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Irradiation and Modified Atmosphere Packaging Effects on Residual Nitrite, Ascorbic Acid, Nitrosomyoglobin, and Color in Sausage

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The present study was undertaken to evaluate the irradiation and modified atmosphere packaging effects on emulsion-type cooked pork sausage during storage for 4 weeks. CO_2 (100%), N_2 (100%), or 25% CO_2/75% N_2 packaged sausage were irradiated at 0, 5, and 10 kGy, and residual nitrite, residual ascorbic acid, nitrosomyoglobin (NO-Mb), color values, and their correlation were observed. Irradiation significantly reduced the residual nitrite content and caused partial reduction of NO-Mb during storage. No difference was observed in ascorbic acid content by irradiation. Irradiation decreased the Hunter color *a* value of sausage. CO_2 or CO_2/N_2 packaging were more effective for reducing residual nitrite and inhibiting the loss of the red color of sausage compared to N_2 packaging. Results indicated that the proper combination of irradiation and modified atmosphere packaging could reduce the residual nitrite in sausage with minimization of color change.

KEYWORDS: Irradiation; sausage; modified atmosphere packaging; residual nitrite; color

INTRODUCTION

Ionizing radiation is well-known as the best method to destroy pathogenic and spoilage microorganisms without compromising the nutritional properties and sensory quality of the foods (*1*), and its use is gradually increasing worldwide. Additionally, new trial applications for reducing toxic or hazardous compounds, such as volatile *N*-nitrosamines (*2*, *3*), and allergenicity of foods (*4*) or for developing a new processing procedure such as production of low-salted fermented foods (*5*, *6*) have been reported, besides the sanitary purpose. Recently, reduction of residual nitrite in pork sausage by γ irradiation was also reported (*7*, *8*). Nitrite plays important roles in meat products to develop typical color, flavor, and texture and to prevent oxidative rancidity and pathogenic microorganism (*9*). Scientific research has cast nitrite and its reaction products in a negative light because of epidemiological suggestions that hot dog consumption is linked to childhood cancer and a risk of leukemia and in a positive light because nitric oxide is synthesized in physiological functions as a biological messenger (*10*, *11*). Cassens (*12*) reported that the use of nitrite to cure meat was seriously questioned in the 1970s; however, no attention was given to residual nitrite in cured meat nowadays. It is because current research demonstrated that the maximum addition of reducing agents, such as ascorbate and erythorbate, provides a drastic decline of residual nitrite in meat products (*12*). Ahn et al. (*8*) suggested that irradiation at 5 kGy or above significantly reduced the residual nitrite levels in sausage, and vacuum packaging was more effective for reducing the nitrite levels compared to aerobic packaging during storage. The authors (*8*) concluded that anaerobic conditions induced the reduced state, which may accelerate the reduction of nitrite to nitric oxide during storage.

This study was designed to evaluate modified atmosphere packaging effects, in an anaerobic environment, on residual nitrite content, its related compounds, and color. By modification of the atmospheric conditions with one or more gases, such as CO_2, N_2, and O_2, product life and desired product quality can be maximized (*13*). Moreover, color changes in irradiated raw meats have been reported in many relevant studies (*14*, *15*), but little information is available on the color changes of irradiated cooked meat products.

The objective of the present study was to investigate the irradiation and modified atmosphere packaging effects on residual nitrite, residual ascorbic acid, nitrosoheme pigments, and color characteristics in emulsion-type cooked pork sausage during refrigerated storage.

MATERIALS AND METHODS

Sample Preparations. Vacuum packaged, refrigerated lean pork and frozen pork backfat were obtained within 48 h of slaughtering from a local meat packer and ground in a grinder (model 160, Fatosa, Barcelona, Spain) twice through 9 and a 3 mm plates, respectively. An emulsion-type pork product was prepared using ground meat, NaCl (1.5% of meat weight), ice/water (20%), pork backfat (20%), trisodium

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phosphate (0.3%), sugar (0.6%), monosodium glutamate (0.03%), spice mix (0.5%), sodium ascorbate (200 ppm), and sodium nitrite ($NaNO_2$, 156 ppm). All ingredients were purchased from Sewoo Co. Ltd. (Seoul, Korea), and the spice mix contained coriander, glucose, red pepper, and onion powder. Lean pork, salt, phosphate, and nitrite were placed in a silent cutter (C-75, Fatosa, Barcelona, Spain) and mixed for about 1 min, after which 50% of the ice was added and mixed at a high speed. When the temperature of the mixture decreased by about 1−2 °C, ground pork backfat was added and mixed until the temperature of the mixture reached 10 °C. The remaining 50% of the ice and the other spices were added and mixed until the temperature of the mixture reached 13 °C. The total emulsification time was about 10 min, and the processing room temperature was about 15 °C. The sausages were stuffed (Patron sausage filler MWF 591, MADO, Oud-Ade, The Netherlands) into a collagen casing (2.5 cm of diameter, Woosung Co. Ltd., Seoul, Korea), dried (45 °C for 30 min), smoked (55 °C for 40 min) by sawdust, and cooked to 70 °C of internal temperature (about 1 h) using a smokehouse (Fracomat 1200, Franke Gm bH & Co., Germany). The cooked sausage was water-spray-cooled for 5 min, dried at room temperature for 30 min, and cut into pieces (about 100 g each).

The produced samples were then flushed with CO_2 (ultrapure CO_2, 99.999%), N_2 (ultrapure N_2, 99.999%), or a CO_2/N_2 mixture (25% CO_2 + 75% N_2) for 9 s into an oxygen-impermeable nylon bag ((2 mL of O_2)(m^{-2})(24 h^{-1}) at 0 °C; 20 cm × 30 cm; Sunkyung Co. Ltd, Seoul, