Comparison of Quality of Bologna Sausage Manufactured by Electron Beam or X-Ray Irradiated Ground Pork

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Abstract

Ground lean pork was irradiated by an electron beam or X-rays to compare the effects of two types of radiation generated by a linear accelerator on the quality of Bologna sausage as a model meat product. Raw ground lean pork was vacuum packed at a thickness of 1.5 cm and irradiated at doses of 2, 4, 6, 8, or 10 kGy by an electron beam (2.5 MeV) or X-rays (5 MeV). Solubility of myofibrillar proteins, bacterial counts, and thiobarbituric acid reactive substance (TBARS) values were determined for raw meat samples. Bologna sausage was manufactured using the irradiated lean pork, and total bacterial counts, TBARS values, and quality properties (color differences, cooking yield, texture, and palatability) were determined. Irradiation increased the solubility of myofibrillar proteins in a dose-dependent manner (p<0.05). Bacterial contamination of the raw meat was reduced as the absorbed dose increased, and the reduction was the same for both radiation types. Differences were observed only between irradiated and non-irradiated samples (p<0.05). X-ray irradiation may serve as an alternative to gamma irradiation and electron beam irradiation.

Keywords: electron beam, x-ray, food irradiation, ground lean pork, bologna sausage

Introduction

Food may be irradiated with three different types of radiation: gamma rays emitted by a radioactive source (cobalt-60 or cesium-137), electrons accelerated to high energies, and X-rays produced from high-energy electrons striking a target material with a high atomic number (Gregoire et al., 2003). The technical advantages of high-energy X-rays are better power utilization, reduced treatment time, and improved dose uniformity. The practical results are higher production rates, lower treatment costs, and better product quality (Cleland and Stichelbaut, 2013). In published research, gamma rays from radioactive isotopes are the most widely used type of ionizing radiation. Nevertheless, there is a general feeling that in the next decade, the majority of industrial radioactive sources will be replaced by the new, powerful electron accelerators as public acceptance grows (Byun et al., 2009; Cleland and Stichelbaut, 2013). One of the advantages of electron accelerators compared with gamma ray sources is that they can be switched from the electron beam mode to the X-ray mode, thereby providing a wide range of dose rates (Miller, 2003). Several reports have indicated that X-ray and electron beam irradiation have similar effects on foods and agricultural commodities (Palou et al., 2007; Van Calenberg et al., 1998, 1999).

To avoid the possibility of measurable food activation via photonuclear reactions, the kinetic energy of an electron beam for direct irradiation is limited to 10 MeV by regulation, while the kinetic energy limit for X-ray irradiation is 5 MeV. Scientific studies have shown that a limit of 7.5 MeV is safe (FAO/IAEA, 1995), and the U.S. Food and Drug Administration has approved the use of 7.5 MeV accelerators (Food and Drug Administration, 2008). High-energy X-rays are suitable for irradiation of materials and products that are too thick to be penetrated by energetic electron beams. Some of the practical applications of high-energy X-rays are the same as those of gamma rays from cobalt-60 sources (Cleland and Stichelbaut, 2013). X-rays have much higher penetration power than high-energy electrons and are suitable for commercial treatment of pallet loads of produce in conveyor systems (McLaughlin, 1999). In some cases, when a product...
can be treated with an electron beam because of high density and thickness or with cobalt-60 because of regulatory restrictions, X-rays may be the only solution. However, with the use of X-rays, the main problems are achieving high-throughput processing with an acceptable dose uniformity ratio in the irradiated object (Lazurik et al., 2007), and the high cost and low energy efficiency compared with gamma or electron beam irradiation (Sadat, 2004). It is expected that the use of X-rays for food irradiation will continue to expand, considering that the supply of accelerators is increasing internationally (Cleland and Stichelbaut, 2013). However, most studies that have compared the effects of different types of ionizing radiation on foods have compared gamma rays and electron beams (Choi et al., 2009; Kim et al., 2010a, 2010b; Park et al., 2010).

In this study, we compared the effects of electron beam and X-ray irradiation on food quality using Bologna sausage as a model meat product. Bologna sausage was manufactured with ground lean pork irradiated with an electron beam or X-rays generated by a linear accelerator, and the quality properties of the sausage were determined.

