Effect of Various Doses of Electron Beam Irradiation Treatment on the Quality Characteristics of Vacuum-Packed Dry Fermented Sausage during Refrigerated Storage

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ABSTRACT: In this study, we investigated the effect of electron beam irradiation on the physicochemical, microbiological, and sensory attributes of dry fermented sausage during 8 weeks of refrigerated storage at 4°C. The five doses of e-beam irradiation applied were: 0 kGy (control) and 1, 2, 3, and 4 kGy. All the experimental treatments led to a significant decrease in pH values during the storage period of 60 days (P<0.05). The 2-kGy treatment caused a significant (P<0.05) decrease in pH value, bacterial growth (total plate count, Listeria monocytogenes, Salmonella Typhimurium, and Escherichia coli), and thiobarbituric acid-reactive substances values than other irradiated fermented sausage batches during the study. Irradiation did not affect the water activity value of the fermented sausage samples. For color characteristics, 3 kGy exhibited significantly lower (P<0.05) L* (lightness) values than other irradiation treatments. A similar trend of significantly lower (P<0.05) a* (redness) and b* (yellowness) values was observed in all irradiated treatments than in the control. All the treatments showed no significant differences (P>0.05) in scores in sensory attributes (color and sourness); however, fermented sausage irradiated with 3 kGy had the highest sensory characteristics (overall acceptability) at the end of the storage period. In conclusion, 1, 2, and 3 kGy irradiation treatments can be beneficial for inhibiting lipid oxidation, controlling microbial growth, and maintaining sensory attributes of fermented sausage during storage, thereby enhancing their food safety and shelf stability.

Keywords: fermented sausage, irradiation, sensory characteristics, storage

INTRODUCTION

Meat products (e.g., cooked ham, dry ham, and dry fermented sausage) are being used as ready-to-eat (RTE) items because of changes in dietary preferences in the last 50 years (Gil-Díaz et al., 2009). Globally, sausages are the most popular RTE meat products because they are delicious, easy to use, affordable, and accessible (Sachindra et al., 2005). Considering the processed food trend globally, there is a significant concern for microbial contaminants in food. Approximately 25% of processed food is believed to be lost due to spoilage (Ghaly et al., 2010). Pathogenic microorganisms can contaminate fermented food products through contact with raw meat and ingredients and manufacturing equipment. Postproduction contamination is also a possibility. Many countries have documented outbreaks of food poisoning related to the consumption of infected fermented sausage (Kuhn et al., 2011).

The application of one of the promising technologies, such as food irradiation, can enhance microbiological safety and shelf-life stability of fermented sausage. In 1895, Wilhelm Conrad, a professor of physics from Germany, discovered X-rays and substantiated their use in microbial control (Nüsslin, 2020). The interest in this method was first oriented toward medicine. In 1980, X-rays were first used in food processing for cereal disinfection in the Soviet Union, and then in 1991, for poultry meat decontamination in France (Farkas, 2006). Irradiation causes chemical changes in food that inactivate bacteria by making changes, such as breaking covalent bonds that affect the structure of food. Food radiation is intended to eradicate bacterial growth, thereby enhancing shelf-life (Harder et al., 2016). Food irradiation is authorized for usage in a wide range of commodities in more than 55 countries (Stefanova et al., 2010; Jin et al., 2012). According to Kong et al. (2017), irradiation is one of the most efficient methods for preservation, and its bacter-
icidal effects in fresh and cooked meat products have been proven by Sommers et al. (2004). Radiation-pasteurized meat and meat products have been in use for many years in Belgium, France, China, Indonesia, The Netherlands, South Africa, and Thailand (Diehl, 1995). The maximum permitted dosage of irradiation is 3 kGy for prolonged shelf-life and protection from parasites and 7 kGy for pathogen control (Monk et al., 1995).

Electron beams (EBs), X-rays, and gamma irradiation are among the most widely examined irradiation methods. Because treatment of food and crops with electron beam irradiation (EBI) at low doses eliminates microbiological contamination, it might be beneficial for both industry and consumers (Lung et al., 2015). EBs are considered to be a safer preservation technique than gamma irradiation because it is generated using electricity and not radioactivity (An et al., 2017). The penetration depth of EBs in food is restricted to 8 cm; therefore, this treatment has been used for items such as grains on a conveyor or low-density meals (Song et al., 2009). The safety and quality of dry fermented sausages that have been exposed to EB radiation were investigated by Cabeza et al. (2009). Their findings revealed that the dosage necessary to accomplish the Foreign Agricultural Service per the United States Department of Agriculture guidelines (1.3 kGy) was fulfilled. Lim et al. (2008) studied the effect of dosage of EB irradiation on changes in the microbiological characteristics of fermented sausage and found that 2 kGy of irradiation was the most effective in the production of fermented sausages. Bouzarjomehri et al. (2020) investigated the effects of EB irradiation on the microbiological and sensory attributes of sausage. The results showed that EBI at a level of 2 kGy could be used for lowering bacterial populations, eradicating Salmonella Typhimurium, and improving the sensory properties of the final product.

Although the effect of irradiation on meat and meat products has been demonstrated in earlier studies, few studies exist on the use of EB irradiation as an antimicrobial and for extending the shelf-life of fermented sausages. Therefore, in this study, we aim to investigate the effect of different doses (1, 2, 3, and 4 kGy) of irradiation on the physicochemical and sensory characteristics of vacuum-packed, dry, and fermented sausages during refrigerated storage.

### MATERIALS AND METHODS

#### Sausage preparation

The sausage formulations used in this study are described in Table 1. To produce fermented sausages, chilled pork lean meat and back fat were cut and chopped using a meat mincer (M-12S, Hankook Fujee Industries Co., Ltd., Suwon, Korea). All the ingredients, including 1 mL/kg (v/w) mixed starter cultures of Lactobacillus sakei and Staphylococcus xylosus, were added to initiate fermentation and mixed well using a rotary slice cutter (SF-2002, Samwoo Industry Co., Daegu, Korea). Subsequently, the batter was stuffed into collagen casings with 24-mm diameter and 150-mm length (IKJIN Co. Ltd., Seoul, Korea) using a vacuum-filling machine (RVF 327, Düker-REX Fleischereimaschinen GmbH, Laufach, Germany). During the process, the temperature was maintained below 14°C.

The sausages were then fermented and aged (ripening) in the chamber (SMK-2000SL, Metatek, Nonsan, Korea). Fermentation (7 days) was performed at 23°C, with relative humidity values ranging from 90% to 95%. Ripening (28 days) was performed at 15°C, relative humidity values ranging from 70% to 75%. All fermented sausages were vacuum-packaged (19/S, Röschwerke GmbH, Hanover, Germany) after ripening was complete and stored at 4°C before irradiation.

#### EBI

Before irradiation was initiated, the fermented sausages were divided into five batches. Four vacuum-packed fermented sausage lots, containing 5 sausages in each pack, were irradiated in EB Tech Co., Ltd. (Daejeon, Korea), with a beam energy power of 10 MeV and 10 kW and a line speed of 0 to 20 min. One of the treatments, packaged in vacuum was non-irradiated and used as 0 dose, control treatment. In this study, we used 0, 1, 2, 3, and 4 kGy of EBI. After irradiation, the samples were stored at 4°C for 8 weeks. The analyses were performed after 1, 15, 30, and 60 days of refrigerated storage.

#### pH value

From each group of fermented sausage samples, 3 g was individually chopped and homogenized in 30 mL of deionized distilled water, using a homogenizer (Polytron®).
PT 2500 E Stand Dispersion Device, Kinematica AG, Malters, Switzerland). The electric probe was dipped into the suspension and pH was measured using a digital pH meter (Mettler Toledo, Columbus, OH, USA).

Water activity
Three grams of finely chopped fermented sausage samples were used to determine the water activity (a_w) of sausages using the LabMaster-aw, Aqualab CX-2 (Novasina AG, Lachen, Switzerland) at 25°C.

Microbial analysis
The microbial count for each fermented sausage batch was performed after 1, 15, 30, and 60 days of refrigerated storage. The bacteriological quality characteristics have been performed for total plate count (TPC), lactic acid bacteria (LAB), Listeria monocytogenes, Escherichia coli, and S. Typhimurium. Approximately, 25 g sample of each fermented sausage sample was collected with a sterile spoon aseptically, blended with 225 mL of 0.1% peptone water, and mixed for 30 s in the Stomacher Lab Blender (400 Circulator, Seward Laboratory System Inc., Suffolk County, NY, USA). Dilution of 1 mL sample in 9 mL of autoclaved distilled water was used for 10-fold diluents (10⁻¹ to 10⁻⁷). Samples were incubated in their respective media: TPC on plate count agar, LAB on de Mane Rogosae Sharpe (Difco Laboratories Inc., Detroit, MI, USA), Listeria spp. on polymyxin acriflavin Li ceftazidime esculin mannitol agar, E. coli on MacConkey sorbitol agar, and Salmonella spp. on bismuth sulfite agar (Kisanbio Co., Ltd., Seoul, Korea). Plates from the appropriate dilution series were used to determine the VBN concentration of the fermented sausage samples. The fermented sausage samples were homogenized with 30 mL distilled water at 1,000 rpm for 1 min. Whatman No. 1 filter paper (GE Healthcare Life Sciences, Pittsburg, PA, USA) was used for the filtration process and the filtrate (1 mL) was pipetted to the outside of the microdiffusion unit Conway chamber. Then, 1 mL (0.01 N) of boric acid and 100 μL (0.066% of bromocresol green and 0.066% methyl red at a 1:1 ratio) of Conway indicators were pipetted into the inner chamber. The outer chamber of the Conway unit was then filled with 1 mL of 50% potassium carbonate and sealed instantly. Materials were incubated at 37°C for 2 h. Total VBN values were obtained from the volume of 0.02 N H₂SO₄ used for titration and the value was expressed in mg%.

Instrumental color
For each type of fermented sausage, 5 samples were sliced into 3-cm-thick slices for color analysis. Reading was performed on the inner part of the sample using a chromameter (CR 300, Konica Minolta Inc., Tokyo, Japan) at room temperature; color parameters were expressed as follows: L* (lightness), a* (redness), and b* (yellowness) using the Commission Internationale de l’Eclairage (CIE) lab system (CIE, 1976). Average L*, a*, and b* values were derived from the mean of five random readings for color analysis.

Texture profile analysis (TPA) and shear force
The TPA analyzer (TA 1, Lloyd Instruments, Largo, FL, USA) was used to determine the texture profile of the fermented sausage samples. The fermented sausage samples were cut into 2-cm-high cylinders for TPA and testing circumstances were: pre- and post-test speeds of 2.0 and 5.0 mm/s, respectively; head speed=2.0 mm/s; distance=8.0 mm; and force=5 g. The values for texture were calculated using the time and force curve graph. The evaluated parameters included hardness (kgf), springiness (m), cohesiveness, and chewiness (kgf). The shear force of fermented sausage was analyzed following the method specified by Bourne (2012). A Warner-Bratzler blade was used for the texture analyzer (TA 1, Lloyd Instruments). The fermented sausage samples were prepared as 3-cm-thick slices. They were cut perpendicular at pre- and post-test speeds set at 1.0 mm/s, with a cross speed of 200 mm/min. Values of maximal shear force (kgf/g) were recorded for each sample.
Sensory analysis

The sensory analysis (color, lactic acid aroma, sourness, and overall acceptability) of the five types of fermented sausages was performed by seven trained panel members, including students in the Department of Animal Resources, Daegu University, Gyeongbuk, Korea. The panel members evaluated the following parameters: color, lactic acid aroma, sourness, and overall acceptability. Scoring was based on a 5-point scale. The attributes scored ranged from 1 (lowest intensity) to 5 (most intense) for each of the attributes listed. The sausages were served as 2-cm-thick slices in a white dish, along with clean water for rinsing the mouth between treatments. The life management committee of Daegu University (approval no. 1040621-201905-HR-004-02) endorsed the sensory assessment process.

Statistical analysis

Data were analyzed through analysis of variance using SAS version 9.4 (SAS Institute, Cary, NC, USA), and a significance level of \( P < 0.05 \) was used for all the assays. Duncan’s multiple range test was used for comparing the differences between the means. In evaluating physico-chemical and microbiological properties, fixed effect factors were included in both treatments and storage periods. Sensory analytical treatments were considered fixed effect variables and panel members as random effect variables (Stanley et al., 2017).

RESULTS AND DISCUSSION

Changes in \( \text{pH} \) and \( \text{aw} \) values

Lower \( \text{pH} \) values are essential during sausage production because they assist in inhibiting the growth of harmful bacteria, increasing the redness of the final product (Lorenzo et al., 2014), and increasing the formation of the distinctive aroma and flavor of the final product (Hugos and Monfort, 1997). The changes in \( \text{pH} \) and \( \text{aw} \) values of fermented sausages treated with and without a different dose of EBI during refrigerated storage are presented in Table 2. A significant difference \(( P < 0.05 \) in \( \text{pH} \) values was exhibited between the irradiated treatments and control. All the samples treated with ionizing irradiation exhibited significantly \(( P < 0.05 \) lower \( \text{pH} \) values than the control (non-irradiated) on the first day of analysis in the following order: 1 kGy and 2 kGy < 3 kGy < 4 kGy < control. However, the control scored the lowest value during the storage period (days 15 ∼ 60). The lowest \( \text{pH} \) value in the control did not correlate with the highest LAB count (Table 2). This might be because of continuous fermentation and acidification, because it was unirradiated. On day 15, \( \text{pH} \) values of fermented sausage that had undergone treatments decreased significantly \(( P < 0.05 \) compared with the initial day of storage. During storage (day 30), control and 3 kGy batches scored higher \( \text{pH} \) values of 5.27 and 5.44 respectively, among all experimental groups. On day 60 of refrigerated storage, irradiated and non-irradiated fermented sausages had significantly \(( P < 0.05 \) lower \( \text{pH} \) values of 5.09, 5.13, 5.15, 5.17, and 5.22 in the following order: control < 2 kGy < 1 kGy < 3 kGy < 4 kGy, respectively. The \( \text{pH} \) values of all dry fermented sausage treatments significantly decreased \(( P < 0.05 \) with increase in storage time. This may be due to the gradual acidification process of LAB with a lower rate of lactic acid production after the application of treatment because the growth of LAB was not completely inhibited with the EB dose applied. The production of free radicals from water \([\text{OH}, \text{H}, \text{H}_2\text{O}^+\text{, and aqueous electrons (}e_{\text{aq}}^-\text{)}]\) after irradiation is much higher in acidic than neutral environments (Spinks and Woods, 1976). Aromatic compounds, carboxylic acids, ketones, aldehydes, and thiols tend to react with \( e_{\text{aq}}^-\text{. Any modification in aqueous electrons can affect the final product because the production of both radical and aqueous electrons is favored by a lower } \text{pH}\) (Brewer, 2009). Moreover,
according to the literature, the pH values of fermented sausages are influenced by irradiation and storage period. Consistently, both irradiated and non-irradiated treatments showed a similar pH-lowering trend, according to Chouliara et al. (2006).

In this study, no substantial difference \((P>0.05)\) was exhibited in \(a_w\) values among treatments with different doses of irradiation and the control. Moreover, the \(a_w\) values of the fermented sausages remained unchanged during the refrigerated storage phase at 4°C. Thus, our finding indicated that irradiation had no effect on the change in the \(a_w\) value of fermented sausage during refrigerated storage (Table 2). This suggests that a stable \(a_w\) value prevents quality changes during the storage of fermented sausages.

### Microbiological quality

The irradiation of meat and meat products is highly efficient in decreasing both microflora and harmful microorganisms. The effect of different doses of EBI on the microbial quality of fermented sausages during the storage period is presented in Table 3. A substantial difference \((P<0.05)\) in TPC was observed between irradiated and non-irradiated fermented sausages on days 1 and 60 of refrigerated storage. The growth of microorganisms concurrently \((P<0.05)\) decreased in sausages with different dose rates of EB irradiation (1, 2, 3, and 4 kGy) at day 1 of storage. The values ranged from 4.87 to 7.42 log CFU/g between control and irradiated, dry fermented sausage samples, respectively. All the treatments exhibited an increase in TPC on day 15 compared with that on day 1, but there was no significant difference \((P>0.05)\) between the control and irradiated fermented sausages at this phase of storage. The TPCs of all experimental batches showed a decreasing trend, and the decrease was substantial for control and 1 kGy-treated samples. Moreover, a decrease in the count was exhibited in the treatments as the dose of irradiation increased on day 15, and the lowest TPC was observed for the irradiating dose of 4 kGy. Total microbial growth was not observed for 2, 3, and 4 kGy on days 30 and 60 of the storage study. The reduction in total aerobic bacterial growth in irradiated meat during storage was due to a post-irradiation effect, in which surviving cells injured by beta rays generated by the electron beam were unable to adapt to the storage environment (Kim et al., 2000). The decline in TPC for 4 kGy-irradiated meat products with storage time was investigated by Chung et al. (2000). In this study, a significant decline \((P<0.05)\) was shown starting 2-kGy dose when the final storage time of 60 days was considered.

