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Effects of irradiation on microbiological safety and physicochemical properties of dry pet food

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Abstract

The objective of this study was to investigate the effects of electron beam (EB) and X-ray (XR) irradiation on dry pet food during long-term storage. The samples were irradiated with EB and XR at doses of 0, 2.5, 5, 10, and 20 kGy, and their microbial safety and quality/oxidation properties were analyzed over 56 days under storage conditions of 25°C and 70% relative humidity. As a result, total aerobic bacteria (TAB) and yeasts and molds (YM) significantly decreased as the doses of EB and XR increased. When treated with 10 kGy for both irradiations, no bacteria were detected in the dry pet food, and this effect remained during the 56-day storage period. While EB and XR were effective in reducing aflatoxin B1 (AFB1) in solution, they showed limited effect on dry pet food. On the other hand, changes in quality traits such as proximate compositions, pH, water activity, color, and volatile basic nitrogen due to EB and XR were negligible. However, both types of irradiation induced lipid and protein oxidation in dry pet food. Also, a significant increase was observed in oxidation-related volatile compounds such as hydrocarbons, aldehydes, and ketones with EB and XR treatment, which suggested these changes could potentially impact the flavor of the dry pet food. The current findings confirm the efficient microbial reduction of dry pet food by EB and XR and the consequent changes in quality and oxidative properties. Future research should focus on sensory evaluations to understand the implications of these oxidized substances on pet preferences and explore potential methods to mitigate negative effects.

Keywords: Dry pet food, Irradiation, X-ray, Electron beam, Microbial safety, Oxidation

Introduction

In recent years, pets have been considered as members of the family [1]. This trend increased the consumers' demand for well-made pet food, and many efforts have been made to develop pet food with a variety of ingredients [2]. Pet food commonly includes a variety of animal and plant-based ingredients, such as chicken, beef, salmon, soy, grains, fats, oils, vitamins, and minerals to provide balanced nutrition and flavor [3].

Although adding different ingredients can provide excellent feed for pets, their involvement can also increase safety concerns for pet food. In the case of dry pet food, the most commonly used type, it undergoes a complex manufacturing process, including grinding, mixing, extrusion, drying, cooling, and packaging [4]. During these processes, the probability of contamination with various raw ingredients, using unhygienic equipment, and cross-contamination, especially by pathogens, can increase [5]. According to the US Food and Drug Administration (FDA) recall database, there were 3,691 pet food recalls in the United States between 2003 and 2022, often due to contamination by *Salmonella* serovars, *Listeria monocytogenes*, fungi, and mycotoxins. Such contamination can lead to symptoms like vomiting, fever, diarrhea, dehydration, and loss of appetite, and in severe cases, pose life-threatening risks on pet animals [6]. Especially, if ingested continuously, even small amounts of mycotoxins can accumulate to high levels in the liver, potentially inducing cancer. Therefore, preventing microbial and mycotoxin contamination in pet food before consumption is essential.

Meanwhile, irradiation may effectively decrease both microorganisms and mycotoxin in food products while minimizing nutritional loss and adverse changes in its quality, as it is conducted without heat [7]. Three different types of irradiation sources, namely gamma-ray, electron beam (EB), and X-ray (XR), can be applied in the food sector. Gamma-ray irradiation,

despite its highest penetration capabilities, involves the use of radioactive isotopes, posing safety concerns [8]. In contrast, EB and XR technologies provide a safer alternative due to their electrical generation methods, ceasing emissions when it is not in operation [9]. This safety advantage drives increasing preference for EB and XR in the food industries and among consumers [10]. EB consist of electrons flowing directly, whereas XRs are generated when the motion of electrons interacts with atoms, transforming into electromagnetic radiation [11]. Generally, there is a difference in their penetration depth [12]. EBs interact directly with materials, causing them to lose energy quickly within the material. On the other hand, XRs are a form of electromagnetic wave with very short wavelengths and possess stronger penetrating power.

Several studies have explored the decontamination effects and physicochemical quality changes in various foods such as fruits, vegetables, grains, meats, seafoods, and dairy products following irradiation with EB and XR [13,14]. However, the impact of irradiation on the quality of pet food remains largely unexplored. Also, the differential effects on pet food quality attributable to the distinct generation mechanisms of EB and XR remain underexplored. Therefore, we evaluated the decontamination effects of EB and XR on the microorganisms and mycotoxins in dry pet food as well as the consequent changes to its physicochemical properties.

Materials and Methods

Sample preparation

The dry pet food in the form of extruded kibble (10 mm in diameter) was supplied by ATbio Co., Ltd. (Namyangju-si, Korea). The samples (100 g) were divided into air-impermeable bags and sealed for EB and XR treatments. Then, sample packs were stacked to a thickness of 5 cm to minimize deviations in the transmittance of the irradiation.

90

91 **Irradiation treatment**

92 Before the irradiation process, two 5 mm alanine dosimeters (Bruker Biospin GmbH,
93 Rheinstetten, Germany) were attached to the front and back of the sample packaging,
94 perpendicular to the direction of irradiation treatment. The dosimeters were analyzed using an
95 electron paramagnetic resonance analyzer (e-scan^{TM} alanine dosimeter reader, Bruker BioSpin
96 GmbH), following International Atomic Energy Agency standardization procedures.

97 EB irradiation was performed at the Advanced Radiation Technology Institute of the Korea
98 Atomic Energy Research Institute using a 10 MeV linear electron accelerator (MB 10-30,
99 Mevex, Stittsville, Ontario, Canada). The beam was maintained at a constant level, and samples
100 were exposed to EB doses of 2.5, 5, 10, and 20 kGy at ambient temperature. XR irradiation
101 was conducted using a high-energy linear accelerator (MB10-8/635, UEL V10–10S, Seoul
102 Radiology Services Co., Eumseong, South Korea) with a beam energy of 7 MeV. Samples were
103 exposed to XR doses of 2.5, 5, 10, and 20 kGy at a temperature of 25°C. A non-irradiated group
104 (0 kGy) was used as the control.

105 After irradiation, the sample bags were opend and stored in aerobic conditions at 25°C and
106 70% relative humidity to mimic the consumer's storing pattern. Each sample was collected for
107 further analysis on days 0, 14, 28, 42, and 56. Since opened dry pet food is typically consumed
108 within 4 to 6 weeks, we set a 56-day maximum to reflect realistic usage conditions.

109

110 **Microbial analysis**

111 After being irradiated, each 5 g sample was aseptically collected. Microorganisms were
112 enumerated following the method by Park et al. [15]. The sample was homogenized for 2 min
113 using a stomacher (BagMixer400P, Interscience, St. Nom, France) in sterile Whirl-Pak bags

with 45 mL of sterile saline solution. The solution was serially diluted, and aliquots were spread onto plate count agar (PCA) and potato dextrose agar (PDA). PCA plates were incubated at 37°C for 48 h, and PDA plates at 25°C for 120 h. Colonies on PCA plates were counted as total aerobic bacteria (TAB) and those on PDA plates as yeast and molds (YM), expressed as colony-forming units per gram (CFU/g). Each distinct single colony was isolated and identified according to the method described by Lee et al. [16].

Aflatoxin B1 (AFB1) decontamination

Inoculation of AFB1

To prepare AFB1 solution sample, AFB1 (≥ 98.0%, Sigma) in powder form was dissolved in acetonitrile to obtain a concentration of 80.00 μg/L. Each 100 mL of this solution was transferred to nylon polyethylene/polypropylene bags and sealed. The bags were then irradiated with electron beam and X-ray at doses of 0, 2.5, 5, 10, and 20 kGy.

To prepare AFB1 spiked dry pet food sample, AFB1 powder was diluted to 0.004 μg/L in acetonitrile, and 1 mL of this solution was used to spike 50 g of dry pet food, reaching a final concentration of 80.00 μg/kg. Each 50 g of sample was then transferred to nylon polyethylene/polypropylene bag (size: 15×20 cm, thickness: 0.07 mm, wire diameter, Inc.) and sealed. The bags were irradiated with EB and XR at doses of 0, 2.5, 5, 10, and 20 kGy.

Analysis of AFB1

Total AFB1 in the samples was determined by HPLC following extraction, purification, and qualitative & quantitative analysis. (i) Extraction: The homogenized dry pet food sample (25 g) was extracted with 100 mL of 70% methanol for 30 min, followed by centrifugation at 3,000 rpm and 4 °C for 15 min. The solution was filtered through a 0.2 μm syringe filter, and

40 mL of 0.1% Tween PBS was added to 10 mL of the filtrate. (ii) Purification: The sample solution (20 mL) was injected into the immunoaffinity column, with flow adjusted to 2-3 mL/min. After passing through, the column was washed with 10 mL of 0.1% Tween PBS and 10 mL of distilled water. To elute the bound AFB1, 1 mL of methanol followed by 1 mL of distilled water was used. (iii) HPLC analysis: The purified sample was injected into the C18 UG120 HPLC column (4.6 × 250 mm, 5 μm). The mobile phase consisted of acetonitrile, methanol, and distilled water in a 1:3:6 (v/v) ratio. The injection volume was 10 μL, with a flow rate of 1.2 mL/min. A fluorescence detector with wavelength of 360 nm for excitation and 450 nm for emission was used. The AFB1 concentration was calculated by comparing peak areas to a standard curve.

Quality properties

pH

The pH was measured as described by Jung et al. [17]. The sample (1 g) was added to 9 mL of distilled water and homogenized for 30 s. After centrifuging the homogenate at 2,265 ×g (Continent 512R, Hanil Co., Ltd., Incheon, Korea), the supernatant was filtered (Whatman No.1, Whatman PLC., Kent, UK), and the pH was measured using a pH meter (Seven2GO, Mettler-Toledo Inc., Schwerzenbach, Switzerland).