Materials and Methods

Preparation of meat samples
Vacuum-packaged and refrigerated lean pork (ham cut) and frozen pork backfat were obtained within 48 h of slaughter from a local meat packer. The lean meat and backfat were ground with a mincer (model 160, Fatosa, Spain) through a 9 mm plate and then through a 3 mm plate. Ground pork (500 g) was vacuum packaged (75 cmHg pulled) in sterilized oxygen-impermeable nylon bags. Ground pork (500 g) was vacuum packaged (75 cmHg pulled) in sterilized oxygen-impermeable nylon plates. Ground pork (500 g) was vacuum packaged (75 cmHg pulled) in sterilized oxygen-impermeable nylon plates.

Electron beam and X-ray irradiation
Packaged lean meat samples were irradiated at doses of 0 (control), 2, 4, 6, 8, and 10 kGy. Electron beam irradiation and X-ray irradiation were performed with an ELV-4 electron accelerator (2.5 MeV) and an X-ray linear accelerator (15 kW, 5 MeV), respectively, at about 20°C at EB Tech Co. (Korea). Dose rates were 20 kGy/h for electron beam irradiation and 5 kGy/h for X-ray irradiation. Actual doses were determined by placing 2 cm² radiochromic dosimetry films (Gafchromic HD-810, International Specialty Products, USA) at two different positions (top and bottom) with each sample. Readings (nine per dose) were made with a spectrophotometer at 560 nm (Van Calen-berg et al., 1999). After irradiation, samples were stored at 4°C and used for subsequent experiments.

Determination of solubility of myofibrillar protein
To determine the effect of electron beam or X-ray irradiation on the solubility of myofibrillar protein of ground pork, we prepared protein solutions from irradiated ground lean pork using a previously described method (Wagner and Anon, 1986) with slight modifications. In brief, 2 g of lean pork was put into a tube and homogenized in 20 mL of cold phosphate-buffered saline (PBS; 0.15 M NaCl, pH 7.4) using a homogenizer (DIAX 900, Heidolph Co., Ltd., Germany) within an ice-water bath. The homogenate was centrifuged at 9,000 g for 20 min at 4°C and the supernatant was discarded. The precipitate was washed with 20 mL of PBS by vortexing for 3 min and centrifuged as described above. The washing process was performed twice. For preparation of salt-soluble myofibrillar protein solution, washed precipitate was put into a tube and homogenized in 20 mL of cold high-salt PBS (0.6 M NaCl, pH 7.4) using the homogenizer within an ice-water bath. The homogenate was centrifuged at 10,000 g for 20 min at 4°C and the supernatant was filtered through Whatman No. 42 filter paper. The filtered supernatant was used as salt-soluble protein solution. The bicinchoninic acid (BCA) method (Hill and Straka, 1988) was used to determine the concentrations of the myofibrillar protein solutions using a BCA protein assay kit (Sigma Chemical Co., USA). Bovine serum albumin (Sigma Chemical Co.) solutions of 1.0 to 10.0 mg/mL were used as standards.

Manufacture of Bologna sausage
Bologna sausage was prepared using ground lean pork irradiated with an electron beam or X-rays at designated doses (2, 4, 6, 8, or 10 kGy) to compare the effects of different forms of accelerator-generated radiation on the qua-
ility of pork products. Bologna sausage was prepared as previously described (Byun et al., 2000; Jo et al., 2001). Sodium tripolyphosphate, sodium nitrite, and spice mix (35% black pepper powder, 25% coriander powder, 19% dextrose, 5% nutmeg powder, 5% caraway powder, 5% chili powder, 5% mustard powder, and 1% garlic powder) were purchased from Woosung Co., Ltd. (Korea), and salt and sugar were purchased from a local market. Lean pork (ham cut; 12 kg), salt (300 g), sodium nitrite (4 g), sodium phosphate (60 g), and ice (2 kg) were placed in a bowl cutter (C-75, Fatosa) and mixed for about 1 min at high speed. When the temperature of the mixture had decreased by about 2°C, ground pork backfat (4 kg) was added and the ingredients were mixed until the temperature reached 10°C. The remaining 2 kg of ice, the spice mix (100 g), and sugar (40 g) were added and mixed in until the temperature of the mixture reached 13°C. The total emulsification time was about 10 min and the temperature of the processing room was 15°C. The meat batters were stuffed (Patron Sausage Filler MWF 591, MADO, Netherlands) into 50-mm cellophane casings (n=40) (Woosung Co., Ltd.). The Bologna sausages were weighed and then cooked in a cooker (Frocomat 1200, Franke GmbH & Co., Germany) to an internal temperature of 70°C. The cooked sausages were water-spray cooled for 5 min and dried at room temperature for 30 min. The sausages were weighed to determine the cooking yield, and the casings were removed from all except five sausages, which were used for sensory evaluation and determination of texture. Dressed Bologna sausages were cut into 1.5 cm thick slices, vacuum packaged, and stored at 5°C for subsequent experiments.

