di Cerbo, A., Morales-Medina, J.C., Palmieri, B., Pezzuto, F., Cocco, R., Flores, G., Iannitti, T., 2017. Functional foods in pet nutrition: focus on dogs and cats. Res. J. Vet. Sci. 161–166. https://doi.org/10.1016/j.rvsc.2017.03.020.

Dodd, S.A., Shoveller, A.K., Fascetti, A.J., Yu, Z.Z., Ma, D.W., Verbrugghe, A., 2021. A comparison of key essential nutrients in commercial plant-based pet foods sold in Canada to American and European canine and feline dietary recommendations. Animals 11 (8), 2348. https://doi.org/10.3390/ani11082348.
European commission, (2021). Evaluation of the EU Legal Framework on Food Irradiation (Directives 1992/EC and 1993/3/EC). ⟨https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52021SC0225⟩. (Accessed 3 March 2025).
Gautam, R.K., Venugopal, V., 2021. Electron beam irradiation to control biohazards in seafood. Food Control 130, 108320. https://doi.org/10.1016/j.foodcont.2021.108320.
Ham, Y.K., Kim, H.W., Hwang, K.E., Song, D.H., Kim, Y.J., Choi, Y.S., Song, B.S., Park, J.H., Kim, C.J., 2017. Effects of irradiation source and dose level on quality characteristics of processed meat products. Radiat. Phys. Chem. 130, 259–264. https://doi.org/10.1016/j.radphyschem.2016.09.010.
Hassanzadeh, P., Tajik, H., Rohani, S.M.R., Moradi, M., Hashemi, M., Aliakbarlu, J., 2017. Effect of functional chitosan coating and gamma irradiation on the shelf-life of chicken meat during refrigerated storage. Radiat. Phys. Chem. 141, 103–109. https://doi.org/10.1016/j.radphyschem.2017.06.014.
Hendriks, W.H., Bakker, E.J., Bosch, G., 2015. Protein and amino acid bioavailability estimates for canine foods. J. Anim. Sci. 93 (10), 4788–4795.
Jung, H.H., 2007. Packaging for Foods Treated with Ionizing Radiation. Blackwell Publishing.
Jung, K., Song, B.S., Kim, M.J., Moon, B.G., Go, S.M., Kim, J.K., Lee, Y.J., Park, J.H., 2015. Effect of X-ray, gamma ray, and electron beam irradiation on the hygienic and physicochemical qualities of red pepper powder. LWT Food Sci. Technol. 63 (2), 846–851. https://doi.org/10.1016/j.lwt.2015.04.030.
Kim, H.J., Kim, H.J., Jeon, J., Nam, K.C., Shim, K.S., Jung, J.H., Kim, K.S., Choi, Y., Kim, S.H., Jang, A., 2020. Comparison of the quality characteristics of chicken breast meat from conventional and animal welfare farms under refrigerated storage. Poult. Sci. J. 99 (3), 1788–1796. https://doi.org/10.1016/j.psj.2019.12.009.
Kim, S.Y., Yong, H.I., Nam, K.C., Jung, S., Yim, D.G., Jo, C., 2018. Application of high temperature (14°C) aging of beef *M. semimembranosus* with low-dose electron beam and X-ray irradiation. Meat Sci. 136, 85–92. https://doi.org/10.1016/j.meatsci.2017.10.016.
Kim, Y.Y., Park, S.J., Kim, J.S., Shin, H.S., 2022. Development of freshness indicator for monitoring chicken breast quality and freshness during storage. Food Sci. Biotechnol. 31 (3), 377–385. https://doi.org/10.1007/s10068-022-01034-x.
Kwon, J.A., Yim, D.G., Kim, H.J., Ismail, A., Kim, S.S., Lee, H.J., Jo, C., 2022. Effect of temperature abuse on quality and metabolites of frozen/thawed beef loins. Food Sci. Anim. Resour. 42 (2), 341 http://doi.org/10.5851/kosfa.2022.e9.
Lacivita, V., Mentana, A., Centonze, D., Chiaravalle, E., Zambrini, V.A., Conte, A., Del Nobile, M.A., 2019. Study of X-Ray irradiation applied to fresh dairy cheese. LWT 103, 186–191. https://doi.org/10.1016/j.lwt.2018.12.073.
Le Moigne, N., Sonnier, R., El Hage, R., Rouif, S., 2017. Radiation-induced modifications in natural fibres and their biocomposites: opportunities for controlled physico-chemical modification pathways? Ind. Crop Prod. 109, 199–213. https://doi.org/10.1016/j.indcrop.2017.08.027.
Lee, H.J., Lee, J.J., 2014. Effects of various kinds of salt on the quality and storage characteristics of tteokgalbi. Korean J. Food Sci. An. 34 (5), 604. https://doi.org/10.5851/kosfa.2014.34.5.604.
Lei, X., Yu, J., Hu, Y., Bai, J., Feng, S., Ren, Y., 2023. Comparative investigation of the effects of electron beam and X-ray irradiation on potato starch: structure and functional properties. Int. J. Biol. Macromol. 236, 123909. https://doi.org/10.1016/j.ijbiomac.2023.123909.
Li, C., He, L., Jin, G., Ma, S., Wu, W., Gai, L., 2017. Effect of different irradiation dose treatment on the lipid oxidation, instrumental color and volatiles of fresh pork and their changes during storage. Meat Sci. 128, 68–76. https://doi.org/10.1016/j.meatsci.2017.02.009.
Mahmoud, B.S., Nannapaneni, R., Chang, S., Wu, Y., Coker, R., 2016. Improving the safety and quality of raw tuna fillets by X-ray irradiation. Food Control 60, 569–574. https://doi.org/10.1016/j.foodcont.2015.08.039.
Matloubi, H., Aflaki, F., Hadjiezadegan, M., 2004. Effect of γ-irradiation on amino acids content of baby food proteins. J. Food Compos. Anal. 17 (2), 133–139. https://doi.org/10.1016/j.jfca.2003.09.005.
Methacanon, P., Weerawatsophon, U., Tanjak, P., Rachtawee, P., Prathumpai, W., 2011. Interleukin-8 stimulating activity of low molecular weight β-glucan depolymerized by γ-irradiation. Carbohydr. Polym. 86 (2), 574–580. https://doi.org/10.1016/j.carbpol.2011.04.075.