Water activity

To measure the water activity of the dry pet food, 3 g of the sample were placed in a water activity meter (HygroPalm HP23-AW-A, Rotronic, Bassersdorf, Switzerland), and the readings were taken after equilibration.

Oxidation properties

Thiobarbituric acid reactive substance (TBARS)

The TBARS value was determined using the methods described by Park et al. [15]. First, 5 g of minced sample was combined with 15 mL of DDW and 50 μL of 7.2% 2,6-Di-tert-butyl-4-methyl-phenol in ethanol, then homogenized at 9,600 rpm for 30 s (T25 basic, IKA Works, Inc.). The homogenate was centrifuged at 2,265 ×g (Continent 512R, Hanil Co., Ltd.), and the supernatant was filtered (Whatman No.4). A 1 mL aliquot of the filtrate was mixed with 2 mL of 20 mM thiobarbituric acid in 15% TCA, heated at 90°C for 30 min, cooled, vortexed, and centrifuged at 2,265 ×g for 15 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (M23, Molecular Devices, USA). TBARS values were expressed as mg of MDA per kg of dry pet food, calculated using a standard curve.

Carbonyl content

The carbonyl content was measured using the method described by Lee et al. [18]. The dry pet food sample (1 g) was homogenized (T25 basic, IKA Works, Inc.) in 10 mL of 0.6 M NaCl in 20 mM sodium phosphate buffer (pH 6.5) at 9,600 rpm for 30 s. The homogenate was divided into 2 test tubes, one for carbonyl content and the other for protein content. Each tube received 0.2 mL of homogenate and 1 mL of 10% TCA, then centrifuged at 1,000 ×g for 10 min, after which the supernatant was removed. For protein content, 1 mL of 2 M HCl was added to the pellet, reacted at room temperature for 1 h, followed by another centrifugation after adding 1 mL of 10 % TCA, and the supernatant was discarded. Then, 2 mL of 6 M guanidine HCl in 20 mM sodium phosphate (pH 6.5) was added and the solution was diluted 5-fold. Absorbance was measured at 280 nm using a spectrophotometer (X-ma 3100, Human Co Ltd., Seoul, Korea), and the protein content was quantified using a standard curve obtained with bovine serum albumin. To determine carbonyl content, 0.2% DNPH in 2 M HCl (1 mL) was added to the pellet, reacted at room temperature for 1 h, then centrifuged with 1 mL of 10 % TCA, and

188 the supernatant was discarded. To wash the DNPH color, 1 mL of ethanol and ethyl acetate
189 (1:1, v/v) solution was added, followed by vortexing and centrifugation at 1,000 ×g, after which
190 the supernatant was removed. This washing process was repeated three times. Then, 2 mL of 6
191 M guanidine HCl in 20 mM sodium phosphate (pH 6.5) was added, and absorbance was
192 measured at 370 nm. Carbonyl content was expressed as nmol carbonyls mg^{-1} using a molar
193 absorptivity of 22,000 M^{-1} cm^{-1}.
194
195 **Volatile compounds analysis**
196 Volatile compounds in dry pet food were analyzed using the solid-phase microextraction
197 and gas chromatography-mass spectrometry (SPME-GC-MS) method described by Ismail et
198 al. [19]. The dry pet food sample (3 g) was placed into a 20-mL headspace vial and sealed with
199 a PTFE-faced silicone septum. For volatile extraction, the vial was warmed to 40°C for 5 min,
200 then a 65 μm polydimethylsiloxane/divinylbenzene fiber (Supelco Inc., Bellefonte, PA, USA)
201 was exposed to the vial's headspace for 60 min. The collected volatiles were desorbed at 270°C
202 in the gas chromatograph's injection port (Trace 1310, Thermo Fisher Scientific, Waltham, MA,
203 USA) in splitless mode. Helium served as the carrier gas at a flow rate of 2 mL/min, facilitating
204 the separation of volatile compounds in a fused silica capillary column (DB-Wax, 60 m × 0.25
205 mm i.d., 0.50 μm film thickness; Agilent Technologies, Santa Clara, CA, USA). The GC oven
206 temperature started at 40°C, increased to 180°C at a rate of 5°C/min, then rose to 200°C at
207 2°C/min and held for 5 min, before increasing to 240°C at 10°C/min, held for 10 min. The
208 triple quadrupole mass spectrometer (TSQ 8000, Thermo Fisher Scientific, Waltham, MA,
209 USA), directly connected to the column, operated in electron ionization mode at 70 eV and
210 250°C. Mass spectra were acquired over a scan range of 35 to 550 m/z at 0.2 s intervals. Volatile
211 compounds were identified by matching their mass spectra with the National Institute of
212 Standards and Technology mass spectral library.

Statistical analysis

For assessing the effect of irradiation treatment on microbial activity and quality attributes, all samples were analyzed in triplicate. Data was analyzed using SAS software (Version 9.4, SAS Institute, Inc., Cary, NC, USA). A one-way ANOVA with Tukey's test was utilized to identify significant differences between the means ($p < 0.05$).

Results and Discussion

Microbial analysis

Total aerobic bacteria (TAB)

The initial count of TAB in the dry pet food was 2.84 log CFU/g (Fig. 1). Irradiation showed a dose-dependent inactivation effect, with TAB significantly reduced from 5 kGy of EB and 2.5 kGy of XR. At 10 kGy, no bacteria were detected in EB- and XR-irradiated samples on day 0. This reduction is due to highly reactive free radicals generated by irradiation, damaging bacterial cell membranes and DNA [20]. Since different bacterial species have varying sensitivities for irradiation, the bacteria present in the samples before and after irradiation were identified (data not shown). From the non-irradiated samples, 14 different bacteria were observed: *Acinetobacter radioresistens, Bacilus cerues, Bacilus glycinifermentans, Bacillus haynesii, Bacillus inaquosorum, Bacillus licheniformis, Bacillus sp. (in: firmicutes), Bacillus sp. THJ-DT1, Bacillus subtilis, Bacillus tequilensis, Priestia megaterium, Rummeliibacillus sp., Rummeliibacillus stabekisii,* and *Staphylococcus sp. BCRC 81404*. Among them, *Bacillus cereus* and *Bacillus licheniformis* are known as pathogenic bacteria. Both pathogens were eliminated from the dry pet food when 2.5 kGy of EB and XR were treated. One and three different bacteria remained in EB- and XR-treated samples up to 5 kGy, respectively, however, all bacteria were sterilized at 10 kGy of EB and XR.

238 On the other hand, there was no significant difference in TAB counts between EB and XR
239 during the whole storage period (Fig. 1). Generally, XR penetrates deeper than EB [12],
240 however, our results did not reflect this, possibly due to the location of TABs in the dry pet
241 food. Both EB and XR may sufficiently penetrate when TABs are at shallow depths. In this
242 study, the height of the samples was 5 cm during irradiation. Additionally, penetration depth
243 does not always correlate with high inactivation, as charged particles from EB are known to
244 interact more intensively with matter than photons from XR [21]. This phenomenon is also
245 supported by other studies, such as Jung et al. [22], which found the D10 value of EB was
246 lower than XR, indicating a higher inactivation effect with EB.

247 In different food resources, TABs can grow with increasing storage period [23]. However,
248 most TAB counts in dry pet food did not change significantly throughout the storage period,
249 except for XR on day 56 (Fig. 1). The initial TAB count was 2.84 log CFU/g and did not exceed
250 2.92 log CFU/g despite of long-term storage. This low TAB level in dry pet food may be
251 attributed to its low water activity (ranged 0.4-0.5, Table 2), as most bacteria require water
252 activity above 0.9 to survive [24].
253
254 **Yeasts and molds (YM)**
255 Before irradiation, the number of YM were 2.17 log CFU/g (Fig. 2). This count was not
256 significantly reduced with 2.5 kGy of EB and XR. However, 5 kGy sterilized all YMs in the
257 dry pet food on day 0, regardless of irradiation type. Previous studies have shown that the
258 inactivation effect on YM is due to an increase in chitinase activity and a decrease in chitin
259 content within fungal cell walls, leading to their collapse [25]. In addition, irradiation can
260 increase intracellular H_2O_2 content, inducing oxidative stress, further contributing to the
261 inactivation of YM. Here, we also confirmed the effect on different YMs. A total of six YMs,

Aspergillus sydowii, Cladosporium parasphaerospermum, Diaporthe eres, Penicillium brevicompactum, Schizophyllum commune, and *Schizophyllum sp.*, were detected in non-irradiated samples (data not shown). However, both EB and XR eliminated all YMs except *Schizophyllum commune*. Similar to the result in TAB (Fig. 1), we also found no significant difference for YMs between EB and XR (Fig. 2).

On the other hand, significant increase in YM counts were observed over the extended storage period. In non-irradiated samples, YM numbers slightly increased on day 14 and decreased thereafter. However, variations were small, with counts mostly ranging from 1.97-2.62 log CFU/g during the whole storage period. In the irradiated dry pet food, YM counts remained lower until day 42, with an increase in EB-treated samples on day 56. This increase in YMs may be due to various factors, including penetration depth, survival condition, and recontamination.