Microbiological evaluation
To determine total aerobic bacterial populations in raw lean pork and sausage samples, we obtained total aerobic plate counts by the method of Oh et al. (2000). Briefly, 10-g portions of sausages were placed aseptically in a sterile nylon bag (10×15 cm; Sunkyung Co., Ltd.) containing 90 mL of 0.1% sterile peptone water (Difco Laboratories, USA) and pommelled for 2 min using a Stomacher 400 lab blender (Seward Medical, UK). Aliquots of the pommelled samples were serially diluted with 0.1% sterile peptone water, and 0.1 mL portions of the dilutions were plated in triplicate on plate count agar (Difco Laboratories). The plates were then incubated at 37°C for 48 h, and visible bacterial colonies on plates were determined as log colony-forming units (CFU) per gram.

2-Thiobarbituric acid reactive substance (TBARS) values
To assess lipid oxidation of raw lean pork and Bologna sausage, we measured TBARS levels using the method of Byun et al. (2000). A sample of raw lean pork or Bologna sausage (5 g) was homogenized in a 50-mL centrifuge tube with 50 µL of butylated hydroxyanisole (7.2% in ethanol) and 15 mL of distilled water, using a homogenizer (DIAx 900). Then, 1-mL portions of the homogenates were mixed with 3 mL of 20 mM 2-thiobarbituric acid (15% in trichloroacetic acid solution), heated in boiling water for 15 min, and centrifuged for 10 min at 2,500 g (Union 5KR, Hanil Science Industrial Co., Ltd., Korea). The absorbance of the supernatant was measured at 532 nm with a spectrophotometer (UV1600 PC, Shimadzu, Japan). Values are reported as micrograms of malondialdehyde per gram.

Quality evaluation of Bologna sausages

Textural properties
All texture analyses were conducted at ambient temperature (20°C) according to the method of Byun et al. (2002), using a texture analyzer system (TA.XT2i, Stable Micro Systems, England) equipped with a probe (1.0 cm thick).

Hunter color values (L*, a*, and b*)
Hunter color values - L* (lightness), a* (redness), and b* (yellowness) - of the sausage surface were measured using a color difference meter (spectrophotometer model CM-3500d, Minolta Co., Japan). Standard values were as follows: L*, 90.5; a*, 0.4; and b*, 11.0.

Sensory evaluation
Sensory evaluation of the sausages was conducted using a 21-member panel trained as described by Byun et al. (2000). Panelists scored each sample using a 7-point descriptive scale, where 1=extremely dislike or extremely weak and 7=extremely like or extremely strong, for color, texture, and flavor. Bologna sausages were steeped in an 85°C water bath until an internal temperature of 65°C was reached (about 20 min), cut into 1 cm thick slices, put on a white dish, and then served to panel members.

Statistical analysis
Data were analyzed by the general linear procedures, least squares means with limited standard deviations, and Duncan’s multiple-range test of SAS software (SAS, 2008).
Results and Discussion

Protein solubility

Table 1 shows the solubility of myofibrillar (salt-soluble) protein of ground lean pork irradiated with an electron beam or X-rays. Irradiation increased the solubility of myofibrillar proteins in a dose-dependent manner \((p<0.05)\). The concentration of the myofibrillar protein solution was 3.11 mg/mL for the non-irradiated sample. For the samples irradiated at 2 kGy, the protein concentrations were 3.32 mg/mL following electron beam irradiation and 3.24 mg/mL following X-ray irradiation. At 10 kGy, the concentration of myofibrillar proteins was 3.82 mg/mL following electron beam irradiation and 3.76 mg/mL following X-ray irradiation. In a previous study (Latreille et al., 1993), the solubility of myofibrillar proteins increased slightly in gamma-irradiated pork and beef, depending on the absorbed dose. Meat compounds are affected by treatment with ionizing radiation directly and indirectly (Fox et al., 1995; Taub et al., 1979). Meat properties such as protein solubility, water-holding capacity, and texture can be changed by radiolytic products generated by high-energy radiolysis of water (International Consultant Group of Food Irradiation, 1996). Changes induced by ionizing radiation may increase the solubility and emulsifying capacity of myofibrillar proteins in meat batter (Byun et al., 2000; Lacroix et al., 2000).