Moosekian, S.R., Jeong, S., Marks, B.P., Ryser, E.T., 2012. X-ray irradiation as a microbial intervention strategy for food. Annu. Rev. Food Sci. 3, 493–510. https://doi.org/10.1146/annurev-food-022811-101306.
Moosekian, S.R., Jeong, S., Ryser, E.T., 2014. Inactivation of sanitizer-injured *Escherichia coli* O157: H7 on baby spinach using X-ray irradiation. Food Control 36 (1), 243–247. https://doi.org/10.1016/j.foodcont.2013.08.024.
Nasab, S.S., Zare, L., Tahmouzi, S., Nematollahi, A., Mollakhalili-Meybodi, N., Abedi, A.S., Delshadian, Z., 2023. Effect of irradiation treatment on microbial, nutritional and technological characteristics of cereals: a comprehensive review. Radiat. Phys. Chem., 111124 https://doi.org/10.1016/j.radphyschem.2023.111124.
Niamnuy, C., Devahastin, S., 2010. 11 Pet foods and their physicochemical properties as affected by processing. In: Devahastin, S. (Ed.), Physicochemical Aspects of Food Engineering and Processing. CRC Press, New York, pp. 327–351.
Pan, L., Xing, J., Luo, X., Li, Y., Sun, D., Zhai, Y., Yang, K., Chen, Z., 2020. Influence of electron beam irradiation on the moisture and properties of freshly harvested and sun-dried rice. Foods 9 (9), 1139. https://doi.org/10.3390/foods9091139.
Park, J.S., Ha, J.W., 2019. X-ray irradiation inactivation of *Escherichia coli* O157: H7, *Salmonella enterica* Serovar Typhimurium, and *Listeria monocytogenes* on sliced cheese and its bactericidal mechanisms. Int. J. Food Microbiol. 289, 127–133. https://doi.org/10.1016/j.ijfoodmicro.2018.09.011.
Pillai, S.D., Shayanfar, S., 2017. Electron beam technology and other irradiation technology applications in the food industry. In: Venturi, M., D'Angelantonio, M. (Eds.), Applications of Radiation Chemistry in the Fields of Industry, Biotechnology and Environment. Springer International Publishing AG, Switzerland, pp. 249–268. https://doi.org/10.1007/978-3-319-54145-7_9.
Rajkumar, R., Chandirasekaran, V., Geetha, M., Muthulakshmi, M., Kumar, K.R., 2022. Preparation of moist pet food using retort process technology and its effect on the hunter laboratory colour values during storage. Pharm. Innov. 11 (8), 2245–2247.
Ricciardi, E.F., Lacivita, V., Conte, A., Chiaravalle, E., Zambrini, A.V., Del Nobile, M.A., 2019. X-ray irradiation as a valid technique to prolong food shelf life: the case of ricotta cheese. Int. Dairy J. 99, 104547. https://doi.org/10.1016/j.idairyj.2019.104547.
Rooijen, C.V., Bosch, G., Van der Poel, A.F., Wierenga, P.A., Alexander, L., Hendriks, W.H., 2014. Reactive lysine content in commercially available pet foods. J. Nutr. Sci. 3, e35. https://doi.org/10.1017/jns.2014.29.
Sahoo, M., Aradwad, P., Panigrahi, C., Kumar, V., Naik, S.N., 2023. Irradiation of food. In: Chhikara, N., Panghal, A., Chaudhary, G. (Eds.), Novel Technologies in Food Science. Scrivener Publishing LLC, Massachusetts, pp. 333–373. https://doi.org/10.1002/9781119776376.ch9.
Shin, D.J., Lee, H.J., Lee, D., Jo, C., Choe, J., 2020. Fat replacement in chicken sausages manufactured with broiler and old laying hens by different vegetable oils. Poult. Sci. J. 99 (5), 2811–2818. https://doi.org/10.1016/j.psj.2020.01.008.
Shin, M.H., Lee, J.W., Yoon, Y.M., Kim, J.H., Moon, B.G., Kim, J.H., Song, B.S., 2014. Comparison of quality of bologna sausage manufactured by electron beam or X-ray irradiated ground pork. Korean J. Food Sci. An. 34 (4), 464. https://doi.org/10.5851/Fkosfa.2014.34.4.464.
Soladoye, O.P., Juárez, M.L., Aalhus, J.L., Shand, P., Estévez, M., 2015. Protein oxidation in processed meat: mechanisms and potential implications on human health. Compr. Rev. Food Sci. Food Saf. 14 (2), 106–122. https://doi.org/10.1111/1541-4337.12127.
Song, H.Y., Kim, K.I., Han, J.M., Park, W.Y., Seo, H.S., Lim, S., Byun, E.B., 2022. Ionizing radiation technology to improve the physicochemical and biological properties of natural compounds by molecular modification: a review. Radiat. Phys. Chem. 194, 110013. https://doi.org/10.1016/j.radphyschem.2022.110013.
Song, W.J., Sung, H.J., Kim, S.Y., Kim, K.P., Ryu, S., Kang, D.H., 2014. Inactivation of *Escherichia coli* O157: H7 and *Salmonella* Typhimurium in black pepper and red pepper by gamma irradiation. Int. J. Food Microbiol 172, 125–129. https://doi.org/10.1016/j.ijfoodmicro.2013.11.017.
Soon, J.M., Seaman, P., Baines, R.N., 2013. *Escherichia coli* O104: H4 outbreak from sprouted seeds. Int. J. Hyg. Environ, 216 (3), 346–354. https://doi.org/10.1016/j.ijheh.2012.07.005.
Stefanova, R., Vasilev, N.V., Spassov, S.L., 2010. Irradiation of food, current legislation framework, and detection of irradiated foods. Food Anal. Methods 3, 225–252. https://doi.org/10.1007/s12161-009-9118-8.
Tahergorabi, R., Matak, K.E., Jaczynski, J., 2012. Application of electron beam to inactivate *Salmonella* in food: recent developments. Food Res. Int. 45 (2), 685–694. https://doi.org/10.1016/j.foodres.2011.02.003.