AFB1 decontamination

AFB1 is a fungal toxin, and pet food, especially dry, is prone to its contamination [26]. When pets consume AFB1 in pet food, it can cause poisoning symptoms and serious liver damage, potentially leading to cancer with long-term exposure [27]. The effects of EB and XR on AFB1 decontamination were examined in both AFB1-inoculated solution and samples (Fig. 3). EB and XR could reduce AFB1 concentration in solution (80.00 μg/L), but were not effective in dry pet food (80.00 μg/kg). In solution, a higher dose resulted in greater AFB1 reduction. When treated with 5 kGy of EB and 10 kGy of XR, AFB1 in the solution was eliminated. Similar to the previous studies, this result showed the potential of these treatments for AFB1 reduction [28,29]. Irradiation can generate free radicals that damage the structure of AFB1, reducing its mutagenicity and cytotoxicity [30,31]. Wang et al. [32] reported that EB

irradiation degraded AFB1 into two different products, $C_{14}H_{12}O_5$ and $C_{17}H_{14}O_5$.

However, both EB and XR did not reduce AFB1 in dry pet food (Fig. 3), possibly due to the low moisture content. Moisture affects mycotoxins degradation, as radiolysis of water during irradiation generates highly reactive hydroxyl radicals (H• and HO•) [33]. Liu et al. [34] found that AFB1 degradation in peanuts increased with moisture content. Woldemariam et al. [35] found no significant AFB1 reduction in red pepper irradiated with 30 kGy of EB. This suggests that AFB1 in dry pet food may be difficult to decontaminate, and the irradiation dose used may not be sufficient to achieve significant reduction. Temcharoen et al. [36] suggested that very high doses, ranging from 50 to 100 kGy, are needed to deactivate aflatoxins in certain foods. Liu et al. [34] observed the degradation of AFB1 in peanut meal with EB up to 300 kGy. However, achieving such high doses of irradiation is impractical for commercial applications due to the cost, potential damage to the food product, and regulatory limitations.

Instead of AFB1, controlling fungal growth in the dry pet food and its ingredients may significantly lower the risk of mycotoxins. Since AFB1 is primarily produced by *Aspergillus flavus* [37], controlling such fungi through irradiation can prevent AFB1 occurrence. For instance, reducing *Aspergillus flavus* in Brazil nuts with 5 kGy and 10 kGy of EB and gamma rays also reduced aflatoxin levels [38]. Zhang et al. [39] used gamma rays at 10, 20, and 30 kGy on soybeans to control *Aspergillus flavus*, achieving significant AFB1 reduction. Therefore, it is essential to deactivate mycotoxin-producing fungi, including those responsible for aflatoxin production like AFB1, through irradiation before toxin formation occurs.

Quality properties

pH

Changes in the pH can affect the flavor, texture, and color of food by altering the acidity

and impacting the structure of components like pigments, fibers, and proteins [40]. In this study, XR did not change the pH value in dry pet food during the whole storage period (Table 1). However, the pH of EB-treated samples increased significantly with higher doses, occasionally surpassing that of XR-treated samples ($P<0.05$). Generally, the pH increase with irradiation is attributed to the influence of free radicals [41]. According to Paul et al. [42], pH changes are attributed to protonation stimulated by radical reactions, potentially affected by ionic interactions. While EB has a shallower penetration compared to XR [43], high-energy charged particles from EB interact more intensively with materials than XR photons [21]. This more intense interaction may result in greater pH increases when using EB compared to XR (Table 1).

Over the storage period, the pH value of non-irradiated samples significantly increased. However, both EB and XR remained stable pH levels during storage, except for EB at 20 kGy. The rise in pH observed during storage could result from protein degradation, forming small nitrogen-containing components with alkaline properties [44]. This increase may also be due to microorganisms in dry pet food degrading proteins and producing nitrogen compounds like ammonia, leading to higher pH levels [45]. Therefore, it can be said that EB and XR irradiation contributed to inhibiting microbial growth, thus helping prevent changes in pH.

The pH changes were practically small, ranging from 6.35 to 6.41, and there were no significant differences in color (Table S1-S3) and volatile basic nitrogen (VBN) value (Table S4) due to irradiation or the storage period. Additionally, the measured range of proximate composition (Table S5), including moisture (5.30-6.58%), crude protein (34.29-34.66%), crude fat (10.49-13.84%), crude fiber (3.34-4.39%), and crude ash (7.55-7.97%), showed minimal differences, indicating that EB and XR up to 20 kGy and a 56-day storage period did not significantly affect the overall quality of the dry pet food.

Water activity

Water activity represents the availability of water for biochemical reactions and is expressed as the ratio of the vapor pressure in a substance to the vapor pressure of pure water [46]. Until day 14, irradiation did not change the water activity in dry pet food, except for XR on day 0 (Table 2). From day 28, water activity varied with irradiation types and doses, but no specific trend was observed. The range of water activity in dry pet food, from 0.437 to 0.560, was not conducive to microbial growth [47]. Bacteria cannot grow below 0.91 [24], and molds cannot grow below 0.80 [48], which explains the lack of significant increase in microorganisms over time as shown in Fig. 1 and Fig. 2. Meanwhile, water activity fluctuated with storage days without any consistent trend, likely due to the variable temperature and humidity conditions at the measurement site during the storage period.

Oxidation properties

Thiobarbituric acid reactive substance (TBARS)

TBARS values measure the level of malondialdehyde (MDA), which is a product of lipid peroxidation [49]. This indicates lipid spoilage progression, which can affect the sensory quality of food, impacting taste, odor, and overall acceptability [50]. On the whole, EB- and XR-treated samples showed higher TBARS values than the control during 56-day storage period (Table 3). Also, their values were largely increased with higher doses ($P<0.05$). Specifically, the non-irradiated sample had 3.58 mg MDA/kg, while 20 kGy of EB and XR increased this to 5.31 mg MDA/kg and 5.33 mg MDA/kg, respectively. This increase is possibly by free radicals produced during the irradiation process [51]. Lipid oxidation by free radicals involves initiation, propagation, and termination stages. In initiation, reactive oxygen species create lipid radicals from unsaturated fatty acids. During propagation, these radicals

form lipid peroxyl radicals that react with other lipids to produce unstable lipid hydroperoxides (ROOH). These hydroperoxides then degrade into aldehydes, ketones, and alcohols, affecting the taste, smell, and overall quality of food, until termination stabilizes the radicals [52]. On the other hand, no significant differences were observed between EB and XR treatments.

In all irradiation doses, TBARS values tended to increase over the storage period, indicating the accumulation of lipid oxidation products [53]. A slight decrease in these values was observed on day 56 (Table 3). This phenomenon could be attributed to microbial metabolism or binding to the other substances [54,55]. In summary, the increase in TBARS values with irradiation was more pronounced than the effects of storage time, as EB and XR can significantly promote lipid oxidation. This should be considered since lipid oxidation can deteriorate to safety and sensory qualities of food [56].

Carbonyl contents

Protein carbonyl usually originates from the oxidation of amino acid side chains or the breakdown of peptide chains with oxidation [13]. During the storage period, protein carbonyl content in irradiated samples was significantly higher compared to the control (Table 4). The content increased with higher irradiation doses ($P<0.05$). Free radicals produced during irradiation can cause protein oxidation, generating protein carbonyl content [57]. Feng et al. [13] reported that raw ground beef treated with EB irradiation develops higher protein carbonyl content than the control. Li et al. [58] also found that irradiation increases protein carbonyl levels in a pork meat emulsion system. Furthermore, it has been reported that many lipid-derived radicals and hydroperoxides also contribute to the formation of carbonyl contents by accelerating protein oxidation [59]. Therefore, the increase in lipid oxidation levels shown in the TBARS results (Table 3) could also be linked to the increased carbonyl contents in EB- and XR-treated samples (Table 4).

384 Comparing EB and XR, their carbonyl contents were not significantly different, except at
385 10 kGy (Table 4). However, as the storage period increased, XR tended to have a greater effect
386 compared to EB ($P<0.05$), with carbonyl content increasing over the storage days. This
387 suggests that free radicals generated from irradiation continued to impact over time.
388 Furthermore, since XR has a deeper penetration depth compared to EB, resulting in a lower
389 scattering at the surface [43], could lead to higher carbonyl content in the XR-treated samples
390 than that in the EB-treated samples. Thus, it can be concluded that over storage time, XR
391 increased protein oxidation more due to deeper penetration in dry pet food and the persistent
392 effect of irradiation-induced radicals.
393
394 **Volatile compounds**
395 Volatile compounds were analyzed to assess the impact of EB and XR on odor changes in
396 dry pet foods (Table 5). Among the many peaks, 33 oxidation-related volatile compounds were
397 identified, including 16 hydrocarbons, 9 aldehydes, 3 ketones, and 5 alcohols. On day 0,
398 significant increases in hydrocarbons, aldehydes, ketones, and alcohols were observed when
399 EB and XR were applied to dry pet food. These increases in volatile compounds are related to
400 oxidation and significantly affect food flavor [60]. It is known that irradiation can generate
401 highly reactive species that accelerate oxidative processes in proteins and lipids, producing
402 many secondary and volatile compounds [61]. In this regard, the increase in volatile
403 compounds aligns with the increase in the TBARS value (Table 3) and the carbonyl content
404 value (Table 4). Moreover, the changes in volatile compounds varied between EB and XR
405 treatments (Table 5), highlighting inconsistent differences between the two irradiation methods.
406 Among the identified hydrocarbons, saturated straight-chain alkanes (n-octane, n-nonane,
407 n-decane, n-dodecane, n-pentadecane, and n-tetradecane) and unsaturated hydrocarbons (1-
408 octene, 1-decene, and 1- undecyne) are known radiolytic products which can be originated

409 from fatty acids [62]. Branched alkanes (2,6,10-timethyldodecane, 5-ethyl-2,2,3-
410 trimethylheptane and 2,6,8-trimethyldecane) significantly increased with irradiation. In
411 addition, several alkane and alkene contents (1-butyl-2-methyl cyclopropane, n-decene, n-
412 octane, n-nonane, 1-decene, and 1-octene) were significantly higher in EB-irradiated samples
413 compared to XR-irradiated samples. The formation of alkanes and alkenes involves ionization
414 and cleavage near carbonyl groups, leading to radical reactions that determine whether alkanes
415 or alkenes are produced based on the cleavage site [21].

