



Application of high temperature (14 °C) aging of beef *M. semimembranosus* with low-dose electron beam and X-ray irradiation



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ABSTRACT

The effects of irradiation source (electron beam [EB] and X-ray [XR]), aging temperature (4 °C and 14 °C), and aging time (0, 3, 7, and 14 days) were evaluated on microbial quality, physicochemical properties, and calpain-1 autolysis in beef *M. semimembranosus*. Regardless of irradiation source, irradiation prior to aging reduced the total number of aerobic bacteria in beef and this reduction was maintained during aging. Irradiation did not affect the pH, b^* value, shear force, or myofibrillar fragmentation index of beef at day 0. Degradation of sarcoplasmic and myofibrillar proteins was greater in beef aged at 14 °C compared with beef aged at 4 °C. EB- or XR-irradiated samples showed slower autolysis of calpain-1; however, beef tenderness was not affected. Therefore, EB or XR irradiation can be applied to beef prior to aging to control microbial growth during high temperature (14 °C) aging, thus shortening the aging time without adversely affecting the physicochemical properties of beef.

1. Introduction

In the meat industry, aging is widely used to improve meat tenderness, which can be impacted by complex changes in muscle metabolism after slaughter (Marino et al., 2013). Aging is generally performed by storing meat for up to 3 weeks at refrigerated temperature (Lee, Sebranek, & Parrish, 1996). However, this conventional aging process has considerable refrigerated space requirements, operational costs, and energy usage (Dransfield, 1994). Higher aging temperatures around 10–15 °C result in the highest degree of meat tenderness, with lowest muscle shortening and maximum aging potential (Devine, Wahlgren, & Tornberg, 1999). The decreasing rates of shear force in beef *M. longissimus thoracis et lumborum* and *M. semitendinosus* are greater at higher incubation temperatures (15 °C and 36 °C) than at 5 °C (Hwang, Park, Cho, & Lee, 2004). This contributes to accelerated protein degradation, owing to the enhanced activity of proteolytic enzymes like calpain or cathepsin at higher aging temperatures (Hwang, Devine, & Hopkins, 2003).

Although high aging temperature can increase meat tenderness with reduced aging time, it may promote the proliferation of microorganisms

in meat, which can lead to a significant reduction in shelf life (Zhu, Mendonca, & Ahn, 2004). For example, total viable bacterial counts increased 2 log CFU/cm² in beef stored at 10 °C for 72 h, compared with a 0.4 log CFU/cm² increase in beef stored at 5 °C (Kinsella et al., 2009). Similarly, in beef samples packaged in polyethylene, *Pseudomonas* species showed faster growth rates with 0.345 log (CFU/cm²)/day at 10 °C compared to 0.090 log (CFU/cm²)/day at 0 °C (Giannuzzi, Pinotti, & Zaritzky, 1998). In this regard, a method to shorten beef aging time, while controlling microbial growth, is ideal for practical use to reduce the aging time and cost.

Irradiation technology, which is approved by the Food and Drug Administration (FDA) for the treatment of food, has been used for decades to ensure the microbial safety of meat without loss of nutritional quality (WHO, 1999). Compared to gamma irradiation, electron-beam irradiation (EB) and X-ray irradiation (XR) are more acceptable to consumers because they are free of radioisotopes (Kong et al., 2017). Park et al. (2010) found that EB up to 10 kGy showed reduced bacterial populations with no adverse effect on quality and most sensory characteristics in beef sausage patties. XR is a relatively new technology for this application. Mahmoud et al. (2015) reported that 2.0 kGy of XR

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reduced the population of *Salmonella* to below the detection limit ($< 1.0 \log \text{CFU/g}$) in chicken fillets.

Lee et al. (1996) reported that aging of EB-irradiated (2 kGy) pre-rigor beef at 30 °C for 2 days resulted in similar shear values as conventional wet-aged beef at 2 °C for 7 or 14 days, without microbial spoilage. However, an irradiation dose of 6.4 kGy on beef muscle decreased the calpain activity by creating highly oxidizing conditions (Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004a). Protein degradation and tenderness improvement during aging is highly associated with calpain-1 activation (Koochmaraie & Geesink, 2006). It can be hypothesized that a low-dose irradiation can be applied to minimize the impact on enzyme activity and meat tenderness, while preventing microbial spoilage. In addition, it is worth investigating the effects of XR irradiation because limited data are currently available. Therefore, the objective of this study was to evaluate the effect of low-dose EB and XR on microbial quality, physicochemical properties, and proteolytic calpain-1 autolysis of beef aged at 4 °C or 14 °C for 14 days.

2. Materials and methods

2.1. Sample preparation and irradiation processing

Beef *M. semimembranosus* (2 h post mortem) were obtained from a commercial slaughterhouse (Daejeon, Korea). Beef muscles were divided into three blocks (300 g each) for non-irradiated control, EB, and XR treatment samples. Each beef sample was vacuum-packaged in a sterilized polyethylene bag (20 cm $\times$ 30 cm; Sunkyoung Co., Ltd., Seoul, Korea) then irradiated. Polyethylene bags were sterilized before use with an EB irradiation dose of 35 kGy.

