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ARTICLE



Combined Effect of Aging and Irradiation on Physicochemical Quality of Pork Shoulder

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Abstract The effect of combined electron-beam irradiation and aging temperature of pork on microbiological and physicochemical properties was investigated. The samples from pork shoulder were irradiated with 0 or 2 kGy, vacuum-packaged, and assigned randomly to an aging temperature (2°C, 10°C, or 25°C) during 8 d. On 4 d of aging at 25°C, total aerobic bacteria of non-irradiated ones reached 7 Log CFU/g which is no salable levels. Shear force values of irradiated meat after aging for 2 and 4 d at 25°C was lower than those aged at 2°C. Irradiated samples at 2°C had lower cooking loss after 2 and 8 d of aging, compared with other aging temperatures. Irradiation did not accelerate 2-thiobarbituric acid reactive substance (TBARS) value when aged up to 4 d. Irradiated samples aged at 10°C and 25°C for 8 d scored significantly higher TBARS values. With an increased aging period, a* and b* in irradiated samples at 2°C slightly increased, but irradiation caused negligible changes in meat color. The highest contents of a desirable nucleotide flavor compounds (inosine-5-phosphate) were observed in pork at 2°C when aged for 4 and 8 d, while the lowest contents were observed at 25°C. Aging in irradiated pork for 8 d at 2°C resulted in optimal condition with improved meat quality and minimal microbiologically negative defect.

Keywords electron beam irradiation, aging temperature, pork, meat quality, nucleotide flavor compounds

Introduction

Irradiation of meat has been considered to be the most efficient technology for inhibiting proliferation of pathogenic microbes without fascinating nutritional traits (WHO, 1999). The WHO, FAO, the International Atomic Energy Agency (IAEA), and the US FDA who are very proficient has given the approval for using the irradiation on wholesomeness of irradiated food (Sohn et al., 2009).

Meat irradiation at low-dose is used to minimize meat quality changes (Zhu et al.,

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Dong-Gyun Yim https://orcid.org/0000-0003-0368-2847 Cheorun Jo https://orcid.org/0000-0003-2109-3798 Ali Mahabbat https://orcid.org/0000-0002-5332-7758 Ji-Young Park https://orcid.org/0000-0001-9680-8685 Seong-Yun Lee https://orcid.org/0000-0003-1361-1962 Ki-Chang Nam https://orcid.org/0000-0002-2432-3045 2004). Along with the irradiation sources, electron beam (EB) processing dose not generate any radioactive wastes, process time is relatively short with minimal temperature expansion, and consumers favors (Black and Jaczynski, 2006). However, it can influence lipid oxidation, color changes, and off-odor of meat, which may generate negative consumer responses (Jo and Ahn, 2000a). Pork shoulder is popular and demanding cuts in Asian markets and the price are much higher compared with other retail cuts (KMTA, 2017). Moreover, irradiation of meat cuts in relation to various quality aspects were studied in terms of nutritional and sensory qualities (Fu et al., 1995; Giroux et al., 2001). However, very few research has been performed on the quality and storability of pork shoulder by EB-irradiation.

Even so, aging improves the tenderness and texture of meat, but also associated with greater loss during cooking (Huff-Lonergan E, 2005; Straadt et al., 2007). Pork has not gained the same popularity of aging techniques due to its relatively tender texture compared to beef. However, aging of pork shoulder can help it to be more tender and juicy. Several factors like temperature and time can be factors affecting aging in meat (Lee et al., 1996). Aging methods, periods, or temperatures during aging have yet to be explored due to production costs, operational efficiencies, and variation in the physicochemical properties of pork (Frenzel et al., 2014). A number of research has been conducted entitled of beef aging and well concerned about the quality and sensory traits of aged meat (DeGeer et al., 2009). To shorten aging time, higher temperature aging technique was applied to beef (Yim et al., 2016). Based on the results of microbiological safety, high temperature aging method was problematic in safety issues (Yim et al., 2016).