416 Irradiation also increases aldehydes and ketones due to free radicals promoting
417 dehydrogenation reactions within molecules. This process includes the oxidation of primary
418 alcohols to aldehydes and secondary alcohols to ketones [63,64]. These oxidation processes
419 increase the content of carbonyl groups (aldehydes and ketones), which aligns with the increase
420 in carbonyl content (Table 4). All 9 detected aldehydes were found in greater quantities in EB-
421 and XR-treated samples compared to non-irradiated ones. Specifically, 2,4-heptadienal, 2-
422 methyl butanal, 3-methyl butanal, hexanal, octanal, and pentanal were higher in XR-treated
423 samples, while 2-heptanal, heptanal, and nonanal were higher in EB-treated samples. The
424 increase in aldehydes indicates lipid oxidation. Aldehydes like heptanal, octanal, nonanal,
425 pentanal, and hexanal are responsible for the unpleasant odors in poultry products [65]. This
426 increase can cause bitter, metallic, and sour taste [61], making the product unpleasant and
427 indicating quality deterioration.

428 The quantities of all 3 detected ketones were higher in EB- and XR-treated samples
429 compared to the control group, with higher levels in XR-treated samples. 2-butanone and 3,5-
430 octadien-2-one maintained this trend after 56 days, while 2-propanone showed no significant
431 difference between EB and XR treatments. The total amount of ketones decreased by day 56,
432 mainly due to a reduction in 2-propanone. It was reported that 3,5-octadien-2-one is a principal

compound causing off-flavor in isolated lentil protein [66]. It is known that this increase in ketone can cause rancid, fruity, acetone-like odor [61]. These odors can give the food a chemical-like smell, which can be unpleasant.

All 5 detected alcohols (6,9-pentadecadien-1-ol, 1-hexanol, 1-octen-3-ol, 1-penten-3-ol, and 2-methyl-2,3-pentanediol) increased significantly with both EB and XR treatments (Table 5). This increase could be due to structural changes in carbohydrates, reduction of aldehydes, and the breakdown of fatty acids during irradiation [62]. These alcohols can serve as precursors to MDA [63]. Also, Mielnik et al. [67] noted that 1-penten-3-ol correlates highly with TBARS values, markers of lipid oxidation. The increase in alcohols due to oxidation can impart an alcoholic or chemical odor, potentially overwhelming the food's original aroma and leading to an unpleasant sensory properties.

Therefore, it is necessary to verify how volatile substances produced by such oxidation actually affect the sense of smell perceived by pets and whether they have any negative effects through sensory evaluation.

Conclusion

Both EB and XR treatments demonstrated excellent efficacy in microbial decontamination of dry pet food without compromising its quality. Furthermore, there were no significant differences between the applications of EB and XR in this study. While higher doses achieved greater decontamination, they also induced oxidation and altered the volatile compounds in the dry pet food. In conclusion, employing EB and XR treatments in dry pet food effectively reduced TAB and YM without compromising its quality. However, given the potential for oxidation, further research is necessary to assess whether these oxidation products adversely

affect the safety and sensory qualities of the food.

Competing interest

The authors declare no conflict of interest.

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Conceptualization: Lee HJ, Jo C..

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Validation: Choi M.

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Writing - review & editing: Park D, Sethukali AK, Choi M, Kim JK, Lee HJ, Jo C.

Ethics Approval and Consent to Participate

This article does not require IRB/IACUC approval because there are no human and animal participants.

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References

1. Laurent-Simpson A. Just like family: How companion animals joined the household. In Just Like Family. New York University Press. 2021. https://doi.org/10.18574/nyu/9781479828852.001.0001.

2. Meeker DL, Meisinger JL. COMPANION ANIMALS SYMPOSIUM: Rendered ingredients significantly influence sustainability, quality, and safety of pet food. J Anim Sci. 2015;93(3):835-847. https://doi.org/10.2527/jas.2014-8524.

3. Remillard RL, Crane SW. Making pet foods at home. Small animal clinical nutrition. 2010;207-223.

4. Le Guillas G, Vanacker P, Salles C, Labouré H. Insights to Study, Understand and Manage Extruded Dry Pet Food Palatability. Animals. 2024;14(7):1095. https://doi.org/10.3390/ani14071095.

5. DeBeer J, Finke M, Maxfield A, Osgood AM, Baumgartel DM, Blickem ER. A Review of Pet Food Recalls from 2003 through 2022. J Food Prot. 2023;100199. https://doi.org/10.1016/j.jfp.2023.100199.

6. Bischoff K. Rumbeiha WK. Pet food recalls and pet food contaminants in small animals: an update. Vet Clin N Am: Small Anim Pract. 2018;48(6):917-931. https://doi.org/10.1016/j.cvsm.2018.07.005.

7. Akhila PP, Sunooj KV, Aaliya B, Navaf M., Sudheesh C, Sabu S, Sasidharan A, Mir SA, George J, Khaneghah AM. Application of electromagnetic radiations for decontamination of fungi and mycotoxins in food products: A comprehensive review. Trends Food Sci Technol. 2021;114:399-409. https://doi.org/10.1016/j.tifs.2021.06.013.

8. Kim YJ, Cha JY, Kim TK, Lee JH, Jung S, Choi YS. The Effect of Irradiation on Meat Products. Food Sci Anim Resour. 2004;44:779-789. https://doi.org/10.5851/kosfa.2024.e35

9. Cleland MR. Advances in gamma ray, electron beam, and X-ray technologies for food irradiation. Food irradiation research and technology. 2006;11-35.

10. INTERNATIONAL ATOMIC ENERGY AGENCY. Development of Electron Beam and X Ray Applications for Food Irradiation. IAEA-TECDOC-2008, IAEA, Vienna. 2022.

11. Nam KC, Jo C, Ahn DU. Irradiation of meat and meat products. Emerging technologies in meat processing: production, processing and technology. 2017;7-36. https://doi.org/10.1002/9781118350676.ch2.

12. GIPA-Gamma Industry Processing Alliance. A Comparison of Gamma, E-Beam, X-Ray and Ethylene Oxide Technologies for the Industrial Sterilization of Medical Devices and Healthcare Products. Whitepaper. 2017.

13. Feng X, Jo C, Nam KC, Ahn DU. Impact of electron-beam irradiation on the quality characteristics of raw ground beef. Innovative Food Sci Emerg Technol. 2019;54:87-92. https://doi.org/10.1016/j.ifset.2019.03.010.

14. Lung HM, Cheng YC, Chang YH, Huang HW, Yang BB, Wang CY. Microbial decontamination of food by electron beam irradiation. Trends Food Sci Technol. 2015;44(1):66-78. https://doi.org/10.1016/j.tifs.2015.03.005.

15. Park D, Lee HJ, Kumar Sethukali A, Yim DG, Park S, Jo C. Effects of Temperature on the Microbial Growth and Quality of Unsealed Dry Pet Food during Storage. Food Sci Anim Resour. 2024. https://doi.org/10.5851/kosfa.2024.e51.

16. Lee HJ, Yoon JW, Kim M, Oh H, Yoon Y, Jo C. Changes in microbial composition on the crust by different air flow velocities and their effect on sensory properties of dry-aged beef. Meat Sci. 2019;153:152-158. https://doi.org/10.1016/j.meatsci.2019.03.019.

17. Jung DY, Lee HJ, Shin DJ, Kim CH, Jo C. Mechanism of improving emulsion stability of emulsion-type sausage with oyster mushroom (Pleurotus ostreatus) powder as a phosphate replacement. Meat Sci. 2022;194:108993. https://doi.org/10.1016/j.meatsci.2022.108993.

18. Lee HJ, Yim DG, Jo C. Effect of plasma-activated organic acids against Salmonella Typhimurium and Escherichia coli O157: H7 inoculated on pork loin and its quality characteristics. Innovative Food Sci Emerg Technol. 2023;88:103455. https://doi.org/10.1016/j.ifset.2023.103455.

19. Ismail A., Lee HJ, Hong SJ, Kim G, Choi M, Jo C. Evaluation of plasma-activated lactic-gallic acid treated chicken meats on the freshness, volatile changes, and metabolites through multi-analytical techniques. Innovative Food Sci Emerg Technol. 2024;91:103544. https://doi.org/10.1016/j.ifset.2023.103544.

20. Al-Masri MR, Al-Bachir M. Microbial load, acidity, lipid oxidation and volatile basic nitrogen of irradiated fish and meat-bone meals. Bioresour Technol. 2007;98(6):1163-1166. https://doi.org/10.1016/j.biortech.2006.05.026.

21. Stewart EM. Food irradiation. Process-Induced Food Toxicants: Occurrence, Formation, Mitigation, and Health Risks. 2009;387-412. https://doi.org/10.1002/9780470430101.ch4b.

22. Jung K, Song BS, Kim MJ, Moon BG, Go SM, Kim JK, Lee YJ, Park JH. Effect of X-ray, gamma ray, and electron beam irradiation on the hygienic and physicochemical qualities of red pepper powder. LWT. 2015;63(2):846-851. https://doi.org/10.1016/j.lwt.2015.04.030.