#### Table 3. Microbial count of e-beam irradiated dry fermented sausages stored at 4°C (unit: log CFU/g)

| Parameter            | Day | Control  | 1 kGy  | 2 kGy  | 3 kGy  | 4 kGy  | SEM  |
|----------------------|-----|----------|--------|--------|--------|--------|------|
| Total plate count    | 1   | 7.42\(\text{a}^\text{b}\) | 6.74\(\text{a}\) | 5.48\(\text{e}\) | 5.14\(\text{d}\) | 4.87\(\text{e}\) | 0.14 |
|                      | 15  | 7.79\(\text{a}\) | 6.96   | 6.76\(\text{c}\) | 6.57   | 6.44\(\text{e}\) | 0.58 |
|                      | 30  | 7.00\(\text{a}\) | 6.90   |        |        |        | 0.14 |
|                      | 60  | 6.33\(\text{a}\) | 6.28\(\text{a}\) |        |        |        | 0.00 |
| SEM                  |     | 0.46     | 0.36   | 0.22   | 0.05   |        | 0.00 |
| Lactic acid bacteria | 1   | 6.88\(\text{a}\) | 6.69\(\text{a}\) | 6.11\(\text{e}\) | 5.95\(\text{e}\) | 4.89\(\text{e}\) | 0.58 |
|                      | 15  | 6.67     | 6.29   | 6.19   |        |        | 0.40 |
|                      | 30  | 7.59\(\text{a}\) | 6.46\(\text{a}\) |        |        |        | 0.20 |
|                      | 60  | 7.14     | 6.78   |        |        |        | 0.50 |
| SEM                  |     | 0.36     | 0.51   | 0.50   | 0.49   | 0.49   | 0.00 |
| Listeria spp.        | 1   | <1.00    | <1.00  |        |        |        | 0.08 |
|                      | 15  | <1.00    | <1.00  |        |        |        | 0.08 |
|                      | 30  |        |        |        |        |        | 0.08 |
|                      | 60  |        |        |        |        |        | 0.08 |
| SEM                  |     |          |        |        |        |        | 0.08 |
| Salmonella spp.      | 1   | <1.00    | <1.00  |        |        |        | 0.08 |
|                      | 15  |        |        |        |        |        | 0.08 |
|                      | 30  |        |        |        |        |        | 0.08 |
|                      | 60  |        |        |        |        |        | 0.08 |
| SEM                  |     |          |        |        |        |        | 0.08 |
| Escherichia coli     | 1   | <1.00    | <1.00  |        |        |        | 0.08 |
|                      | 15  |        |        |        |        |        | 0.08 |
|                      | 30  |        |        |        |        |        | 0.08 |
|                      | 60  |        |        |        |        |        | 0.08 |
| SEM                  |     |          |        |        |        |        | 0.08 |

Means with different small letters \((a-d)\) within a row and capital letters \((A,B)\) within a column are significantly different \((P<0.05)\). CFU, colony forming unit; SEM, standard error of the mean; −, not determined.
A considerable difference (P<0.05) in LAB count was noted between irradiated and control fermented sausages batches on days 1 and 30 of storage. Moreover, a significantly higher (P<0.05) LAB count was observed in the control than in irradiated fermented sausage samples on the initial day of storage. A significantly (P<0.05) low LAB count (4.89 log CFU/g) was exhibited in the 4 kGy EB-irradiated sample on day 1 of storage. There was no significant difference (P>0.05) between control and irradiated treatments on day 15 of storage. LAB growth was undetermined for 3 and 4 kGy from 15~60 days. This trend of LAB count was similar to that of TPC for the corresponding days. This could be because of the EBI dose, which caused the same effect on TPC and LAB. The LAB counts significantly increased (P<0.05) for control and 1 kGy-treated samples on day 30 compared with day 15 of the storage period, and the control exhibited a higher count than the 1 kGy-treated sample.

Among pathogenic microorganisms, L. monocytogenes showed growth only for control and 1 kGy-treated samples for days 1 and 15 of storage; growth within this period was very limited (<1.00 log CFU/g). No growth was observed for Listeria spp. for the rest of the storage time (days 30 and 60) in all experimental treatments. Badr (2005) reported that EBI at 1 and 2 kGy dosages reduced the number of L. monocytogenes in sausages. In this study, the application of different doses of EBI had a similar trend in the enumeration of Salmonella spp. and E. coli during the study period. Limited growth of <1.00 log CFU/g for both Salmonella spp. and E. coli was exhibited in only the control and 1 kGy-treated samples at day 1 of storage. Thereafter, no counts were detected for Salmonella spp. and E. coli in all treatment groups from day 15 of storage to the final storage period of 60 days. EBI is more effective than gamma-ray irradiation in decreasing the counts of Bacillus cereus and E. coli O157:H7 (Ibrahim, 2013). Consequently, our findings indicate that EBI starting at a dose of 2 kGy can successfully control microorganism growth and may help to improve the shelf-life of fermented sausages. Vural et al. (2007) reported that irradiation (2 kGy) could be effective against pathogens during the preservation of fermented sausages. Furthermore, Samelis et al. (2005) discovered that a 4-kGy radiation dose could eradicate pathogenic microorganisms in meat products.

**TBARS and VBN**

The TBARS and VBN values of dry fermented sausages treated with different doses of ionizing EBI compared with the control sample are shown in Table 4. Lipid oxidation, assessed by TBARS, is an important indicator of the meat product’s quality and acceptability and the oxidation-associated deterioration of processed meat products (Morrissey et al., 1998). Lipid oxidation is one of the key issues with irradiated raw meat and RTE meat products. There is no consistent trend in TBARS value of treatments at different storage time studies. TBARS values were significantly lower (P<0.05) for 1 kGy- and 2 kGy-treated samples at day 1 of storage, 2 kGy- and 3 kGy-treated samples at 15 days of storage, control and 3 kGy-treated samples at 30 days of storage, and 1 kGy- and 2 kGy-treated samples at 60 days of storage. As the initial and final storage time were considered, significantly lower values were exhibited for 1- and 2-kGy dose treatments. The application of different doses of EBI had a significant effect (P<0.05) on TBARS values of dry fermented sausages across the storage period. The TBARS values of all treatments significantly increased (P<0.05) from the initial to the final day of the storage period. The respective values of irradiating samples ranged from 0.72 to 3.58 during refrigerated storage. Thus, there were minor but statistically significant (P<0.05) differences in TBARS values demonstrated between control and irradiated (1, 2, 3, and 4 kGy) fermented sausage samples on day 60 of storage. All the batches initially displayed elevated TBARS values after day 1 of refrigerated storage.

