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

So Yeon Kim[a], Hae In Yong[a], Ki Chang Nam[b], Samooel Jung[c], Dong-Gyun Yim[d], Cheorun Jo[a,e,*]

[a] Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Research Institute of Agriculture and Life Science, Seoul National University, Seoul 08826, Republic of Korea
[b] Department of Animal Science and Technology, Sunchon National University, Suncheon 57922, Republic of Korea
[c] Division of Animal and Dairy Science, Chungnam National University, Daejeon 34134, Republic of Korea
[d] Department of Health Administration and Food Hygiene, Jinju Health College, Jinju 52655, Republic of Korea
[e] Institute of Green Bio Science and Technology, Seoul National University, Pyeongchang 25354, Republic of Korea

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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/cm^2 in beef stored at 10 °C for 72 h, compared with a 0.4 log CFU/cm^2 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/cm^2)/day at 10 °C compared to 0.090 log (CFU/cm^2)/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

* Corresponding author at: Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Republic of Korea.
E-mail addresses: lo0703ve@snu.ac.kr (S.Y. Kim), awsm_y@naver.com (H.I. Yong), kichang@scnu.kr (K.C. Nam), samooel@cnu.ac.kr (S. Jung), tousa0994@naver.com (D.-G. Yim), cheorun@snu.ac.kr (C. Jo).

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reduced the population of *Salmonella* to below the detection limit (< 1.0 log 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 (Koohmaraie & 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 × 30 cm; Sunkyung 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 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 × *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 × 1.5 × 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 × *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 × *g* for 20 min and the supernatant was collected and mixed with an equal volume of 2 × 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

loaded onto the gel. Protein standards (Precision Plus Protein™ Unstained Standards, Bio-Rad, CA, USA) were included in each electrophoretic run to determine molecular size. Electrophoresis was performed using a Mini-slab Size Electrophoresis System AE-6531 (Atto Corporation, Tokyo, Japan) at 20 mA for 70 min. Gels were stained for 30 min in 0.1% Coomassie Brilliant Blue R-250 solution, containing 30% methanol and 10% acetic acid. After staining, gels were destained for 90 min using a solution containing 30% methanol and 10% acetic acid.

2.7.3. Western blot

Autolysis of calpain-1 was studied by western blot according to the method of Towbin and Gordon (1984). After electrophoresis, gels were electroblotted onto polyvinylidene difluoride membranes (Bio-Rad, CA, USA) at 4 °C for 90 min at 90 V using a mini trans-blot cell (Bio-Rad, CA, USA). The transfer buffer (pH 8.3) consisted of 0.025 M Tris, 0.192 M glycine, and 20% methanol. After transfer, membranes were incubated in blocking solution (5% skim milk in 0.05% Tween 20 [TBS-T]) at room temperature for 1 h on a shaker and washed in TBS-T. Membranes were then incubated overnight at 4 °C on a shaker with primary antibodies (Mu-calpain antibody, 9A4H8D3, Alexis Crop., CA, USA) diluted 1:5000 with 1% skim milk. After incubation, membranes were washed three times with TBS-T for 5 min each and incubated at room temperature for 1 h with HRP-conjugated secondary antibody (Goat Anti-mouse IgG-HRP, sc-2005, Santa Cruz Biotechnology Co. Ltd., CA, USA) diluted 1:5000 with TBS-T. Membranes were washed three times with TBS-T for 5 min each and then bound antibodies were detected using Clarity Western ECL Substrate (Bio-Rad, CA, USA). The signal intensity was determined using Image Lab Software Version 5.2.1 (Bio-Rad, CA, USA).

2.8. Statistical analysis

All experiments were performed in triplicate. Using SAS software (version 9.3, SAS Institute Inc., NC, USA), a multifactorial analysis of variance using the general linear model was applied to investigate the effect of irradiation (EB and XR), aging temperature (4 °C and 14 °C), and aging time (0, 3, 7, and 14 days). The differences among the mean values were identified using the Tukey's multiple range test at a confidence level of $P < 0.05$. The mean values and standard errors of the means were recorded.