23. Giannuzzi L, Pinotti A, Zaritzky N. Mathematical modelling of microbial growth in packaged refrigerated beef stored at different temperatures. Int J Food Microbiol. 1998;39(1-2):101-110. https://doi.org/10.1016/S0168-1605(97)00127-X.

24. Sperber WH. Influence of water activity on foodborne bacteria—a review. J Food Prot. 1983;46(2):142-150. https://doi.org/10.4315/0362-028X-46.2.142.

25. Li L, Fan L, Shang F, Zhang Y, Shuai L, Duan Z. Antifungal Activity and Mechanism of Electron Beam Irradiation Against Rhizopus oryzae. J Food Prot. 2023;86(5):100070. https://doi.org/10.1016/j.jfp.2023.100070.

26. Castaldo L, Graziani G, Gaspari A, Izzo L, Tolosa J, Rodríguez-Carrasco Y, Ritieni A. Target analysis and retrospective screening of multiple mycotoxins in pet food using UHPLC-Q-Orbitrap HRMS. Toxins. 2019;11(8):434. https://doi.org/10.3390/toxins11080434.

27. Macías-Montes A, Rial-Berriel C, Acosta-Dacal A, Henríquez-Hernández LA, Almeida-González M, Rodríguez-Hernández Á, Zumbado M, Boada LD, Zaccaroni A, Luzardo OP. Risk assessment of the exposure to mycotoxins in dogs and cats through the consumption of commercial dry food. Sci Total Environ. 2020;708:134592. https://doi.org/10.1016/j.scitotenv.2019.134592.

28. Guo Y, Zhao L, Ma Q, Ji C. Novel strategies for degradation of aflatoxins in food and feed: A review. Food Res Int. 2021;140:109878. https://doi.org/10.1016/j.foodres.2020.109878.

29. Hojjati M, Shahbazi S, Askari H, Makari M. Use of X-Irradiations in Reducing the Waste of Aflatoxin-Contaminated Pistachios and Evaluation of the Physicochemical Properties of the Irradiated Product. Foods. 2023;12(16):3040. https://doi.org/10.3390/foods12163040.

30. Liu R, Wang R, Lu J, Chang M, Jin Q, Du Z, Wang S, Li Qiu, Wang X. Degradation of AFB1 in aqueous medium by electron beam irradiation: Kinetics, pathway and toxicology. Food Control. 2016;66:51-157. https://doi.org/10.1016/j.foodcont.2016.02.002.

31. Wang F, Xie F, Xue X, Wang Z, Fan B, Ha Y. Structure elucidation and toxicity analyses of the radiolytic products of aflatoxin B1 in methanol–water solution. J Hazard Mater. 2011;192(3):1192-1202. https://doi.org/10.1016/j.jhazmat.2011.06.027.

32. Wang SQ, Huang GQ, Li YP, Xiao JX, Zhang Y, Jiang WL. Degradation of aflatoxin B 1 by low-temperature radio frequency plasma and degradation product elucidation. Eur Food Res Technol. 2015;241:103-113. https://doi.org/10.1007/s00217-015-2439-5.

33. Le Caër S. Water radiolysis: influence of oxide surfaces on H2 production under ionizing radiation. Water. 2011;3(1):235-253. https://doi.org/10.3390/w3010235.

34. Liu R, Lu M, Wang R, Wang S, Chang M, Jin Q, Wang X. Degradation of aflatoxin B1 in peanut meal by electron beam irradiation. Int J Food Prop. 2018:21(1):892-901. https://doi.org/10.1080/10942912.2018.1466321.

35. Woldemariam HW, Kießling M, Emire SA, Teshome PG, Töpfl S, Aganovic K. Influence of electron beam treatment on naturally contaminated red pepper (Capsicum annuum L.) powder: Kinetics of microbial inactivation and physicochemical quality changes. Innovative Food Sci Emerg Technol. 2021;67:102588. https://doi.org/10.1016/j.ifset.2020.102588.

36. Temcharoen P, Thilly WG. Removal of aflatoxin B1 toxicity but not mutagenicity by 1 megarad gamma radiation of peanut meal. J Food Saf. 1982;4(4):199-205. https://doi.org/10.1111/j.1745-4565.1982.tb00445.x.

37. Reddy KRN, Raghavender CR, Salleh B, Reddy CS, Reddy BN. Potential of aflatoxin B1 production by Aspergillus flavus strains on commercially important food grains. International J Food Sci Technol.

2011;46(1):161-165. https://doi.org/10.1111/j.1365-2621.2010.02468.x.

38. Assuncao E, Reis TA, Baquiao AC, Correa B. Effects of gamma and electron beam radiation on Brazil nuts artificially inoculated with Aspergillus flavus. J Food Prot. 2015;78(7):1397-1401. https://doi.org/10.4315/0362-028X.JFP-14-595.

39. Zhang ZS, Xie QF, Che LM. Effects of gamma irradiation on aflatoxin B1 levels in soybean and on the properties of soybean and soybean oil. Appl Radiat Isot. 2018;139:224-230. https://doi.org/10.1016/j.apradiso.2018.05.003.

40. Andrés-Bello A, Barreto-Palacios V, García-Segovia P, Mir-Bel J, Martínez-Monzó J. Effect of pH on color and texture of food products. Food Eng Rev. 2013;5:158-170. https://doi.org/10.1007/s12393-013-9067-2.

41. Ruzza P, Honisch C, Hussain R, Siligardi G. Free radicals and ros induce protein denaturation by uv photostability assay. Int J Mol Sci. 2021;22(12):6512. https://doi.org/10.3390/ijms22126512.

42. Paul A, Stösser R, Zehl A, Zwirnmann E, Vogt RD, Steinberg CE. Nature and abundance of organic radicals in natural organic matter: effect of pH and irradiation. Environ Sci Technol. 2006;40(19):5897-5903. https://doi.org/10.1021/es060742d.

43. Kroc TK. Monte Carlo simulations demonstrating physics of equivalency of gamma, electron-beam, and X-ray for radiation sterilization. Radiat Phys Chem. 2023;204:110702. https://doi.org/10.1016/j.radphyschem.2022.110702.

44. Zhang JY, Liu SL, Wang Y, Ding YT. Chemical, microbiological and sensory changes of dried Acetes chinensis during accelerated storage. Food Chem. 2011;127(1):159-168. https://doi.org/10.1016/j.foodchem.2010.12.120.

45. Casaburi A, Piombino P, Nychas GJ, Villani F, Ercolini D. Bacterial populations and the volatilome associated to meat spoilage. Food microbial. 2015;45:83-102. https://doi.org/10.1016/j.fm.2014.02.002.

46. Mathlouthi M. Water content, water activity, water structure and the stability of foodstuffs. Food control. 2001;12(7):409-417. https://doi.org/10.1016/S0956-7135(01)00032-9.

47. Tapia MS, Alzamora SM, Chirife J. Effects of water activity (aw) on microbial stability as a hurdle in food preservation. Water activity in foods: Fundamentals and applications. 2020;323-355. https://doi.org/10.1002/9781118765982.ch14.

48. Leistner L. Basic aspects of food preservation by hurdle technology. Int J Food Microbiol. 2000;55(1-3):181-186. https://doi.org/10.1016/S0168-1605(00)00161-6.

49. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-358. https://doi.org/10.1016/0003-2697(79)90738-3.

50. Sasse A, Colindres P, Brewer MS. Effect of natural and synthetic antioxidants on the oxidative stability of cooked, frozen pork patties. J Food Sci. 2009;74(1):S30-S35. https://doi.org/10.1111/j.1750-3841.2008.00979.x.

51. Li YZ, Cai KZ, Hu GF, Nie W, Liu XY, Xing W, Xu B, Chen CG. γ -Ray irradiation reduces the formation of polycyclic aromatic hydrocarbons during the baking of sausage. Radiat Phys Chem. 2021;183:109406. https://doi.org/10.1016/j.radphyschem.2021.109406.

52. Yin H, Xu L, Porter NA. Free radical lipid peroxidation: mechanisms and analysis. Chem Rev. 2011;111(10):5944-5972. https://doi.org/10.1021/cr200084z.

53. Arshad MS, Amjad Z, Yasin M, Saeed F, Imran A, Sohaib M, Anjum FM, Hussain S. Quality and stability evaluation of chicken meat treated with gamma irradiation and turmeric powder. Int J Food Prop. 2019;22(1):154-172. https://doi.org/10.1080/10942912.2019.1575395.

54. Gomez-Sanchez A, Hermosín I, Maya I. Influence of malondialdehyde on the Maillard degradation of Amadori compounds. Carbohydr Res. 1992;229(2):307-322. https://doi.org/10.1016/S0008-6215(00)90577-9.

55. Shin YG, Rathnayake D, Mun HS, Dilawar MA, Pov S, Yang CJ. Sensory attributes, microbial activity, fatty acid composition and meat quality traits of Hanwoo cattle fed a diet supplemented with stevioside and organic selenium. Foods. 2021;10(1):129. https://doi.org/10.3390/foods10010129.

56. Grebenteuch S, Kroh LW, Drusch S, Rohn S. Formation of secondary and tertiary volatile compounds resulting from the lipid oxidation of rapeseed oil. Foods. 2021;10(10):2417. https://doi.org/10.3390/foods10102417.

57. Estévez M. Protein carbonyls in meat systems: A review. Meat sci. 2011;89(3):259-279. https://doi.org/10.1016/j.meatsci.2011.04.025.

58. Li X, Gao K, Jinfeng B, Wu X, Li X, Guo C. Investigation of the effects of apple polyphenols on the chromatic values of weakly acidic lysine-fructose maillard system solutions. LWT. 2020;125:109237. https://doi.org/10.1016/j.lwt.2020.109237.