Although numerous studies have been conducted to find out the effect of irradiation on meat quality, very limited data is available on combined effect of aging and irradiation on the pork quality. Therefore, the objective of this study was not only to establish optimal conditions for aging by examining the impacts of aging at different temperatures and times of pork shoulder, but also to establish a pork resulted in a safe and quality product guide with proper physicochemical quality using EB irradiation.

Materials and Methods

Sample preparation and irradiation

Pork shoulder (mainly M. *supraspinatus*) was selected from 6 different pig carcasses for replication sample design from a carcass processing center (Daejeon, Korea) within 2 days from the time of slaughtering. After visible fats trimmed out, meat samples were sliced into a thick piece of 1 cm, which is enough to be irradiated over the whole sample. After vacuum packaged, there were stored at 5°C overnight and irradiated next day.

Using a Linear Accelerator each prepared sample was irradiated at 2 kGy which is checked by an alanine dosimeter and then meat samples (irradiated and non-irradiated) were returned to a 4°C room without any delay and aged for 8 d. The microbiological, shear force, cooking loss, lipid oxidation, color and nucleotides analyses were performed at different aging temperatures (2°C, 10°C, or 25°C) on 0, 2, 4, and 8 d.

Microbial and physicochemical analysis

Ten grams of sample were aseptically homogenized after adding 90 mL of sterile solution in a sterile Stomacher bag for 2 min (BagMixer[®] 400, Interscience, France). Consequently the diluents were planted onto aerobic plated count agar (Difco Laboratories), incubated at 37°C for 45 h. The total number of colonies observed on plate of each sample after incubation was counted and expressed as log of colony forming units per gram (Log CFU/g).

Shear force (kg·f) was measured by the method (Yim et al., 2016). After cooking samples sliced with 2 cm-thickness in an electric grill (EMG-533, AIJIA, Hong Kong, China) up to 70°C, cooking loss was determined as follows: cooking loss (%)=100×(raw meat weight – final cooked meat weight)/raw meat weight. Lipid oxidation was determined as 2-thiobarbituric acid reactive substance (TBARS) values, following the slightly modified method (Ahn et al., 1998). Color parameters of sample surfaces were evaluated using a portable colorimeter (CR-200, Konica Minolta, Japan) from three randomly different locations. Flavor-related nucleotides in meats were measured according to the modified method described by (Yim et al., 2016). The peaks for individual nucleotides of the samples were branded with the retention time for the subsequent standards: inosine-5-phosphate (inosine monophosphate, IMP), inosine, hypoxanthine (Sigma, Agilent, USA); using the area of each peak, the concentration of nucleotides were calculated.

Statistical analysis

The experiment was performed with 3 independent trials having 3 observations for each treatment combination following in each trial. Data from each trial of experiment were analyzed using SAS software (SAS, 2003). Two-way analysis of variance (ANOVA) was performed. The differences among the mean values were determined by the Duncan's multiple comparison test at p<0.05, when the significant differences were detected. The mean values with standard error of the means (SEM) were reported.

Results and Discussion

Aging temperature, periods, and irradiation treatment influenced TAB of pork shoulder (Table 1). As aging temperature and

Table 1. Effect of different aging temperature on total aerobic bacterial counts (Log CFU/g) in pork shoulder treated by electron beam
irradiation

Steward (lear)	T (9C)	Irradiation dose (kGy)					
Storage (day)	Temperature (°C)	0	2	SEM ¹⁾			
0	-	3.22 ^x	2.71 ^y	0.06			
2	2	3.60°	3.58 ^b	0.08			
	10	3.99 ^b	3.90 ^a	0.04			
	25	6.18 ^{ax}	3.90 ^{ay}	0.09			
	SEM ²⁾	0.08	0.07				
4	2	4.22°	4.17°	0.05			
	10	5.07 ^{bx}	4.45 ^{by}	0.04			
	25	7.12 ^{ax}	4.58 ^{ay}	0.02			
	SEM	0.04	0.04				
8	2	4.42 ^{cx}	4.31 ^{by}	0.02			
	10	5.12 ^{bx}	4.51 ^{ay}	0.05			
	25	7.28 ^{ax}	4.60 ^{ay}	0.03			
	SEM	0.02	0.05				

 $^{1)}$ n=9, $^{2)}$ n=24.