Tapia, M.S., Alzamora, S.M., Chirife, J., 2020. Effects of water activity (a_w) on microbial stability as a hurdle in food preservation. In: Barbosa-Cánovas, G.V., Fontana, A.J., Schmidt, S.J., Labuza, T.P. (Eds.), Water Activity in Foods: Fundamentals and Applications. Wiley-Blackwell, New Jersey, pp. 323–355. https://doi.org/10.1002/9781118765982.ch14.

Tatum Jr, D., 2016. The Effect of Labeling on Mitigating Cognitive Biases about Food Irradiation: An Empirical Evaluation of Effects on Consumers' Attitudes and Purchase Intent (Doctoral dissertation). Georgia State University. https://doi.org/10.57709/15010762.

Taylor, B.J., Quinn, A.R., Kataoka, A., 2019. *Listeria monocytogenes* in low-moisture foods and ingredients. Food Control 103, 153–160. https://doi.org/10.1016/j.foodcont.2019.04.011.

Thakur, B.R., Singh, R.K., 1994. Food irradiation-chemistry and applications. Food Rev. Int. 10 (4), 437–473. https://doi.org/10.1080/87559129409541012.

Tian, F., Decker, E.A., Goddard, J.M., 2013. Controlling lipid oxidation of food by active packaging technologies. Food Funct. 4 (5), 669–680. https://doi.org/10.1039/C3FO30360H.

Trindade, R.A., Mancini-Filho, J., Villavicencio, A.L.C.H., 2010. Natural antioxidants protecting irradiated beef burgers from lipid oxidation. LWT - Food Sci. Technol. 43 (1), 98–104. https://doi.org/10.1016/j.lwt.2009.06.013.

Watson, P.E., Thomas, D.G., Bermingham, E.N., Schreurs, N.M., Parker, M.E., 2023. drivers of palatability for cats and dogs—what it means for pet food development. Animals 13 (7), 1134. https://doi.org/10.3390/ani13071134.

Zehi, Z.B., Afshari, A., Noori, S., Jannat, B., Hashemi, M., 2020. The effects of X-ray irradiation on safety and nutritional value of food: a systematic review article. Curr. Pharm. Biotechnol. (10), 919–926. https://doi.org/10.2174/1389201021666200219093834.

Zhao, L., Zhang, Y., Guo, S., Xiong, W., Xia, H., Liu, W., Pan, Z., Venkitasamy, C., 2017. Effect of irradiation on quality of vacuum-packed spicy beef chops. J. Food Qual. 2017. https://doi.org/10.1155/2017/1054523.

Zicker, S.C., 2008. Evaluating pet foods: how confident are you when you recommend a commercial pet food? Top. Companion Anim. 23 (3), 121–126. https://doi.org/10.1053/j.tcam.2008.04.003.