59. Fritz KS, Petersen DR. Exploring the biology of lipid peroxidation-derived protein carbonylation. Chem Res Toxicol. 2011;24(9):1411-1419. https://doi.org/10.1021/tx200169n.

60. Gray JI, Monahan FJ. Measurement of lipid oxidation in meat and meat products. Trends Food Sci Technol. 1992;3:315-319. https://doi.org/10.1016/S0924-2244(10)80019-6.

61. Zianni R, Mentana A, Tomaiuolo M, Campaniello M, Iammarino M, Centonze D, Palermo C. Volatolomic approach by HS-SPME/GC–MS and chemometric evaluations for the discrimination of X-ray irradiated mozzarella cheese. Food Chem. 2023;423:136239. https://doi.org/10.1016/j.foodchem.2023.136239.

62. Nawar WW. Volatiles from food irradiation. Food Rev Int. 1986;2(1):45-78.

https://doi.org/10.1080/87559128609540788.

63. Feng X, Ahn DU. Volatile profile, lipid oxidation and protein oxidation of irradiated ready-to-eat cured turkey meat products. Radiat Phys Chem. 2016;127:27-33. https://doi.org/10.1016/j.radphyschem.2016.05.027.

64. Mexis SF, Badeka AV, Chouliara E, Riganakos KA, Kontominas MG. Effect of γ-irradiation on the physicochemical and sensory properties of raw unpeeled almond kernels (Prunus dulcis). Innovative Food Sci Emerg Technol. 2009;10(1):87-92. https://doi.org/10.1016/j.ifset.2008.09.001.

65. Mancinelli AC, Silletti E, Mattioli S, Dal Bosco A, Sebastiani B, Menchetti L, Koot A, Ruth S, Castellini C. Fatty acid profile, oxidative status, and content of volatile organic compounds in raw and cooked meat of different chicken strains. Poult Sci. 2021; 100(2):1273-1282. https://doi.org/10.1016/j.psj.2020.10.030.

66. Chang C, Stone AK, Green R, Nickerson MT. Reduction of off-flavours and the impact on the functionalities of lentil protein isolate by acetone, ethanol, and isopropanol treatments. Food Chem. 2019;277:84-95. https://doi.org/10.1016/j.foodchem.2018.10.022.

67. Mielnik MB, Olsen E, Vogt G, Adeline D, Skrede G. Grape seed extract as antioxidant in cooked, cold stored turkey meat. LWT. 2006;39(3):191-198. https://doi.org/10.1016/j.lwt.2005.02.003.

Figure captions

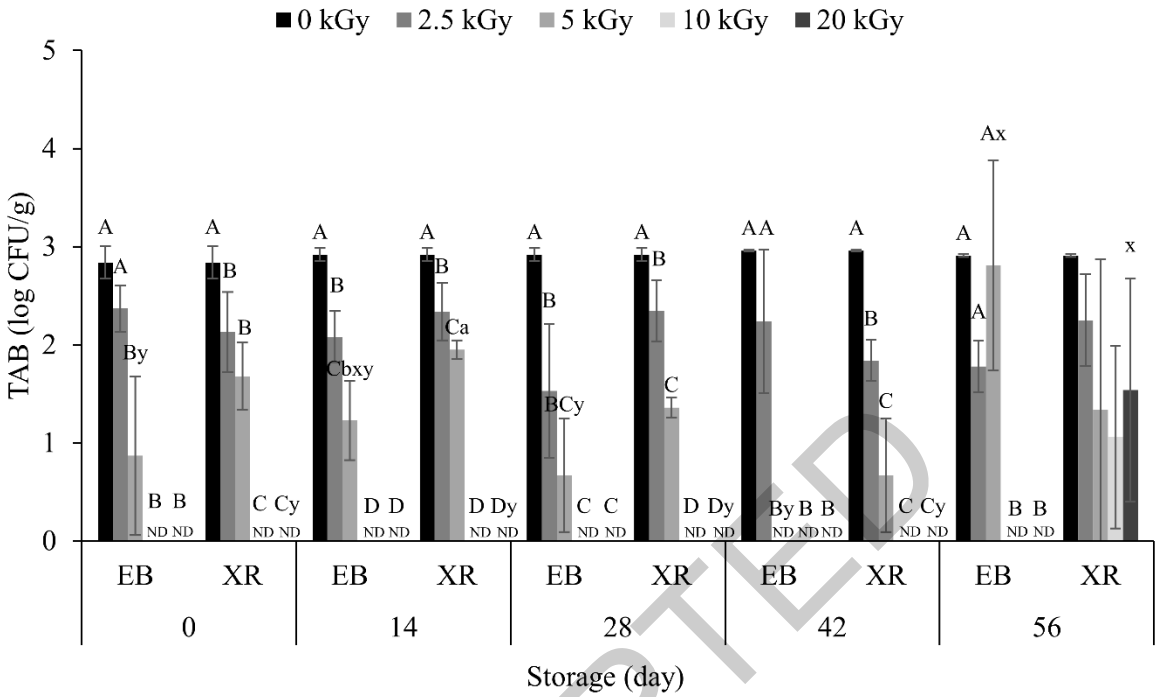
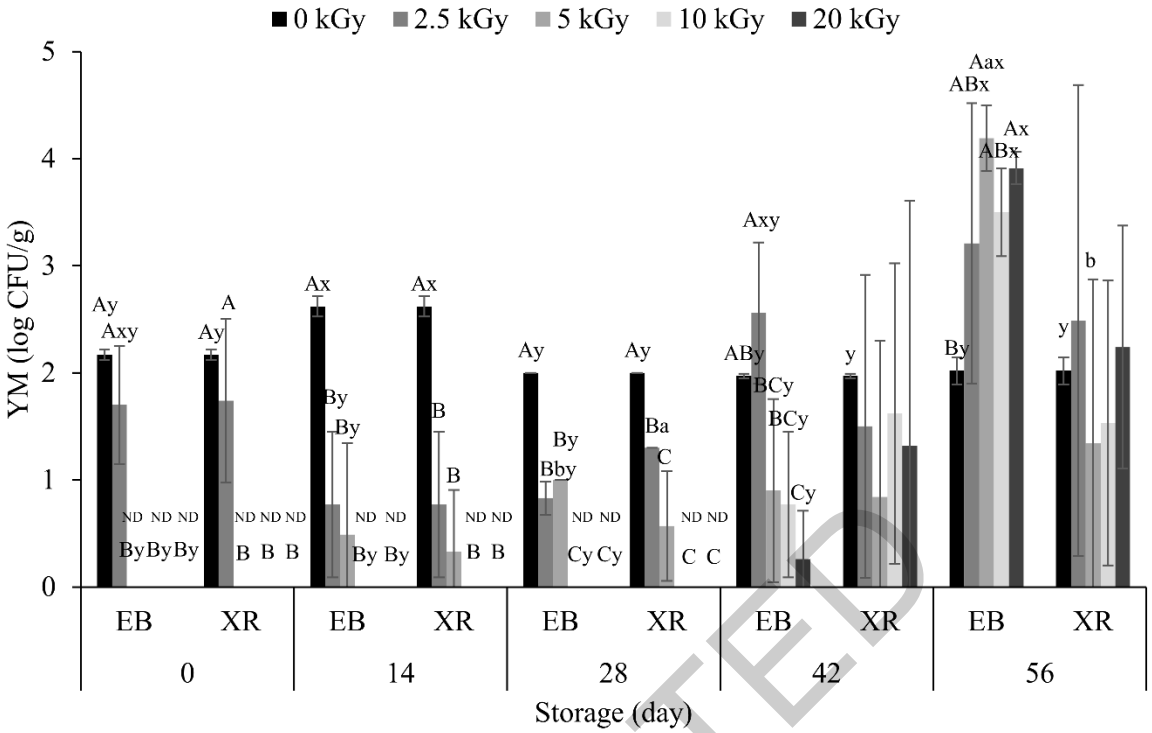


Fig. 1. Inactivation effect of electron beam (EB) and X-ray (XR) irradiation on total aerobic bacteria (TAB) counts (log CFU/g) of dry pet food with different doses and storage. $^{A\text{-}D}$Different letters indicate significant differences ($P < 0.05$) between different irradiation dose treatments. ^{a,b}Different letters indicate significant differences ($P < 0.05$) between different type of irradiation treatments. ^{x,y}Different letters indicate significant differences ($P < 0.05$) between different storage days treatments. ND, not detected.



669

670 Fig. 2. Inactivation effect of electron beam (EB) and X-ray (XR) irradiation on total aerobic bacteria (TAB) counts
671 (log CFU/g) of dry pet food with different doses and storage. [A-C]Different letters indicate significant differences
672 ($P < 0.05$) between different irradiation dose treatments. [a,b]Different letters indicate significant differences ($P <$
673 0.05) between different type of irradiation treatments. [x,y]Different letters indicate significant differences ($P < 0.05$)
674 between different storage days treatments. ND, not detected.