Bacterial evaluation of raw lean pork and Bologna sausage

Table 2 shows the survival of bacteria in raw lean pork irradiated with an electron beam or X-rays and Bologna sausage made with irradiated raw lean pork. The bacterial population in non-irradiated raw lean pork was 4.91 Log CFU/g. Both electron beam and X-ray irradiation reduced the total bacterial counts of raw meat, and the reduction was dependent upon the dose. No bacterial growth was observed in samples irradiated with a dose over 8 kGy. At 2 kGy, X-ray irradiation was more effective at reducing the initial bacterial contamination level than electron beam irradiation. However, the differences between radiation types were indistinguishable at doses of 4 kGy and 6 kGy \((p>0.05)\). The bacterial population was reduced to about 1.39 Log CFU/g after manufacturing the sausage, and no bacterial growth was observed from the sausages made with lean pork irradiated with doses over 6 kGy. In their comparison of X-rays and electron beams, Van Calenberg et al. (1999) reported that the microbiological quality of minced chicken meat was the same regardless of radiation type. Our results show that electron beams and X-rays were equally effective for inactivating bacteria in the tested minced lean pork and may serve as an alternative to gamma irradiation. However, it is also important to consider the differences in penetration depth between the radiation types (Woods and Pikaev, 1994).

TBARS levels of raw lean pork and Bologna sausage

Table 3 shows the TBARS values for raw lean pork irradiated with an electron beam or X-rays and Bologna sausage made with irradiated pork. TBARS levels in raw lean pork were increased by irradiation \((p<0.05)\), which is
consistent with the general observation that ionizing radiation increases lipid oxidation (Smith et al., 1960; Thayer, 1994). Lipid oxidation is generally higher in irradiated meat than in non-irradiated meat and is dependent upon the irradiation dose (Byun et al., 2000; Lee et al., 1999). However, there appears to be very little effect of irradiation on meat lipids at doses in the range of 0-10 kGy (Hampson et al., 1996). Our results indicate that irradiation of lean meat with a dose below 10 kGy did not strongly induce lipid oxidation of the final meat product (Bologna sausage in this study).

Quality properties of Bologna sausage

Color
Electron beam or X-ray irradiation did not affect the color of Bologna sausage made from irradiated meat compared with sausage made from non-irradiated meat (Table 4). No significant differences in color were observed among samples (p>0.05), regardless of radiation dose or type. Ionizing radiation can induce changes in meat color by promoting oxidation of myoglobin and denaturation of globins (Clarke and Richards, 1971). In a previous study (Byun et al., 2002), the redness of gamma-irradiated pork loin was increased by myoglobin oxidation induced by ionizing energy, and the increased redness was reduced during storage. The effects of radiation on the color of ground meat have not been well defined, and comparisons among radiation types have not been reported. Even though the color of ground meat can be improved by irradiation, it is difficult to maintain this improvement in processed meat products (Byun et al., 2000). However, the nitrites added to the manufactured Bologna sausage might help to maintain the redness of the final product.

Cooking yield
Radiation type and dose did not significantly affect the cooking yield of Bologna sausage manufactured from irradiated lean pork (Table 4). Cooking yield ranged from 95.14% (2 kGy, electron beam irradiation) to 96.82% (8 kGy, X-ray irradiation) and was 96.75% for the control. Denaturation of meat proteins can induce changes in the processing properties of meat, and emulsifying capability can also be changed by ionizing radiation. In a previous study (Lacroix et al., 2000), pork loins irradiated at a dose of 20 kGy had a higher emulsifying capacity. The higher emulsifying capacity of irradiated samples indicated a slight radiolysis-related increase in the emulsifying capacity of pork proteins. It was expected that the higher emulsifying capability would result in a higher cooking yield, but no effects of ionizing radiation on cooking yield were observed in this study.

Texture
Radiation type (electron beam or X-ray) and dose had no significant effect on the textural properties of Bologna sausage made from gamma-irradiated beef compared with sausages made from non-irradiated meat. (Table 4). Byun et al. (2000) also reported no differences in the textural properties of Bologna sausage made from gamma-irradiated beef compared with sausages made from non-irradiated meat.