3. Results and discussion

3.1. Total aerobic bacteria

The initial number of total aerobic bacteria was reduced in irradiated samples when compared to non-irradiated controls ($P < 0.05$; Table 1). The efficiency between EB and XR irradiation on microbial inactivation was not different. Reductions were 1.40 and 2.07 log CFU/g for EB and XR, respectively, compared with 4.73 log CFU/g for non-irradiated samples. In most cases, irradiated samples showed significantly lower total aerobic bacterial counts than non-irradiated samples during 14 days of storage ($P < 0.05$). EB irradiation doses of 5 kGy resulted in approximately a 0.5 log CFU/g reduction in initial total aerobic bacterial counts in cooked beef patties (Park et al., 2010). Prendergast, Crowley, McDowell, and Sheridan (2009) also found that irradiation doses of 5 kGy gave a 2–3 log reduction in total aerobic bacterial counts in vacuum-packaged beef. Microbial inactivation by irradiation is mainly caused by the generation of free radicals such as $OH\cdot$, $H\cdot$, and e^- resulting from water hydrolysis. Free radicals also generate hydrogen peroxide, which is an effective antimicrobial agent (Manas & Pagán, 2005). As a result, bacterial cell walls and DNA can be damaged and malfunction of microorganisms can occur.

Beef samples aged at 14 °C had higher total aerobic bacterial counts than samples aged at 4 °C, indicating that storage temperature had a significant effect on microbial growth rate ($P < 0.0001$). Similar results were reported by Yim, Jo, Kim, Seo, and Nam (2016). In their study, EB irradiation resulted in a significant decrease in initial total aerobic bacterial counts, but bacteria proliferated rapidly in beef loins aged at 10 °C and 25 °C compared to those aged at 2 °C. In our results, the total aerobic bacterial counts of non-irradiated samples stored at 14 °C for 3 days were approximately 7 log CFU/g, which is considered the upper microbial limit for good quality fresh meat (ICMFS, 1986). However, the microbial counts of both irradiated samples aged at 14 °C for 7 days were below 7 log CFU/g. These results indicate that EB and XR can extend the shelf life of beef aged at high temperature, with a maximum shelf life of 7 days at 14 °C in this experiment.

3.2. pH

EB and XR did not change the initial pH values of beef samples, which had a mean value of 5.79 (Table 2). This result agrees with that reported by Lee et al. (1996) that the pH of beef steaks is not affected by EB up to 7 days, irrespective of storage temperature. However, in our study, pH values decreased at both temperatures during aging ($P < 0.0001$), except for a slight pH increase in samples aged at 14 °C for 7–14 days ($P < 0.05$). The decrease in pH at early storage times can be explained by the accumulation of inorganic phosphoric acid resulting from the depletion of muscle adenosine triphosphate (ATP) (Scherer et al., 2005) and the production of lactic acid resulting from the degradation of glycogen (Kozioł, Maj, & Bieniek, 2015). Meanwhile, the increase in pH of samples stored at 14 °C for 14 days may be attributed to the production of ammonia, amines, and other basic substances from the degradation of proteins by microorganisms and endogenous enzymes in beef (Muela, Sañudo, Campo, Medel, & Beltrán, 2010). This would be expected, given the changes seen in aerobic bacterial count during storage (Table 1).

3.3. Surface color

Meat color is an important visual factor that determines how consumers perceive product quality and it significantly influences the consumer's purchasing decisions (Carpenter, Cornforth, & Whittier, 2001). XR significantly decreased the initial L^* value of beef when compared to the other groups, but there were no differences in the L^* value between control and irradiated samples after 3 days of storage. The a^* value of beef was initially lower in irradiated samples, but was not different after aging for 14 days. The irradiation treatments and aging temperatures had no effect on the b^* value of beef samples. Therefore, we conclude that EB and XR could affect the color of beef initially, but the difference may disappear with extended storage. Several studies have shown that irradiating fresh beef results in undesirable color changes (Brewer, 2004). Nam et al. (2003) reported that beef appears to be the meat most susceptible to irradiation-induced color changes, often changing to an unattractive greenish or brownish gray color (Kim, Nam, & Ahn, 2002). However, this study showed that 5 kGy of EB and XR had no effect on the overall color of beef after 3 days of storage, although a decrease in L^* and a^* values were detected at day 0 in irradiated samples when compared to non-irradiated samples. EB and XR irradiation treatments did not affect the surface color of beef samples at the end of the storage period (Table 3). Color changes in irradiated fresh meat occur because of the inherent susceptibility of the myoglobin molecule to energy input and alterations in the chemical environment, with heme iron being particularly susceptible (Brewer, 2004).