^{a-c} Values with different letters within the same column differ significantly (p<0.05).

xy Values with different letters within the same row differ significantly (p<0.05).

periods increased, TAB increased. This agrees with (Borch et al., 1996), who observed as refrigeration temperatures decreased, TAB decreased. However, TAB of irradiated pork shoulder samples was lower compared with the non-irradiated ones under the same aging tempeature. TAB of irradiated shoulders showed irradiation effectively controlled the growth of microorganisms during aging at 25°C. TAB of non-irradiated ones at 25°C was in the range 6 Log CFU/g at 2 d and these microorganisms reached a level of 7 Log CFU/g after 4 d of storage which is no salable levels. The results showed TAB multiplied hastily in case of non-irradiated and aged samples at 25°C after 2 d. Our investigation suggested that irradiation improved the microbiological safety of pork at 10°C and 25°C by reducing the TAB after 4 d of storage. However, TAB increased slightly in aged samples up to 8 d at 2°C. This result was agreed with the study reported by (Frenzel et al., 2014).

The consequence of different aging temperature on shear force values in non- and irradiated pork shoulders during the aging time is reported in Table 2. It was found that regardless of irradiation, the shear force values decreased as the aging time and temperature increased. In particular, shear force values of irradiated meat after aging for 2 and 4 d at 2°C was higher than those aged at 25°C (p<0.05). The decrease in shear force values in aging could be due to the degradation of myofibrillar components (Ba et al., 2014). Frenzel et al. (2014) suggested that aging temperatures did not affect the shear force values, but shear force values decreased as the aging periods increased. Recently, (Kim et al., 2018) reported however, the dilapidation of sarcoplasmic and myofibrillar proteins were greater in beef with aging temperatures of 14°C than that of 4°C. In addition, EB and X-ray irradiated beef showed slower autolysis of calpain-1 but this did not affect tenderness. Results from our study also showed that, as pork aged, the shear force values decreased.

As shown in Table 3, with an increased aging time and temperature, cooking loss increased. In particular, the cooking loss of irradiated meat after aging for 2 and 8 d at 2°C was lower than those aged at 10°C and 25°C (p<0.05). The cooking loss showed no differences among the irradiated and non-irradiated samples after aging for 4 d. (Frenzel et al., 2014) noted that

Starrage (1)	T	Shear force values (kg·f)						
Storage (day)	Temperature (°C)	Control	Irradiated (2 kGy)	SEM ¹⁾				
0		2.17 ^y	3.27 ^x	0.18				
2	2	1.83 ^{ay}	2.25 ^{ax}	0.07				
	10	1.83ª	1.93 ^b	0.08				
	25	1.58 ^{by}	1.91 ^{bx}	0.06				
	SEM ²⁾	0.06	0.08					
4	2	1.57 ^y	1.90 ^{ax}	0.08				
	10	1.48	1.63 ^{ab}	0.12				
	25	1.47	1.44 ^b	0.07				
	SEM ²⁾	0.08	0.09					
8	2	1.40	1.43	0.08				
	10	1.39	1.37	0.06				
	25	1.37	1.36	0.13				
	SEM	0.10	0.09					

Table 2. Effect of different aging temperature on shear force values of pork shoulder treated by electron beam irradiation

¹⁾ n=12, ²⁾ n=24.

 a^{-c} Values with different letters within the same column differ significantly (p<0.05).

x,y Values with different letters within the same row differ significantly (p<0.05).