**Table 4. TBARS and VBN of e-beam irradiated dry fermented sausages stored at 4°C**

| Parameter | Day | E-beam irradiation | SEM |
|-----------|-----|-------------------|-----|
|           |     | Control | 1 kGy | 2 kGy | 3 kGy | 4 kGy |
| TBARS (mg MDA/kg) | 1   | 0.90<sup>BC</sup> | 0.72<sup>CD</sup> | 0.85<sup>BC</sup> | 0.94<sup>CD</sup> | 0.95<sup>CD</sup> | 0.01 |
|           | 15  | 1.29<sup>CD</sup> | 1.14<sup>CD</sup> | 0.77<sup>BC</sup> | 1.01<sup>CD</sup> | 1.18<sup>CD</sup> | 0.01 |
|           | 30  | 3.07<sup>BC</sup> | 3.34<sup>BC</sup> | 3.59<sup>BC</sup> | 3.02<sup>BC</sup> | 3.55<sup>BC</sup> | 0.03 |
|           | 60  | 3.20<sup>BC</sup> | 2.98<sup>BC</sup> | 3.09<sup>BC</sup> | 3.35<sup>BC</sup> | 3.58<sup>BC</sup> | 0.06 |
| VBN (mg%) | 1   | 8.49<sup>BC</sup> | 8.30<sup>BC</sup> | 8.49<sup>BC</sup> | 8.40<sup>BC</sup> | 9.52<sup>BC</sup> | 0.17 |
|           | 15  | 8.86<sup>BC</sup> | 8.40<sup>BC</sup> | 8.77<sup>BC</sup> | 8.40<sup>BC</sup> | 8.12<sup>BC</sup> | 0.29 |
|           | 30  | 13.54<sup>BC</sup> | 10.27<sup>BC</sup> | 12.61<sup>BC</sup> | 7.65<sup>BC</sup> | 12.51<sup>BC</sup> | 0.79 |
|           | 60  | 6.81<sup>BC</sup> | 11.86<sup>BC</sup> | 7.46<sup>BC</sup> | 7.74<sup>BC</sup> | 8.02<sup>BC</sup> | 0.88 |

Means with different small letters (a-e) within a row and capital letters (A-D) within a column are significantly different (P<0.05). TBARS, thiobarbituric acid-reactive substances; VBN, volatile basic nitrogen; MDA, malondialdehyde; SEM, standard error of mean.
The increased oxidation potential of radiation energy that results in the production of a significant number of free radicals within the sausage batter might contribute toward greater TBARS values to irradiated samples resulting in undesirable flavors in the product. The result of the study showed that lipid oxidation increased for all the irradiated and control treatments as the storage period increased. Physicochemical alterations in beef patties at 10 kGy, generated by lipid oxidation and surface discoloration, increased due to EBI, according to Gill et al. (1998) and Poon et al. (2004). Typically, irradiation stimulates lipid oxidation and accelerates the process during storage (Kanatt et al., 1998). According to another study by Ham et al. (2017), gamma rays cause an increase in TBARS values in cooked beef patties, whereas in cooked pork sausages, X-rays have been responsible for the higher oxidation levels. According to Ahn et al. (1999) irradiation in meat and meat products increases lipid oxidation depending on the radiation dose and the state of the packing.

Analysis of VBN content is usually used to assess the freshness of raw meats, as well as the shelf-life and microbiological quality of processed meat products (Ba et al., 2018). A significant variation (P<0.05) was exhibited in VBN values of treatments receiving different doses of ionizing EBI (Table 4). Treatment with a 4-kGy dose had the highest (P<0.05) VBN value on day 1 and the lowest value at 15 days of storage when other treatments were similar during these storage times. At 30 days of storage, the same DFS sample showed an elevated value again with the control and 2 kGy treatment and 3 kGy presented a significantly low (P<0.05) value at this storage time. On day 1 of storage, 1 kGy had a lower VBN value of 8.30 mg% than other irradiated and control sausage groups; nevertheless, its VBN substantially (P<0.05) increased with the increase in storage period (days 15 ~ 60). At the final storage time of 60 days, the 1-kGy dose-treated batch exhibited the highest value, and no variation was exhibited by other treatment groups. The control fermented sausages presented a substantially (P<0.05) higher value compared with irradiated sausage samples after days 15 and 30 of storage. After the completion of two weeks of storage, 1 and 3 kGy treatment had similar lower VBN values than the irradiated 2 kGy and control samples. The VBN values of all fermented sausages treated increased significantly (P<0.05) during day 30 of storage, except for those treated with 3 kGy, which exhibited the lowest value. Considering the final storage day (day 60), treatments are ordered as follows: 1 kGy < 4 kGy < 3 kGy < 2 kGy < control. The VBN content significantly (P<0.05) increased with the increase in the storage period. A significant difference (P<0.05) was exhibited in the VBN values for the experimental fermented sausage groups during storage. The irradiated (2, 3, and 4 kGy) and control fermented sausage samples presented a significant (P<0.05) decrease in the TVBN value on the final day of storage (day 60). However, the 1 kGy-treated group exhibited an increase in VBN content, at 11.86 mg%. During storage, the decrease in the development of VBN was driven by a drop in the initial levels of commonly found bacteria in dry fermented sausages. The higher VBN of irradiated dry fermented sausages is explained by the accelerated degradation of protein due to the application of a high dose of irradiation. These findings are congruent with those of Javanmard et al. (2006).

**Instrumental color**

Color is responsible for the quality of meat products, formation and the stability of color are important for sausage products. CIE color values of fermented sausages treated with different doses of ionizing EBI during refrigerated storage of 60 days are shown in Table 5. A significant difference (P<0.05) was observed in the lightness (L*) values of fermented sausages on the days 15 and 60 of storage but the application of beta rays on fermented sausages did not show any difference in the lightness (L*) color attributes compared to control on days 1 and 30 of refrigerated storage. The lightness value for control treatment was higher on day 15 and lowest on the final storage day. Slighter significant (P<0.05) differences in L* values between control and irradiated (1, 2, 3, and 4 kGy) samples were observed in this study on day 15, and lightness values exhibited in irradiated samples are lower than the control treatment. With the increase in EBI dose, the lightness of fermented sausages decreased on day 15 of storage and 1 kGy showed the highest lightness value of 44.74 among other irradiated sausage samples. Fermented sausage sample treated with 4-kGy dose irradiation showed the lowest L* value on day 15 and the highest value on the final storage (day 60). The dry fermented sausages treatments (control, 1 kGy, and 3 kGy) showed a significant decrease at the end of the storage period but L* values for 2 and 4 kGy increased during this period. At the end of the storage study, the treatments were ordered as 2 kGy > 4 kGy > 1 kGy > 3 kGy > control. Park et al. (2010) discovered that EB and gamma irradiation did not affect the lightness of beef patties, regardless of irradiation doses of 0 to 20 kGy.

A significant difference (P<0.05) was observed in redness (a*) values of fermented sausages on days 15 and 60 of storage but no significant differences in the a* value between irradiated and non-irradiated fermented sausage samples days 1 and 30 of refrigerated storage. The dry fermented sausages treated with 3 kGy have demonstrated the highest a* value compared with other experimental groups on days 1 and 15 of the storage period but the lowest value among other batches on day 30. The a*
Table 5. Color parameters of e-beam irradiated dry fermented sausages stored at 4°C

| Parameter       | Day | Control | 1 kGy | 2 kGy | 3 kGy | 4 kGy | SEM  |
|-----------------|-----|---------|-------|-------|-------|-------|------|
| L* (lightness)  | 1   | 46.33A  | 46.45A| 45.70AB| 48.97A| 47.40A| 2.39 |
|                 | 15  | 44.97AB | 44.74AB| 40.48BC| 41.50AB| 40.62BC| 1.99 |
|                 | 30  | 44.79AB | 42.88B | 43.43B | 43.29B | 42.73B | 1.22 |
|                 | 60  | 41.29ab | 42.82ab| 46.75aA| 41.79bB| 46.39ab | 2.55 |
| SEM             |     | 2.81    | 1.59  | 1.83  | 2.22  | 2.86  |      |
| a* (redness)    | 1   | 9.56A   | 9.17a | 9.94A | 10.35A| 9.24A | 1.61 |
|                 | 15  | 8.32abA | 8.97abA| 9.48abA| 10.04abA| 9.12abA| 1.12 |
|                 | 30  | 7.68B  | 7.40B  | 8.29AB | 7.05B | 8.02B | 1.00 |
|                 | 60  | 9.83A  | 8.96abB| 7.50bcB| 8.45abAB| 6.71abB| 1.22 |
| SEM             |     | 1.22    | 1.00  | 1.27  | 1.50  | 1.25  |      |
| b* (yellowness) | 1   | 9.48AB  | 10.38dA| 10.99abA| 12.44abA| 11.40abA| 1.98 |
|                 | 15  | 12.12abA| 8.41abB| 11.53abA| 11.88abA| 11.42abA| 1.17 |
|                 | 30  | 9.41ab  | 8.05abB| 8.43abB| 7.74abB| 8.06abA| 1.10 |
|                 | 60  | 10.22abA| 9.68abAB| 7.68abB| 9.29abAB| 8.34abB| 1.44 |
| SEM             |     | 1.33    | 1.23  | 1.19  | 1.85  | 1.56  |      |

Means with different small letters (a-c) within a row and capital letters (A-C) within a column are significantly different (P<0.05). SEM, standard error of mean.

Values for irradiated and control sausage samples significantly decreased (P<0.05) on day 15 in comparison with the initial day. The control treatment exhibited a lower a* value than irradiated treatments during the same storage phase. Regarding the final refrigerated storage day, the redness value significantly decreased (P<0.05) for the irradiated fermented sausage sample compared with that on the initial day, but control treatment has shown an increase in a* value. The treatments were in the following order on the final storage day, control> 1 kGy> 3 kGy> 2 kGy> 4 kGy. It might be associated with a nitrosyl hemochrome breakdown. Our finding agrees with the suggestion by Ham et al. (2017) that irradiation with up to 10 kGy reduced redness in emulsified sausages. According to many preliminary investigations, irradiation-induced color changes vary based on animal species, muscle type, irradiation dose, meat product, and packaging method. Dark meat, such as beef, turn a variety of colors, ranging from brown to greenish-brown following irradiation; however, light meat, such as pork loin, turn to a pink hue (Ahn et al., 1998; Kim et al., 2002; Nam and Ahn, 2003). Brewer (2004) reported a drop in a* values (red-green) caused by the synthesis of green pigments due to the irradiation of meat. EBI application on fermented sausages significantly affected (P<0.05) their yellowness value (Table 4). Control treatment had a significantly lower b* value (P<0.05) on the initial storage day than irradiated samples but exhibited higher b* values at other storage times (15~60 days). The highest yellowness values were observed in the 3 kGy-treated group on days 1 and 15 of storage, and the lowest values were observed on day 30. This increase in yellowness may be associated with the increase in lipid oxidation (TBA value) during storage (Table 4). On day 30, the b* value significantly decreased (P<0.05) for all the treatments, in contrast to the first two weeks of refrigerated storage. Compared with that on day 30, b* values increased for all experimental groups on the final storage day (day 60). However, the irradiated, fermented sausages exhibited a lower yellowness value than the control in the following order: 1 kGy> 3 kGy> 4 kGy> 2 kGy. This trend of lower b* values in irradiated treatments than control was similar to that of CIE a* values of fermented sausages. The irradiation treatment had a noticeable and comparable effect on all the color attributes L*, a*, and b* values in this study.

TPA and shear force

The textural qualities of sausages are related to the extraction of salt-soluble proteins, which significantly impacts water content, fat content, gel-formation, and viscosity (Ruusunen and Puolanne, 2005). The changes in TPA and shear force values of irradiated and control fermented sausages during 60 days of refrigerated storage are documented in Table 6. The hardness value obtained from TPA did not show a significant difference (P>0.05) in control and irradiated sausages on days 1, 30, and 60 of refrigerated storage. In this study, an increase in hardness was exhibited by the control and 3 kGy-treated groups at 15 days of storage. The lowest hardness was observed in the 2 kGy-treated group during the storage days. All sausage samples with or without EBI showed similar springiness during the storage period (P>0.05); however, for day 15 of storage, the 1 and 2 kGy-treated groups showed significantly lower (P<0.05) values than the other experimental groups. During refrigerated storage, no significant difference was detected in cohesiveness between irradiated and control dry fermented sau-
Table 6. Texture profile analysis and shear force of e-beam irradiated dry fermented sausages stored at 4°C

| Parameter          | Day | Control | 1 kGy | 2 kGy | 3 kGy | 4 kGy | SEM |
|--------------------|-----|---------|-------|-------|-------|-------|-----|
| Hardness (kgf)     | 1   | 7.38    | 6.01  | 6.22  | 6.96  | 7.45  | 1.58|
|                    | 15  | 8.84    | 5.70  | 5.07  | 7.92  | 6.35  | 1.26|
|                    | 30  | 8.12    | 5.56  | 8.69  | 8.47  | 7.42  | 1.98|
|                    | 60  | 9.00    | 9.65  | 8.42  | 6.84  | 5.30  | 3.47|
| SEM                |     | 2.07    | 2.10  | 3.42  | 1.03  | 1.91  |     |
| Springiness (m)    | 1   | 0.83    | 0.87  | 0.94  | 0.88  | 0.84  | 0.11|
|                    | 15  | 0.95    | 0.90  | 0.94  | 0.95  | 0.95  | 0.02|
|                    | 30  | 0.86    | 0.93  | 0.87  | 0.88  | 0.90  | 0.06|
|                    | 60  | 0.90    | 0.93  | 0.82  | 0.91  | 0.91  | 0.07|
| SEM                |     | 0.10    | 0.05  | 0.08  | 0.04  | 0.06  |     |
| Cohesiveness       | 1   | 0.34    | 0.32  | 0.37  | 0.33  | 0.33  | 0.05|
|                    | 15  | 0.11    | 0.36  | 0.31  | 0.22  | 0.12  | 0.11|
|                    | 30  | 0.33    | 0.38  | 0.32  | 0.28  | 0.25  | 0.06|
|                    | 60  | 0.33    | 0.33  | 0.42  | 0.37  | 0.37  | 0.05|
| SEM                |     | 0.09    | 0.05  | 0.06  | 0.09  | 0.07  |     |
| Chewiness (kgf)    | 1   | 2.06    | 1.68  | 2.19  | 2.06  | 2.02  | 0.28|
|                    | 15  | 0.95    | 1.86  | 1.50  | 1.64  | 0.76  | 0.92|
|                    | 30  | 2.23    | 1.94  | 2.36  | 2.13  | 1.73  | 0.45|
|                    | 60  | 2.69    | 3.08  | 2.45  | 2.35  | 6.13  | 3.57|
| SEM                |     | 0.90    | 0.71  | 0.48  | 0.70  | 3.91  |     |
| Shear force (kgf)  | 1   | 7.38    | 5.98  | 4.21  | 5.89  | 5.50  | 0.56|
|                    | 15  | 6.18    | 7.65  | 6.15  | 6.40  | 7.86  | 0.60|
|                    | 30  | 6.66    | 7.08  | 6.27  | 5.62  | 6.44  | 0.61|
|                    | 60  | 6.76    | 6.30  | 7.56  | 6.71  | 6.10  | 0.80|
| SEM                |     | 7.89    | 0.88  | 0.50  | 0.77  | 0.46  |     |

Means with different small letters (a-c) within a row and capital letters (A-C) within a column are significantly different (P<0.05). SEM, standard error of mean.

Sage samples on days 1 and 60. For cohesiveness, the control and 4 kGy-treated groups showed the lowest values on day 15. The 1 kGy-treated group showed a higher value on days 15 and 30 of storage. Similar investigations have shown that irradiation does not influence the textural characteristics of processed meat products (Ham et al., 2017). A significant difference between irradiated and control treatment in chewiness was exhibited only on day 15 of storage. The chewiness for the treatments on day 15 of storage are as follows: 1 kGy> 3 kGy> 2 kGy> control> 4 kGy. The reduction in chewiness was exhibited by all treatments except 1 kGy, which showed a gradual increase after 15 days of storage. Storage period affected the TPA chewiness attribute of the 4 kGy-treated and control sample, increasing for 30 and 60 days of storage. The 4 kGy-treated sausage sample revealed chewiness, with a maximum value of 6.13 kgf on the final day of refrigerated storage. This was likely due to a loss in moisture content and integrity among ingredients of fermented sausages during the storage study. Thus, our results indicate that irradiation dosage does not have much influence on the textural characteristics of fermented sausage. Herrero et al. (2007) reported texture profile values that were quite close. A significant variation (P<0.05) in shear force values was observed for all the experimental batches of fermented sausage treatments that received a different dose of EBI. There was a significant difference (P<0.05) in shear force between irradiated and control fermented sausages during 60 days of refrigerated storage (Table 6). Lower shear force values were observed in the irradiated fermented sausage compared with the control and the 2 kGy-treated group exhibited a lower shear force value compared with the other irradiated samples on day 1 of storage. On day 15 of storage, the shear force values significantly increased (P<0.05) for the EBI-treated dry fermented sausage samples. The maximum value was noted at 4 kGy; however, the control showed a decrease in value. On day 30, 1 kGy treatment showed the highest shear force value than other experimental batches. A gradual increase (P<0.05) in shear force values was witnessed in all irradiated samples during the study period of 60 days. This may be related to the drying-induced weight loss, because evaporation from the products led to an increase in hardness. The 2 kGy-treated sample initially (day 1) exhibited the lowest shear force value compared with other treatments, but a significant increase (P<0.05) with the highest value was observed at the end of storage (day 60). The shear force value for the control sample steadily decreased (P<0.05) with the increase in storage period, ranging from 7.38 to 6.76 kgf. The find-
ings of this study indicate that the non-irradiated control was less likely to lose water than the irradiated dry fermented sausage.