3.4. Shear force and myofibrillar fragmentation index (MFI)

EB- and XR-irradiated beef showed no differences in shear force values compared with non-irradiated controls at all aging times (Table 4). Davis, Sebranek, Huff-Lonergan, Ahn, and Lonergan (2004)

Table 1
Total aerobic bacterial counts (log CFU/g) of the EB- and XR-irradiated beef samples aged at 4 °C or 14 °C during 14 days of aging.

Aging temperature (°C)	Irradiation[A]	Aging time (days)				SEM[C]
		0	3	7	14	
4	Control	4.73^{bx}	4.99^{bx}	5.17^{bx}	6.15^{ax}	0.170
	EB	3.33^{by}	3.40^{by}	3.79^{aby}	4.06^{ay}	0.105
	XR	2.66^{y}	2.96^{y}	3.25^{y}	3.60^{y}	0.242
	SEM[B]	0.159	0.240	0.168	0.140	
14	Control	4.73^{cx}	6.57^{bx}	8.16^{ax}	8.41^{ax}	0.204
	EB	3.33^{cy}	4.24^{bcy}	4.98^{by}	7.31^{axy}	0.277
	XR	2.66^{cy}	3.40^{bcy}	4.50^{by}	6.37^{ay}	0.286
	SEM[B]	0.159	0.253	0.223	0.357	
	Temp.	Irradiation	Aging	Temp. × irradiation	Temp. × aging	Irradiation × aging
P-value	< 0.0001	< 0.0001	< 0.0001	0.0768	< 0.0001	0.2747

[a–c]Values with different letters within the same row differ significantly ($P < 0.05$).
[x,y]Values with different letters within the same column differ significantly ($P < 0.05$).
[A] Control, non-irradiated; EB, electron beam irradiation; XR, X-ray irradiation.
[B] Standard error of the means ($n = 9$).
[C] Standard error of the means ($n = 12$).

Table 2
pH of the EB- and XR-irradiated beef samples aged at 4 °C or 14 °C during 14 days of aging.

Aging temperature (°C)	Irradiation[A]	Aging time (days)				SEM[C]
		0	3	7	14	
4	Control	5.80^{a}	5.61^{b}	5.59^{b}	5.54^{b}	0.039
	EB	5.78^{a}	5.54^{b}	5.52^{b}	5.49^{b}	0.052
	XR	5.80^{a}	5.59^{ab}	5.54^{b}	5.49^{b}	0.054
	SEM[B]	0.043	0.083	0.030	0.032	
14	Control	5.80^{a}	5.47^{bc}	5.40^{cy}	5.72^{ab}	0.054
	EB	5.78^{a}	5.53^{b}	5.53^{bx}	5.68^{ab}	0.043
	XR	5.80^{a}	5.58^{bc}	5.54^{bcx}	5.66^{ab}	0.034
	SEM[B]	0.043	0.051	0.024	0.054	
	Temp.	Irradiation	Aging	Temp. × irradiation	Temp. × aging	Irradiation × aging
P-value	0.0577	0.7739	< 0.0001	0.1545	0.6367	0.6592

[a–c]Values with different letters within the same row differ significantly ($P < 0.05$).
[x,y]Values with different letters within the same column differ significantly ($P < 0.05$).
[A] Control, non-irradiated; EB, electron beam irradiation; XR, X-ray irradiation.
[B] Standard error of the means ($n = 9$).
[C] Standard error of the means ($n = 12$).

reported that EB irradiation (4.4 kGy) had no effect on the shear force values of fresh pork loins. However, Rowe, Maddock, Lonergan, and Huff-Lonergan (2004b) reported that irradiated (6.4 kGy) beef steaks showed significantly higher shear force values than non-irradiated steaks. Rowe et al. (2004b) also demonstrated that higher carbonyl content was associated with the higher shear force seen in irradiated beef. Irradiation of beef at an early postmortem stage induces oxidative conditions that increase protein oxidation. Oxidized amino acids cause denaturation and aggregation of myofibrillar protein and loss of proteolytic enzyme activity (Rowe et al., 2004a). Thus, application of low-dose irradiation on meat is important to avoid extensive oxidizing conditions that can cause meat toughness. Lee et al. (1996) showed that 2 kGy of electron beam irradiation did not affect shear force or myofibrillar fragmentation index in beef. The current study used a relatively low dose (5 kGy) of EB and XR irradiation compared with previous studies (6.4 kGy), and it had no adverse effect on beef tenderness. Our preliminary study indicated that 2 kGy of EB or XR could not induce microbial inactivation during storage at 14 °C (data not shown). Aging temperature had a significant effect on shear force values (Table 4). The decreasing rate of shear force was more evident in samples stored at 14 °C than in samples stored at 4 °C. The shear force values of samples stored at 14 °C for 7 days are similar to the values of samples stored at 4 °C for 14 days. This may be attributed to the accelerated destruction of muscle structure and increased protease activity at high storage temperatures. It has been reported that rigor temperatures around 10–15 °C give the greatest improvement in tenderness by reducing muscle shortening and lessening the impact on enzyme activity (Devine et al., 1999).

MFI is an indicator of the extent of myofibrillar protein degradation during the postmortem aging of meat (Li et al., 2012). It represents the degradation of structural proteins in the I-band of the myofibril and the weakening of myofibril linkages (Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995). Irradiation treatment had no effect on MFI (Table 5). MFI values significantly increased over storage time ($P < 0.0001$) and they were significantly affected by aging temperature. Samples aged at 14 °C had higher MFI values compared to samples aged at 4 °C ($P < 0.0001$). Lee et al. (1996) reported that MFI increases in beef with increasing post-aging time, regardless of EB treatment. This may be attributed to *Z*-disk collapses in the beef intramuscular fibers as the post-mortem aging time increases. Li et al. (2012) reported that muscles kept at 14 °C showed increased MFI values and suggested that accelerated proteolysis is the reason for improved tenderness.

3.5. SDS-PAGE

SDS-PAGE analysis showed no changes in the sarcoplasmic and myofibrillar protein patterns between non-irradiated and EB- or XR-irradiated samples at 0 and 14 days of aging (Fig. 1). Lee et al. (2000) also found no change in sarcoplasmic and myofibrillar proteins in

Table 3
Surface color of the EB- and XR-irradiated beef samples aged at 4 °C or 14 °C during 14 days of aging.

Aging temperature (°C)	Irradiation[A]	Aging time (days)				SEM[C]
		0	3	7	14	
L^*						
4	Control	37.18^{x}	36.37	35.54	33.43	0.852
	EB	35.56^{xy}	35.35	33.93	34.44	0.830
	XR	34.74^{y}	33.11	34.43	33.32	0.908
	SEM[B]	0.621	0.958	0.926	0.907	
14	Control	37.18^{ax}	31.28^{b}	33.17^{ab}	33.03^{ab}	0.936
	EB	35.56^{xy}	32.64	33.50	34.70	1.036
	XR	34.74^{y}	33.83	32.24	32.75	0.804
	SEM[B]	0.621	0.928	1.135	0.784	
a^*						
4	Control	17.34^{x}	18.14^{x}	16.06	15.59	0.843
	EB	14.88^{y}	15.40^{y}	14.73	14.97	0.711
	XR	14.42^{y}	15.01^{y}	13.86	13.42	0.448
	SEM[B]	0.553	0.404	0.903	0.778	
14	Con	17.34^{x}	17.23	16.68	14.99	1.134
	EB	14.88^{y}	17.32	14.94	16.63	0.607
	XR	14.42^{ay}	16.48^{ab}	17.57^{a}	17.31^{a}	0.550
	SEM[B]	0.553	0.568	1.139	0.839	
b^*						
4	Control	5.15	5.44^{x}	5.77	5.91	0.367
	EB	5.31	5.67^{x}	5.16	5.95	0.243
	XR	4.94	4.25^{y}	4.99	5.60	0.337
	SEM[B]	0.344	0.191	0.415	0.288	
14	Control	5.15^{ab}	5.10^{b}	5.44^{ab}	6.72^{a}	0.358
	EB	5.31	5.67	5.16	6.42	0.396
	XR	4.94	4.58	5.99	6.29	0.400
	SEM[B]	0.344	0.371	0.513	0.269	
	Temp.	Irradiation	Aging	Temp. × irradiation	Temp. × aging	Irradiation × aging
L^*						
P-value	0.0046	0.0375	0.0002	0.2121	0.0705	0.2281
a^*						
P-value	0.0016	0.0007	0.0466	0.0093	0.1922	0.0546
b^*						
P-value	0.1432	0.2657	< 0.0001	0.1047	0.3572	0.0606

[a,b]Values with different letters within the same row differ significantly ($P < 0.05$).
[x,y]Values with different letters within the same column differ significantly ($P < 0.05$).
[A] Control, non-irradiated; EB, electron beam irradiation; XR, X-ray irradiation.
[B] Standard error of the means ($n = 9$).
[C] Standard error of the means ($n = 12$).

gamma-irradiated (up to 10 kGy) beef round by SDS-PAGE. Similarly, no changes were detected in protein patterns between non-irradiated and gamma-irradiated beef, pork, and chicken meats below 10 kGy of irradiation dose (Yook, Kim, Kim, Lim, & Byun, 1998). These results indicate that EB and XR irradiation at 5 kGy does not destroy or degrade protein structures in beef samples. The intensity of the 43-kDa band in sarcoplasmic protein patterns decreased in beef aged for 14 days at 14 °C, compared to beef aged for 14 days at 4 °C (Fig. 1). This result shows that high aging temperature enhances the degradation of this protein. Okayama, Fukumoto, Nakagawa, Yamanoue, and Nishikawa (1992) reported that meat tenderness during postmortem aging is significantly correlated with the intensity of the 43-kDa

Table 4
Shear force values (N) of the EB- and XR-irradiated beef samples aged at 4 °C or 14 °C during 14 days of aging.

Aging temperature (°C)	Irradiation[A]	Aging time (days)				SEM[C]
		0	3	7	14	
4	Control	90.73^{a}	89.92^{a}	75.59^{b}	69.18^{b}	3.040
	EB	92.15^{a}	94.35^{a}	79.28^{b}	71.76^{b}	1.825
	XR	96.96^{a}	94.82^{a}	79.19^{b}	71.15^{c}	1.717
	SEM[B]	1.816	2.041	2.979	2.086	
14	Control	90.73^{a}	84.43^{a}	65.98^{b}	49.65^{c}	2.396
	EB	92.15^{a}	85.64^{a}	65.39^{b}	53.86^{c}	2.473
	XR	96.96^{a}	87.20^{b}	66.16^{c}	54.96^{d}	1.555
	SEM[B]	1.816	1.848	2.925	1.940	
	Temp.	Irradiation	Aging	Temp. × irradiation	Temp. × aging	Irradiation × aging
P-value	< 0.0001	0.0040	< 0.0001	0.8032	< 0.0001	0.7355

[a–d]Values with different letters within the same row differ significantly ($P < 0.05$).
[A] Control, non-irradiated; EB, electron beam irradiation; XR, X-ray irradiation.
[B] Standard error of the means ($n = 9$).
[C] Standard error of the means ($n = 12$).

Table 5
Myofibrillar fragmentation index (MFI) of the EB- and XR-irradiated beef samples aged at 4 °C or 14 °C during 14 days of aging.

Aging temperature (°C)	Irradiation[A]	Aging time (days)				SEM[C]
		0	3	7	14	
4	Control	42.74^{c}	47.56^{c}	73.58^{b}	100.78^{a}	4.502
	EB	45.44^{c}	50.66^{c}	74.93^{b}	97.27^{a}	4.451
	XR	44.20^{c}	44.78^{c}	73.20^{b}	95.36^{a}	4.204
	SEM[B]	2.848	2.798	5.086	5.932	
14	Control	42.74^{c}	55.65^{c}	95.21^{b}	157.79^{a}	4.811
	EB	45.44^{c}	55.83^{c}	92.35^{b}	150.93^{a}	5.312
	XR	44.20^{c}	57.56^{c}	90.59^{b}	152.40^{a}	5.191
	SEM[B]	2.848	3.524	5.517	7.311	
	Temp.	Irradiation	Aging	Temp. × irradiation	Temp. × aging	Irradiation × aging
P-value	< 0.0001	0.8191	< 0.0001	0.7171	< 0.0001	0.9220

[a–c]Values with different letters within the same row differ significantly ($P < 0.05$).
[A] Control, non-irradiated; EB, electron beam irradiation; XR, X-ray irradiation.
[B] Standard error of the means ($n = 9$).
[C] Standard error of the means ($n = 12$).