	Tomporatura (%C) —	Cooking loss (%)						
Storage (day)	Temperature (°C)	Control	Irradiated (2 kGy)	SEM ¹⁾				
0		10.21	10.46	0.27				
2	2	9.62	10.40 ^b	0.67				
	10	13.02	13.24ª	1.05				
	25	13.15	14.32ª	0.95				
	SEM ²⁾	1.03	0.77					
4	2	15.07	14.52	0.94				
	10	15.83	15.22	1.21				
	25	18.00	16.85	0.87				
	SEM ²⁾	0.87	1.12					
8	2	18.77	19.80 ^b	0.92				
	10	20.48 ^y	24.86 ^{ax}	1.16				
	25	21.88 ^y	27.39 ^{ax}	0.77				
	SEM	0.96	0.97					

Table 3. Effect of different aging temperature on cooking loss of pork shoulder treated by electron beam irradiation

¹⁾ n=12, ²⁾ n=24.

^{a-c} Values with different letters within the same column differ significantly (p<0.05).

^{x,y} Values with different letters within the same row differ significantly (p < 0.05).

cooking loss was not significantly affected by aging periods and temperature and the explanation of the variances between cooking loss and aging was not clearly obvious. Shin et al. (2014) demonstrated that the different doses of X-ray did not impinge on the cooking loss of irradiated pork sausage because the meat of proteins were segregated by X-ray. However, rradiated samples after aging for 8 d were higher than non-irradiated ones at 10°C and 25°C (p<0.05).

Lipid oxidation, as determined by TBARS values, is presented in Table 4. As the time and temperature of aging went up, TBARS scores slightly increased regardless of irradiation conditions. The initial TBARS values did not change at irradiated samples until day 4, but drastically increased at 8 d. This is due to the fact that samples were packaged under vacuum conditions. Vacuum-packaging is normally better than aerobic for storage of irradiated meat because vacuum-packaging minimizes lipid oxidation in meat (Du et al., 2001). The storage temperature was a major factor of the rate of lipid oxidation as TBARS values were the lowest for samples at 2°C after aging for 8 d while the values were highest at 25°C (p<0.05). Generally, irradiation forwarded lipid oxidation of meat during storage only under the aerobic conditions (Ahn et al., 1998; Ahn and Nam, 2004; Davis et al., 2004; Jo and Ahn, 2000b). Irradiation did not increase lipid oxidation up to 4 d. This findings are accordant with the previous study (Li et al., 2017) reporting irradiation would not induce immediate TBARS surge at the beginning of pork aging of 3 d. Irradiation cannot increase lipid oxidation in dry-cured shoulder hams and low-dose irradiation is efficient for falling lipid oxidation (Jin et al., 2012). Mattison et al. (1986) also reported low dose irradiation on pork loin had no effect on TBARS values. The irradiated samples showed only a little higher TBARS values than the non-irradiated control at 10°C and 25°C after aging for 8 d (p<0.05). From our study, lipid oxidation might be compacted at low aging temperature (2°C) and these results were consistent with a prior study (Le et al., 1996). Thus, our results showed that aging pork shoulders up to 8 d at 2°C resulted in relatively safe in terms of lipid oxidation.

Color changes in aging periods, temperature and irradiation were not consistent (Table 5). Thus, it indicates that low-dose

Stamora (dava)	Tommorphyne (%C)	TBARS (mg malonedialdehyde/kg meat)					
Storage (days)	Temperature (°C) —	Control	Irradiated (2 kGy)	SEM ¹⁾			
0		0.22	0.24	0.01			
2	2	0.28°	0.30 ^b	0.01			
	10	0.32 ^b	0.31 ^b	0.00			
	25	0.36ª	0.38ª	0.02			
	SEM ²⁾	0.01	0.01				
4	2	0.31 ^b	0.31 ^b	0.01			
	10	0.31 ^b	0.33 ^b	0.01			
	25	0.37ª	0.38ª	0.01			
	SEM ²⁾	0.01	0.01				
8	2	0.31°	0.33°	0.01			
	10	0.36 ^{by}	0.40 ^{bx}	0.01			
	25	0.41 ^{ay}	0.44 ^{ax}	0.01			
	SEM	0.01	0.01				

Table 4. Effect of different aging temperature on thiobarbituric acid reactive substance (TBARS, mg malonedialdehyde/kg meat) values of pork shoulder treated by electron beam irradiation

¹⁾ n=12, ²⁾ n=24.

a-c Values with different letters within the same column differ significantly (p<0.05).

x,y Values with different letters within the same row differ significantly (p<0.05).