Palatability
The palatability of Bologna sausage was organoleptically evaluated (for color, texture, and flavor) by the panels (Table 4). Color and texture were indistinguishable among all samples, but there were differences in flavor. In a previous study, no differences were observed between beef patties treated by gamma irradiation and those treated by electron beam irradiation (Park et al., 2010). However, in the current study, the flavor scores decreased as the irradiation dose increased, and there were significant differences between samples treated with electron beam or X-ray irradiation at 8 or 10 kGy and the non-irradiated lean pork and Bologna sausage manufactured from non-irradiated lean pork (0 kGy) were used as controls.

Table 3. Thiobarbituric acid values (µg malondialdehyde/g) in raw lean pork irradiated by an electron beam or X-rays and Bologna sausage manufactured with irradiated meat

| Samples       | Radiation source | 0 kGy | 2 kGy | 4 kGy | 6 kGy | 8 kGy | 10 kGy |
|---------------|-----------------|------|------|------|------|------|-------|
| Raw lean pork | Electron beam   | 0.17±0.01 | 0.20±0.01 | 0.21±0.01 | 0.22±0.01 | 0.23±0.02 | 0.24±0.02 |
|               | X-rays          | 0.22±0.02 | 0.21±0.09 | 0.23±0.01 | 0.23±0.01 | 0.24±0.01 | 0.24±0.01 |
| Bologna sausage | Electron beam | 0.22±0.01 | 0.20±0.01 | 0.21±0.01 | 0.22±0.01 | 0.23±0.02 | 0.24±0.01 |
|               | X-rays          | 0.22±0.02 | 0.24±0.01 | 0.24±0.02 | 0.24±0.01 | 0.24±0.01 | 0.24±0.05 |

Values are means±standard deviation (n=5). For raw lean pork samples, means in the same row with different lowercase letters are significantly different (p<0.05).

1Non-irradiated lean pork and Bologna sausage manufactured from non-irradiated lean pork (0 kGy) were used as controls.
Effect of X-Ray Irradiation on Meat Quality

Diated sample (p>0.05). The panel detected an off-flavor (“wet dog” flavor) in samples treated with ionizing radiation at doses above 6 kGy. Sulfur-containing proteins can be denatured and/or broken down by the attack of radiolytic radicals with strong oxidation power (Dogbevi et al., 1999; Oh et al., 2004), and unexpected volatiles can be generated by the deamination of irradiated meat (Latreille et al., 1993).

Conclusions

Electron beam and X-ray irradiation had equivalent effects on the inactivation of bacteria and on lipid oxidation in the tested ground lean pork. Based on these results, Bologna sausage can be manufactured from irradiated meat, as its processing properties were unaffected by electron beam or X-ray irradiation at absorbed doses below 10 kGy. High-energy X-ray irradiation may serve as an alternative to gamma or electron beam irradiation.

Table 4. Color differences, cooking yield, texture, and palatability of Bologna sausages manufactured with lean pork irradiated by an electron beam or X-rays

| Radiation source | Irradiation dose (kGy) | Lightness (L) | Redness (a) | Yellowness (b) | Cooking yield (%) | Texture | Palatability |
|------------------|------------------------|---------------|-------------|---------------|------------------|---------|-------------|
|                  | 0^1                    | 72.91±0.87    | 6.32±0.32   | 11.10±0.75    | 96.75±0.61       |         |             |
|                  | 2                      | 72.51±1.17    | 6.53±0.63   | 11.12±0.75    | 96.17±1.08       |         |             |
|                  | 4                      | 69.71±1.72    | 6.68±0.76   | 10.81±0.87    | 95.96±0.73       |         |             |
|                  | 6                      | 71.73±1.94    | 6.27±0.86   | 11.15±1.06    | 96.43±0.69       |         |             |
|                  | 8                      | 70.22±1.56    | 6.55±0.36   | 11.21±1.30    | 96.78±0.71       |         |             |
|                  | 10                     | 70.32±1.35    | 6.83±0.07   | 11.13±0.55    | 95.91±0.87       |         |             |

Values are means±standard deviation (n=5). Means in the same row with different lowercase letters are significantly different (p<0.05). kgf, kilogram-force.

1. Bologna sausage manufactured from non-irradiated lean pork (0 kGy) was used as a control for both irradiation treatments.
2. Cooking yield is the weight of Bologna sausage after cooking and cooling as a percentage of the weight of uncooked sausage.
3. Palatability was assessed by a panel on a scale of 1 (very poor) to 7 (very good).

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