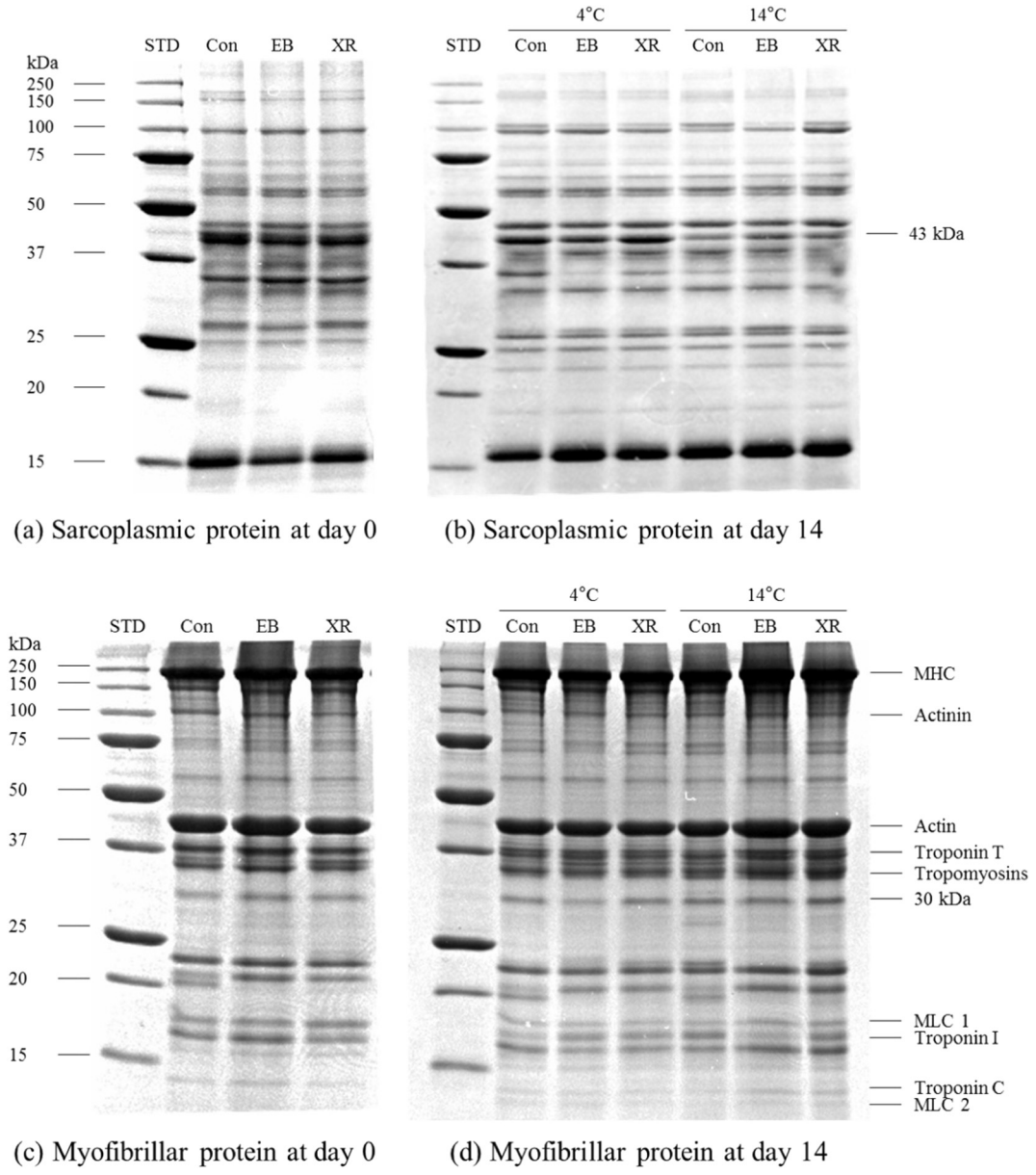

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the EB- and XR-irradiated beef samples aged for 0 and 14 days at 4 °C or 14 °C. (STD, standard molecular bands; Control, non-irradiated; EB, electron beam; XR, X-ray.)