EB or XR was performed within 4 h after slaughter, using a linear electron beam RF accelerator (ELV-8, 10 MeV, EB-Tech Co., Ltd., Daejeon, Korea) or the ELV-8 accelerator with an X-ray converter attached (7.5 MeV, EB Tech Co., Ltd.), respectively. The beam current was 1 mA, and the dose rate was 2.95 kGy/s. The average absorbed dose was 5 kGy and was calculated using a cellulose triacetate dosimeter system (FTR-125, Fujifilm Co., Tokyo, Japan). All experiments were performed in triplicate, with three observations for each experiment. After irradiation, the beef samples were stored at refrigerated temperature (4 °C) or elevated temperature (14 °C) until further analysis. Samples were collected after 0, 3, 7, and 14 days of aging. An elevated temperature of 14 °C was selected because the highest tenderness and lowest cold shortening of beef has been shown at this temperature (Devine et al., 1999).

2.2. Microbial analysis

Five gram beef samples were blended with 45 mL of 0.85% sterile saline solution for 2 min using a stomacher (BagMixer® 400, Interscience Ind., St. Nom, France). Samples for microbial testing were prepared in a series of decimal dilutions using sterile saline. Each diluent (0.1 mL) was spread on total plate count agar (Difco Laboratories, MI, USA) in triplicate, and the agar plates were incubated at 37 °C for 48 h. The number of colonies was counted and expressed as colony forming units per gram ($\log \text{CFU/g}$).

2.3. pH

pH was measured by blending 1 g beef samples with 9 mL of distilled water (DW) for 30 s at 10,000 rpm using a homogenizer (T10 basic, Ika Works, Staufen, Germany). The homogenates were filtered by filter paper (No. 4, Whatman International Ltd., Kent, UK) after centrifugation at $2265 \times g$ for 10 min (Continent 512R, Hanil Co., Ltd., Incheon, Korea). The pH of the filtrate was measured using a pH meter (SevenGo, Mettler-Toledo International Inc., Schwerzenbach, Switzerland).

2.4. Instrumental color measurement

The lightness (L^*), redness (a^*), and yellowness (b^*) of the beef samples were measured by a spectrophotometer (CM-5, Konica Minolta Censing Inc., Osaka, Japan) (Yong et al., 2017). The instrument was calibrated with a standard black and white plate before measurement. Measurements were taken in triplicate at different locations within each sample.

2.5. Shear force measurement

Beef samples were vacuum-packaged and cooked in a water bath at 85 °C for 30 min to achieve a core temperature of approximately 75 °C (Jayasena, Nam, Kim, Ahn, & Jo, 2015). Samples were then cooled at 4 °C and three core samples (1.0 $\times$ 1.5 $\times$ 3 cm) were taken in the longitudinal direction of muscle fibers. Each sample was cut at a speed of 120 mm/min at 20 N force using a Warner-Bratzler blade attached to a texture analyzer (LLOYD instruments, Ametek, Fareham, UK), with a maximum cell load of 10 kg and a target load of 10 g. The shear force value was calculated as the mean of the maximum force required to shear each set of core samples.

2.6. Myofibrillar fragmentation index (MFI)

MFI was determined by turbidity methods, as described by Hopkins, Littlefield, and Thompson (2000), with some modifications. MFI is an indicator of measuring the extent of myofibrillar protein degradation of meat during aging (Olson, Parrish, & Stromer, 1976). For each sample, 0.5 g of minced beef was homogenized with 30 mL of MFI buffer containing 0.1 M KCl, 0.001 M EDTA, 0.001 M sodium azide (NaN_3), 0.025 M potassium phosphate (0.007 M KH_2PO_4 and 0.018 M K_2HPO_4 giving a pH 7.0 at 4 °C) at 10,000 rpm for 30 s. After homogenization, the mixture was left to rest for 30 s and then re-homogenized for 30 s. The resulting homogenate was filtered with a 1-mm mesh strainer to remove the connective tissues and washed with 10 mL of MFI buffer. The filtered homogenate was centrifuged at $10,000 \times g$ for 10 min (HM-150IV, Hanil Co. Ltd., Seoul, Korea) and then the supernatant was removed. The remaining pellet was mixed with 10 mL of MFI buffer and vortexed. This step was repeated five times. After removal of the supernatant, 10 mL of MFI buffer was added to the pellet and samples were vortexed. Aliquots of the resulting suspension were diluted with MFI buffer to 0.5 mg/mL of protein concentration, and the absorbance was measured at 540 nm using a spectrophotometer (X-ma 3100, Human Co. Ltd., Seoul, Korea). MFI values were calculated as absorbance units multiplied by 200.

2.7. SDS-PAGE and western blotting

2.7.1. Preparation of meat extraction

Beef samples were minced and 0.5 g of the minced sample was blended with 5 mL of Tris-EDTA buffer (0.05 M Tris and 0.01 M EDTA) at pH 8.3 using a homogenizer (T10 basic) for 1 min. The homogenized solution was centrifuged at $10,000 \times g$ for 20 min and the supernatant was collected and mixed with an equal volume of $2 \times$ SDS sample buffer (0.125 M Tris-HCl buffer at pH 6.8, containing 20% glycerol, 2% SDS, 2% β -mercaptoethanol, and 0.02% bromophenol blue). Samples were boiled at 95 °C for 10 min and cooled at 4 °C for 2 min. The total protein concentration in meat extracts was 1 mg/mL, determined using the Lowry, Rosebrough, Farr, and Randall (1951) method. The prepared meat extracts were used for SDS-PAGE and western blot.

2.7.2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE method of Laemmli (1970) was used with some modifications. The stacking gel and separating gel contained 4.5% and 12.5% polyacrylamide, respectively and 20 μL of meat extract was

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