**Sensory analysis**
The overall quality of meat products is defined by consumer acceptance. In sausages, the sensory index includes acceptability, color, texture, flavor, taste, saltiness, and sourness (Robbins et al., 2003). In addition, customer expectations are substantially impacted by visual features (Brewer and Novakofski, 2008). The irradiation of food generally leads to the formation of odd smells and tastes. These smells are related to the production of extremely volatile sulfur compounds (Galán et al., 2011). The tastes in the meat and meat products are produced by the amino acid breakdown and are characterized as sweet, ancient, pungent, or sour (Ahn et al., 2000). The results from the sensory evaluation of irradiated, fermented sausages during 60 days of refrigerated storage at 4°C are presented in Table 7. Sensory analysis (color, lactic acid aroma, sourness, and overall acceptability) of five kinds of fermented sausages was investigated. Irradiation did not significantly ($P>0.05$) affect sensory attributes, except for overall acceptability at day 1 of storage, both for control and irradiated fermented sausage samples. The storage period did not affect the color attribute for all experimental treatments. However, storage period had a substantial effect ($P<0.05$) on the lactic acid aroma of the 2 kGy-treated sample and the sourness score of the control treatment. Furthermore, these scores increased significantly ($P<0.05$) with the increase in storage time. This may be due to the gradual build-up of lactic acid by LAB. A significant variation ($P<0.05$) was observed in sourness by panelist’s score only in the control group during storage (1~60 days), but irradiation did not show any effect on other experimental groups. There was a significant difference ($P<0.05$) in overall acceptability at initial and final storage days between control and irradiated treatments, and the 3 kGy-treated group exhibited the highest score during this phase. The overall acceptability score for the irradiated and control batches (scored by the panelists) were as follows: 3 kGy> 2 kGy> 4 kGy > 1 kGy> control on day 1 of storage. On the final refrigerated storage day (day 60), the drop in overall acceptability score of all the fermented sausage groups with the extension in storage period (days 1~60) but the overall acceptability of sensory characteristics was higher in irradiated treatments (1, 2, 3, and 4 kGy) than the control treatment. Therefore, 3 kGy was observed to maintain the overall acceptability by panel scores than other treated fermented sausage samples in the experimental study during refrigerated storage time. The finding of this study indicated that the use of EBI had a minimal effect on the sensory attributes of fermented sausages. Similarly, Arvanitoyannis et al. (2009) found that low-dose EBI of meat products had no influence on the meat’s quality or nutritional value.

**Application of EBI in dry fermented sausages at a 2-kGy**

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### Table 7. Sensory analysis of e-beam irradiated dry fermented sausages stored at 4°C

| Parameter          | Day | Control | 1 kGy | 2 kGy | 3 kGy | 4 kGy | SEM  |
|--------------------|-----|---------|-------|-------|-------|-------|------|
| Color              | 1   | 2.96    | 3.26  | 3.54  | 3.50  | 3.28  | 0.74 |
|                    | 15  | 3.24    | 3.20  | 3.50  | 3.60  | 3.68  | 0.74 |
|                    | 30  | 2.60    | 2.50  | 2.88  | 2.88  | 3.00  | 0.48 |
|                    | 60  | 3.04    | 3.00  | 3.32  | 3.30  | 3.36  | 0.75 |
|                    | SEM | 0.79    | 0.76  | 0.50  | 0.77  | 0.58  |      |
| Lactic acid aroma  | 1   | 3.40    | 2.50  | 1.92ab| 3.02  | 3.06  | 1.18 |
|                    | 15  | 3.54    | 3.12  | 3.40a | 2.88  | 2.84  | 0.71 |
|                    | 30  | 2.38    | 2.60  | 2.26ab| 2.22  | 2.32  | 1.12 |
|                    | 60  | 2.44    | 2.12  | 2.20ab| 2.80  | 2.39  | 0.86 |
|                    | SEM | 0.92    | 1.04  | 1.00  | 0.97  | 1.00  |      |
| Sourness           | 1   | 2.50ab  | 2.36  | 2.92  | 2.84  | 2.56  | 1.08 |
|                    | 15  | 3.54a   | 2.86  | 3.18  | 2.58  | 2.48  | 0.84 |
|                    | 30  | 1.90a   | 2.06  | 2.10  | 2.06  | 2.46  | 1.05 |
|                    | 60  | 2.10a   | 2.38  | 1.94  | 2.78  | 2.68  | 0.76 |
|                    | SEM | 0.83    | 0.97  | 0.97  | 0.88  | 1.04  |      |
| Overall acceptability | 1   | 3.04a   | 3.18b | 3.70ab| 4.40a | 3.18b | 0.81 |
|                    | 15  | 3.55    | 3.58  | 3.78  | 4.12  | 3.96  | 0.52 |
|                    | 30  | 3.60    | 3.30  | 4.04  | 3.80  | 3.94  | 0.80 |
|                    | 60  | 2.69    | 2.95ab| 3.84ab| 4.01a | 3.06bc| 0.65 |
|                    | SEM | 0.84    | 0.78  | 0.68  | 0.57  | 0.57  | 0.62 |

Means with different small letters (a-c) within a row and capital letters (A,B) within a column are significantly different ($P<0.05$). SEM, standard error of mean.
dose rate was most efficient for the survival of LAB and the reduction in microbial growth and lipid oxidation during refrigerated storage. At this dose, VBN content was lower than that in other treatments. Irradiation did not affect the $a_\text{w}$ values of the fermented sausage samples according to the findings of this study. The 2-kGy dose showed the lowest pH values among all sausage batches. For color characteristics, 3 kGy exhibited lower $L^*$ values than other treatments. A similar trend of lower $a^*$ and $b^*$ was observed for irradiated treatment than for non-irradiated treatment. Furthermore, 3 kGy treatment was better for different sensory qualities (highest lactic acid aroma and overall acceptability) and lower chewiness property in TPA characteristics than other treated fermented sausage samples. Thus, we recommend the use of 1 kGy irradiation to prevent lipid oxidation, 2 kGy for microbial control, and 3 kGy for the maintenance of sensory attributes to enhance food safety and shelf stability of fermented sausages during storage.

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The authors declare no conflict of interest.

**AUTHOR CONTRIBUTIONS**

Concept and design: all authors. Analysis and interpretation: SNK, SS. Data collection: SS, AA. Writing the article: all authors. Critical revision of the article: SNK, SS. Final approval of the article: all authors. Statistical analysis: SNK, SS. Obtained funding: SNK. Overall responsibility: SNK.

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