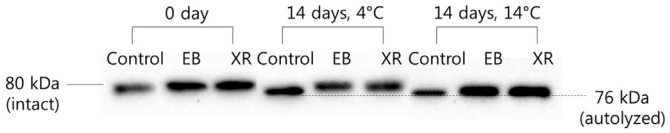

Fig. 2. Western blot of calpain-1 in the sarcoplasmic fraction of the EB- and XR-irradiated beef samples aged for 0 and 14 days at 4 °C or 14 °C. (Control, non-irradiated; EB, electron beam; XR, X-ray.)

sarcoplasmic protein band identified as creatine kinase. Similarly, in our results, lower shear force values were observed in beef aged for 14 days at 14 °C compared to beef aged at 4 °C. This was accompanied by decreasing intensity of the 43-kDa band in SDS-PAGE. This result suggests that sarcoplasmic protein degradation may reflect increased proteolysis due to high aging temperature and this could be used as a marker for beef tenderness (Bowker, Fahrenholz, Paroczay, & Solomon, 2008). Degradation of myofibrillar proteins, especially troponin T, myosin light chain 1, and troponin I, is observed during 14 days of aging. However, no differences were found in the myofibrillar protein pattern between non-irradiated controls and irradiated beef samples. Using western blot analysis, Rowe et al. (2004b) reported that oxidative conditions caused by 6.4 kGy of irradiation have a negative effect on troponin T degradation, indicating a significant decrease in beef tenderness. This result can be explained by the increased sensitivity of western blot analysis to detect very low amounts of myofibril fragments. Therefore, samples were further analyzed by western blot.

3.6. Western blot

EB and XR did not affect calpain-1 activity in sarcoplasmic extracts from beef samples at day 0 (Fig. 2). Calpain-1 is one of the proteolytic enzymes present in meat that acts on the cytoskeletal proteins in the myofibrils, resulting in a decrease in the binding force between the myofibrils (Koohmaraie & Geesink, 2006). Autolysis of calpain-1 is associated with enzyme activity in post-mortem muscle. Oxidation of the cysteine residue in the active site of calpain-1 results in loss of activity (Lametsch, Lonergan, & Huff-Lonergan, 2008). Rowe et al. (2004b) found that strong oxidative conditions caused by irradiation decreased sarcoplasmic and myofibril-bound calpain-1 activity and reduced the degradation of nebulin, titin, desmin, and troponin-T in beef steaks. In addition, the irradiated beef steaks showed higher shear force compared to non-irradiated samples. Similarly, the present study showed a slower rate of sarcoplasmic calpain-1 autolysis in irradiated beef compared to non-irradiated controls at 14 days. Calpain-1 consists of 80, 78, and 76 kDa bands and degradation of the intact 80 kDa (inactive form) band to autolyzed 76 kDa (active form) occurs during aging. The result indicates that calpain-1 autolysis was less extensive and there was lower enzyme activity in irradiated samples compared to non-irradiated controls. However, the effect of 5 kGy of irradiation on calpain-1 activity may be negligible to meat tenderness, because there was no significant difference in shear force values or MFI between non-irradiated and irradiated samples.

4. Conclusion

These results suggest that low-dose EB and XR irradiation of beef prior to aging are effective in decreasing microbial growth during aging, with no undesirable changes in physicochemical properties. The low-dose EB and XR irradiation (5 kGy) of beef can be combined with high temperature (14 °C) aging to significantly decrease aging time. EB and XR irradiation may reduce calpain-1 autolysis during beef aging to some extent; however, these effects are not strong enough to reduce final meat tenderness. Therefore, the low-dose EB and XR irradiation of beef could be an effective technique for high temperature aging.

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