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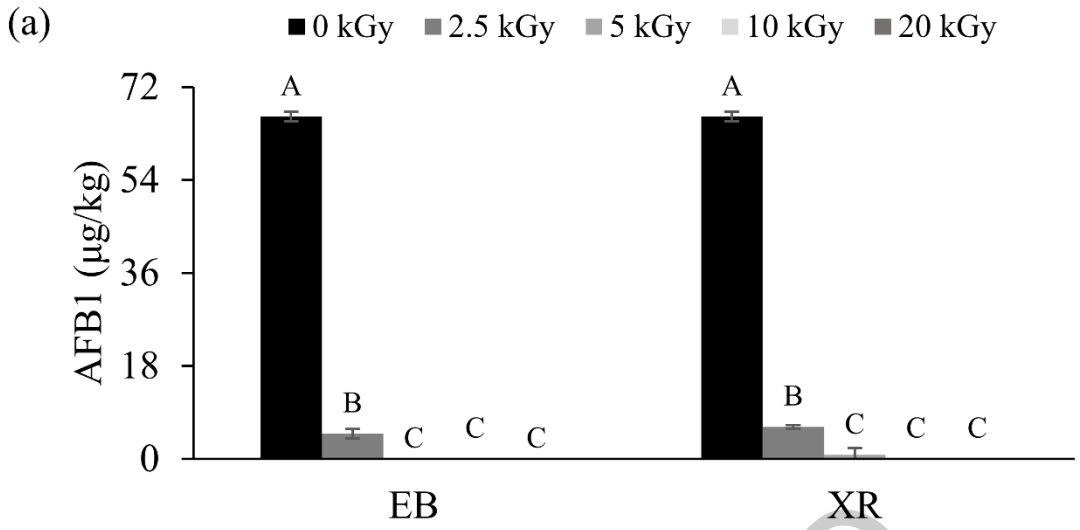

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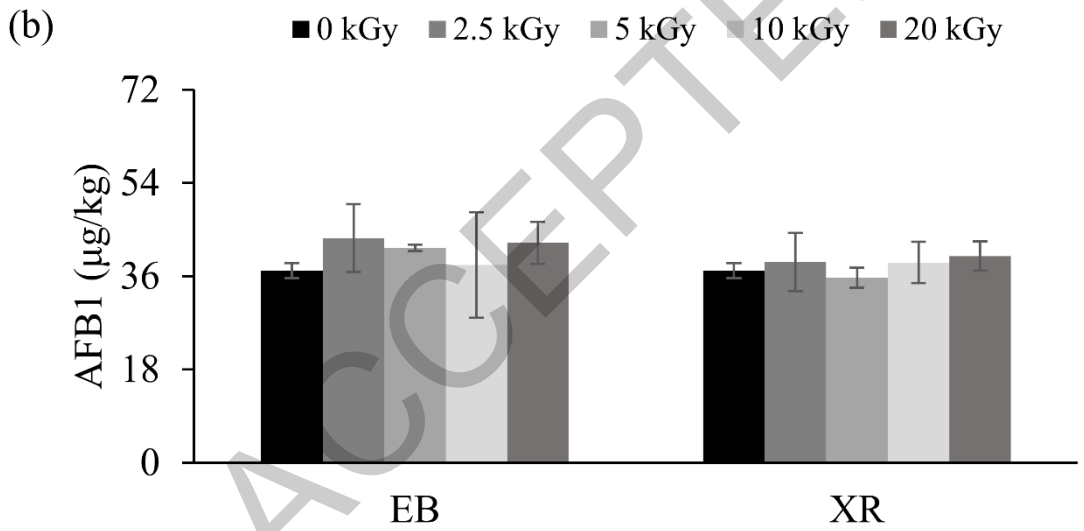

678

679 Fig. 3. Effect of electron beam (EB) and X-ray (XR) irradiation with different doses on aflatoxin B1 (AFB1, μg/kg)
680 of (a) acetonitrile solution and (b) dry pet food. $^{A-C}$Different letters indicate significant differences ($P < 0.05$)
681 between different irradiation dose treatments.

682

683 Table 1. Effect of electron beam (EB) and X-ray (XR) irradiation on pH of dry pet food with different doses and
684 storage days

Storage (Days)	Type	Irradiation dose (kGy)					SEM[1)]
		0	2.5	5	10	20	
0	EB	6.35^{By}	6.38^{AB}	6.40^{Aa}	6.40^{Aa}	6.39^{Ay}	0.006
	XR	6.35^{y}	6.36	6.37^{b}	6.37^{b}	6.37	0.005
	SEM[2)]	0.000	0.005	0.006	0.007	0.005	
14	EB	6.35^{By}	6.37^{AB}	6.40^{AB}	6.40^{Aa}	6.40^{Aaxy}	0.010
	XR	6.35^{y}	6.35	6.36	6.36^{b}	6.36^{b}	0.008
	SEM[2)]	0.000	0.007	0.014	0.010	0.005	
28	EB	6.35^{By}	6.37^{AB}	6.38^{A}	6.39^{A}	6.39^{Aaxy}	0.008
	XR	6.35^{y}	6.36	6.37	6.36	6.36^{b}	0.006
	SEM[2)]	0.000	0.007	0.011	0.008	0.005	
42	EB	6.37^{x}	6.37	6.39	6.39	6.39^{xy}	0.005
	XR	6.37^{x}	6.35	6.38	6.37	6.38	0.015
	SEM[2)]	0.000	0.006	0.018	0.013	0.009	
56	EB	6.38^{Bx}	6.37^{B}	6.40^{AB}	6.41^{Aa}	6.41^{Aax}	0.006
	XR	6.38^{x}	6.37	6.38	6.37^{b}	6.37^{b}	0.006
	SEM[2)]	0.000	0.008	0.006	0.005	0.002	

685 [1]Standard error of the mean (n = 15),[2] (n = 6).

686 [A,B]Different letters indicate significant differences ($P < 0.05$) between different irradiation dose treatments.

687 [a,b]Different letters indicate significant differences ($P < 0.05$) between different type of irradiation treatments.

688 [x,y]Different letters indicate significant differences ($P < 0.05$) between different storage days treatments.

689

690 Table 2. Effect of electron beam (EB) and X-ray (XR) irradiation on water activity of dry pet food with different
691 doses and storage days

Storage (Days)	Type	Irradiation dose (kGy)					SEM[1)]
		0	2.5	5	10	20	
0	EB	0.458^{y}	0.451^{z}	0.458^{z}	0.457^{z}	0.460^{z}	0.0023
	XR	0.458^{ABy}	0.453^{By}	0.454^{Bz}	0.456^{By}	0.464^{Az}	0.0013
	SEM[2)]	0.0000	0.0006	0.0036	0.0012	0.0018	
14	EB	0.458^{x}	0.490^{y}	0.488^{y}	0.487^{y}	0.479^{y}	0.0026
	XR	0.458^{x}	0.491^{x}	0.488^{y}	0.483^{x}	0.484^{y}	0.0016
	SEM[2)]	0.0000	0.0038	0.0010	0.0013	0.0016	
28	EB	0.441^{Bz}	0.438^{Bz}	0.441^{Bz}	0.464^{Aaz}	0.465^{Az}	0.004
	XR	0.441^{Bz}	0.437^{Bz}	0.443^{Bz}	0.442^{Bbz}	0.464^{Az}	0.003
	SEM[2)]	0.0000	0.0046	0.0052	0.0021	0.0027	
42	EB	0.560^{Av}	0.530^{Bbx}	0.538^{Bx}	0.531^{Bx}	0.528^{Bw}	0.0027
	XR	0.560^{Av}	0.563^{Aav}	0.546^{Bw}	0.539^{BCw}	0.529^{Cw}	0.0024
	SEM[2)]	0.0000	0.0016	0.0032	0.0033	0.0020	
56	EB	0.525^{Aw}	0.529^{Ax}	0.526^{Ax}	0.530^{Ax}	0.508^{Bbx}	0.0032
	XR	0.525^{ABCw}	0.534^{Aw}	0.522^{BCx}	0.530^{ABw}	0.518^{Cax}	0.0024
	SEM[2)]	0.0000	0.0022	0.0032	0.0049	0.0010	

692 [1]Standard error of the mean (n = 15),[2] (n = 6).

693 $^{A\text{-}C}$Different letters indicate significant differences ($P < 0.05$) between different irradiation dose treatments.

694 ^{a,b}Different letters indicate significant differences ($P < 0.05$) between different type of irradiation treatments.

695 $^{v\text{-}z}$Different letters indicate significant differences ($P < 0.05$) between different storage days treatments.

696

Table 3. Effect of electron beam (EB) and X-ray (XR) irradiation on TBARS (mg MDA/kg) of dry pet food with different doses and storage days

Storage (Days)	Type	Irradiation Dose (kGy)					SEM[1)]
		0	2.5	5	10	20	
0	EB	3.58^{D}	3.89^{Cz}	4.07^{Cz}	4.65^{B}	5.31^{A}	0.060
	XR	3.58^{D}	3.82^{CDz}	4.04^{Cz}	4.44^{Bz}	5.33^{Ay}	0.064
	SEM[2)]	0.000	0.051	0.056	0.067	0.094	
14	EB	3.66^{D}	4.05^{CDxyz}	4.11^{Cyz}	4.82^{B}	5.61^{A}	0.089
	XR	3.66^{D}	3.99^{Cyz}	4.20^{Cyz}	4.61^{Byz}	5.67^{Axy}	0.051
	SEM[2)]	0.000	0.059	0.056	0.104	0.058	
28	EB	3.70^{D}	4.19^{Cxy}	4.47^{Cx}	4.86^{B}	5.65^{A}	0.075
	XR	3.70^{D}	4.29^{Cx}	4.54^{Cx}	5.07^{Bx}	5.95^{Ax}	0.074
	SEM[2)]	0.000	0.047	0.115	0.054	0.087	
42	EB	3.59^{D}	4.23^{Cx}	4.45^{Cxy}	4.81^{B}	5.70^{A}	0.066
	XR	3.59^{E}	4.10^{Dxy}	4.44^{Cxy}	4.82^{By}	5.84^{Ax}	0.055
	SEM[2)]	0.000	0.072	0.024	0.071	0.080	
56	EB	3.61^{D}	3.96^{Cyz}	4.27^{Caxyz}	4.76^{B}	5.42^{A}	0.073
	XR	3.61^{D}	3.98^{Cyz}	4.07^{Cbz}	4.57^{Bz}	5.31^{Ay}	0.023
	SEM[2)]	0.000	0.047	0.001	0.056	0.096	

[1]Standard error of the mean (n = 15),[2] (n = 6).