TBARS, thiobarbituric acid reactive substance.

Storage	Temperatura	L*			a*			b*		
(days)	(°C)	Control	Irradiated (2 kGy)	SEM	Control	Irradiated (2 kGy)	SEM	Control	Irradiated (2 kGy)	SEM ¹⁾
0		50.98	50.25	0.69	18.64	19.22	0.26	5.01	5.34	0.18
2	2	55.73 ^{ax}	47.63 ^y	0.63	17.54 ^{by}	18.98 ^x	0.19	5.18 ^b	5.01	0.20
	10	50.53 ^{bx}	46.10 ^y	0.75	18.94ª	19.00	0.27	6.32 ^{ax}	4.91 ^y	0.34
	25	49.68 ^b	47.57	0.90	19.19 ^a	18.79	0.24	5.11 ^b	4.94	0.30
	SEM ²⁾	0.64	0.87		0.22	0.25		0.16	0.37	
4	2	48.62 ^{by}	52.71 ^{ax}	1.04	19.18 ^a	19.44	0.31	6.16 ^b	6.52 ^b	0.57
	10	48.70 ^b	49.36 ^b	0.95	19.84 ^{ax}	18.80 ^y	0.18	5.68 ^{by}	7.14 ^{abx}	0.34
	25	56.59ª	52.82ª	1.62	17.39 ^{by}	18.89 ^x	0.29	8.36ª	8.09ª	0.35
	SEM	1.46	0.97		0.29	0.24		0.52	0.33	
8	2	45.66 ^b	48.86	1.07	19.87ª	19.78 ^a	0.41	5.76 ^y	6.60 ^x	0.21
	10	50.72 ^{ax}	46.56 ^y	0.90	18.80 ^{ab}	19.17ª	0.34	6.57	5.62	0.34
	25	52.21ª	48.63	1.44	17.57 ^b	17.25 ^b	0.65	6.65	5.97	0.49
	SEM	1.17	1.14		0.56	0.38		0.26	0.44	

¹⁾ n=9, ²⁾ n=24.

^{a-c} Values with different letters within the same column differ significantly (p<0.05).

x,y Values with different letters within the same row differ significantly (p < 0.05).

irradiation did not influence the meat color and cause discoloration problem of pork shoulder from all stages of aging, affirming the previous results (Nam and Ahn, 2003; Shin et al., 2014). L* in control at 2°C gradually decreased whereas a* in both groups and b* in irradiated groups slightly increased as aging time increased. This result agrees with (Li et al., 2017) who reported that a* and b* of pork increased between day 1 and 8 with aging period. Especially, a* were highest for samples at 2°C after aging for 8 d, regardless of irradiation. This coincides with the finding of (Kim et al., 2012) who reported that storage at 4°C tended to decline the L* of non-irradiated sausages, while a* and b* tended to increase. The changes of a* in the irradiated sausages during aging could be linked to the demolition of NO-myoglobin by irradiation progression (Kim et al., 2012).

Flavor-linked nucleotide compounds are generated from the decay of adenosine-5-triphosphate (ATP) (Flores et al., 1999). Before slaughter, ATP is the foremost compound in muscle, while huge amounts of IMP and little amounts of ATP, adenosine-5-monophosphate (AMP), and adenosine-5-diphosphate ADP were found in muscles after slaughtered (Lee and Lee, 2001). ATP converted into AMP by dephosphorylation and is then become to IMP, which contributes to the good taste and imparts flavor to the meat. IMP changes to inosine, which has a acerbic feel and then to hypoxanthine, which has a bitter taste (Tikk et al., 2006). The nucleotides of pork were influenced by irradiation, aging periods, and temperature (Table 6). From these results, the IMP contents were higher for samples at 2°C or 10°C after aging for 4 d than at 25°C, regardless of irradiation (p<0.05). On the other hands, the hypoxanthine contents were lower for samples at 2°C or 10°C after aging for 4 d than at 25°C.