[A-D]Different letters indicate significant differences ($P < 0.05$) between different irradiation dose treatments.

[a,b]Different letters indicate significant differences ($P < 0.05$) between different type of irradiation treatments.

[x-z]Different letters indicate significant differences ($P < 0.05$) between different storage days treatments.

704 Table 4. Effect of electron beam (EB) and X-ray (XR) irradiation on carbonyl contents (nmol/mg protein) of dry
705 pet food with different doses and storage days

Storage (Days)	Type	Irradiation Dose (kGy)					SEM[1)]
		0	2.5	5	10	20	
0	EB	0.14^{Cy}	0.15^{BCy}	0.16^{ABy}	0.17^{Aaz}	0.18^{Az}	0.005
	XR	0.14^{By}	0.15^{ABz}	0.15^{ABz}	0.16^{ABby}	0.17^{Az}	0.008
	SEM[2)]	0.000	0.005	0.011	0.003	0.006	
14	EB	0.14^{y}	0.16^{xy}	0.16^{y}	0.16^{z}	0.18^{z}	0.010
	XR	0.14^{By}	0.17^{AByz}	0.17^{AByz}	0.18^{ABy}	0.20^{Az}	0.011
	SEM[2)]	0.000	0.013	0.014	0.006	0.014	
28	EB	0.15^{Bxy}	0.16^{Bxy}	0.20^{Axy}	0.21^{Aby}	0.21^{Ayz}	0.008
	XR	0.15^{Dxy}	0.18^{Cxyz}	0.21^{Bx}	0.24^{Aax}	0.23^{ABy}	0.007
	SEM[2)]	0.000	0.008	0.006	0.006	0.007	
42	EB	0.18^{Bx}	0.18^{Bx}	0.20^{ABxy}	0.22^{Axy}	0.23^{Abxy}	0.008
	XR	0.18^{Cx}	0.20^{BCxy}	0.20^{BCxy}	0.23^{ABx}	0.26^{Aax}	0.009
	SEM[2)]	0.000	0.010	0.010	0.010	0.003	
56	EB	0.18^{Bx}	0.18^{Bbx}	0.23^{Ax}	0.23^{Abx}	0.25^{Abx}	0.006
	XR	0.18^{Cx}	0.21^{Bax}	0.24^{ABx}	0.25^{Aax}	0.27^{Aax}	0.007
	SEM[2)]	0.000	0.004	0.011	0.003	0.004	

706 [1]Standard error of the mean (n = 15),[2] (n = 6).

707 [A-D]Different letters indicate significant differences ($P < 0.05$) between different irradiation dose treatments.

708 [a,b]Different letters indicate significant differences ($P < 0.05$) between different type of irradiation treatments.

709 [x-z]Different letters indicate significant differences ($P < 0.05$) between different storage days treatments.

Table 5. Effect of 20 kGy of electron beam (EB) and X-ray (XR) irradiation on volatile compounds (area unit × 10^6) in dry pet food on storage days 0 and 56

Compound	Day 0			SEM[1)]	Day 56			SEM[1)]
	Control	EB 20kGy	XR 20kGy		Control	EB 20kGy	XR 20kGy	
Total alkane	22.41	40.11	33.30	2.363	51.08	50.04	49.38	1.258
Cyclopropane, 1-butyl-2-methyl	ND^{C}	0.51^{A}	0.30^{B}	0.023	ND^{C}	0.61^{A}	0.48^{B}	0.108
Decane, 2,6,8-trimethyl	4.02^{B}	6.46^{A}	5.74^{A}	0.387	10.03^{A}	9.03^{B}	8.92^{B}	0.150
Decane, 2-methyl	3.44^{B}	4.33^{A}	4.20^{A}	0.174	7.03^{A}	6.43^{B}	6.52^{AB}	0.156
n-Decane	0.60^{C}	1.22^{A}	0.85^{B}	0.033	0.88^{B}	1.12^{A}	0.99^{AB}	0.056
Dodecane, 2,6,10-trimethyl	2.24^{B}	6.08^{A}	4.72^{AB}	0.921	9.56	9.13	9.43	0.279
Dodecane, 2,7,10-trimethyl	0.34^{B}	0.82^{A}	0.61^{AB}	0.092	1.12	1.03	1.03	0.027
n-Dodecane	1.50^{B}	2.30^{A}	2.20^{A}	0.104	2.37	2.84	2.54	0.224
Heptane, 2,4-dimethyl	3.88^{AB}	4.91^{A}	3.65^{B}	0.311	2.60^{A}	2.01^{B}	2.01^{B}	0.105
Heptane, 5-ethyl-2,2,3-trimethyl	4.86^{B}	10.57^{A}	8.55^{AB}	1.199	15.70	14.77	15.03	0.339
n-Octane	0.23^{C}	0.68^{A}	0.39^{B}	0.039	0.41^{C}	0.80^{A}	0.61^{B}	0.021
n-Nonane	0.07^{C}	0.39^{A}	0.20^{B}	0.011	0.07^{C}	0.39^{A}	0.26^{B}	0.008
n-Pentadecane	0.88^{B}	1.34^{A}	1.32^{A}	0.072	0.78^{C}	1.26^{A}	1.00^{B}	0.053
n-Tetradecane	0.36^{B}	0.51^{A}	0.56^{A}	0.002	0.52	0.62	0.56	0.031
Total alkene, alkyne	0.18	3.42	2.29	0.117	0.38	3.77	3.04	0.049
1-Decene	0.06^{C}	2.11^{A}	1.44^{B}	0.043	0.13^{C}	2.22^{A}	1.80^{B}	0.023
1-Octene	0.07^{C}	1.07^{A}	0.62^{B}	0.071	0.19^{C}	1.26^{A}	0.97^{B}	0.028
1-Undecyne	0.04^{B}	0.25^{A}	0.23^{A}	0.008	0.07^{B}	0.29^{A}	0.27^{A}	0.009
Total aldehyde	19.65	49.15	52.34	1.412	33.30	52.79	60.72	1.080
2,4-Heptadienal, (E,E)	0.84^{C}	1.83^{B}	2.00^{A}	0.028	0.87^{B}	1.71^{A}	1.78^{A}	0.036

2-Heptenal	0.29^{C}	1.89^{A}	1.68^{B}	0.033	0.33^{B}	0.83^{A}	0.88^{A}	0.027
Butanal, 2-methyl	1.91^{C}	3.86^{B}	4.38^{A}	0.118	4.42^{B}	6.95^{A}	7.39^{A}	0.230
Butanal, 3-methyl	4.09^{C}	8.44^{B}	9.58^{A}	0.266	8.96^{B}	12.76^{A}	14.12^{A}	0.431
Heptanal	0.96^{C}	3.14^{A}	2.67^{B}	0.062	1.39^{C}	2.96^{B}	3.17^{A}	0.052
Hexanal	4.95^{B}	16.62^{A}	15.54^{A}	0.490	7.64^{C}	13.67^{B}	16.27^{A}	0.396
Nonanal	0.70^{C}	2.23^{A}	1.84^{B}	0.057	0.61^{B}	2.14^{A}	2.03^{A}	0.040
Octanal	1.09^{B}	1.98^{A}	1.90^{A}	0.036	1.31^{C}	1.91^{B}	2.21^{A}	0.035
Pentanal	4.80^{C}	9.15^{B}	12.75^{A}	0.413	7.77^{C}	9.87^{B}	12.86^{A}	0.237
Total ketones	11.87	55.61	71.30	1.488	13.03	45.53	47.00	1.147
2-Butanone	0.62^{C}	2.44^{B}	3.72^{A}	0.077	1.01^{C}	2.84^{B}	3.32^{A}	0.097
2-Propanone	7.24^{C}	48.81^{B}	62.10^{A}	1.514	7.15^{B}	37.97^{A}	37.34^{A}	1.112
3,5-Octadien-2-one	4.01^{B}	4.35^{B}	5.48^{A}	0.138	4.87^{B}	4.71^{B}	6.35^{A}	0.083
Total alcohols	9.71	24.25	28.12	4.210	13.16	15.17	18.07	5.288
6,9-Pentadecadien-1-ol	ND^{C}	0.64^{A}	0.45^{B}	0.011	ND^{C}	0.71^{A}	0.57^{B}	0.006
1-Hexanol	2.06^{B}	3.69^{A}	3.47^{A}	0.064	1.56^{B}	2.08^{A}	2.25^{A}	0.046
1-Octen-3-ol	0.26^{B}	0.47^{A}	0.44^{A}	0.011	0.31^{B}	0.45^{A}	0.47^{A}	0.012
1-Penten-3-ol	5.66^{C}	15.85^{B}	18.83^{A}	0.597	9.32^{C}	10.31^{B}	12.44^{A}	0.253
2-Methyl-2,3-pentanediol	1.73^{C}	3.60^{B}	4.93^{A}	0.085	1.97^{B}	1.62^{C}	2.34^{A}	0.050

[1]Standard error of the mean (n = 15).

[A-C]Different letters indicate significant different ($p < 0.05$) between control and different type of irradiation treatments.

ND, not detected.