Storage Temperature		IM	P (mg/100 g	g)	Ino	sine (mg/10	0 g)	Нурох	anthine (mg	/100 g)
(days)	(°C)	Control	Irradiated (2 kGy)	SEM	Control	Irradiated (2 kGy)	SEM	Control	Irradiated (2 kGy)	SEM
0		121.84 ^x	87.82 ^x	2.42	47.27 ^x	36.00 ^y	0.24	20.87 ^x	12.69 ^y	0.46
2	2 ¹⁾	65.27ª	68.56 ^b	4.12	40.19 ^a	40.25 ^b	0.58	26.1°	20.76 ^b	1.82
	10	61.29 ^{by}	88.9 ^{ax}	0.69	40.49 ^{ay}	48.78 ^{ax}	0.47	29.67 ^{bx}	15.65 ^{cy}	0.24
	25	66.14 ^{ax}	42.73 ^{cy}	0.53	25.08 ^{by}	36.36 ^{ac}	0.41	34.20ª	33.16 ^a	0.32
	SEM	1.13	3.25		0.54	0.45		0.35	1.48	
4	2 ¹⁾	78.99ª	76.38ª	0.70	55.17 ^{ax}	49.52 ^{ay}	0.48	16.46 ^{by}	19.27 ^{cx}	0.23
	10	52.92 ^{bx}	42.42 ^{by}	1.19	30.51 ^{by}	35.31 ^{bx}	0.92	34.14 ^{ay}	38.22 ^{bx}	0.97
	25	13.79 ^{cx}	7.67 ^{cy}	0.68	53.79 ^{ax}	29.69 ^{cy}	1.05	34.89 ^{ay}	48.26 ^{ax}	0.60
	SEM	0.94	0.84		0.83	0.87		0.70	0.64	
8	21)	66.17 ^{ax}	64.61 ^{ay}	0.35	57.17 ^{ax}	47.29 ^{by}	0.22	18.32 ^{cy}	28.67 ^{bx}	0.24
	10	49.85 ^{by}	57.93 ^{bx}	0.36	46.65 ^{by}	57.8 ^{ax}	0.44	33.13 ^{bx}	27.92 ^{by}	0.35
	25	4.33°	4.78°	0.13	1.62 ^{cy}	11.43 ^{cx}	0.22	70.16 ^a	69.45 ^a	0.90
	SEM	0.18	0.38		0.29	0.33		0.70	0.42	

Table 6. Effect of different aging temperature on nucleotide-related compounds of pork shoulder treated by electron beam irradiation

¹⁾ n=24, ²⁾ n=9.

^{a-c} Values with different letters within the same column differ significantly (p<0.05).

x, y Values with different letters within the same row differ significantly (p<0.05).

IMP, inosine monophosphate.

Conclusion

An optimizing aging conditions of pork shoulders could be recommended at 2°C up to 8 d. The results of the present study concluded low-dose irradiating of EB (less than 2 kGy) followed by aging could inhibit microbial growth, promote generation of flavor compounds and tenderizing texture, and provide minimal detrimental effects of color and lipid oxidation.

Conflicts of Interest

The authors declare no potential conflict of interest.

Author Contributions

Conceptualization: Nam KC. Data curation: Park JY, Lee SY. Formal analysis: Park JY. Methodology: Lee SY. Validation: Jo C. Writing - original draft: Yim DG, Mahabbat A. Writing - review & editing: Yim DG, Jo C, Mahabbat A, Park JY, Lee SY, Nam KC.

Ethics Approval

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

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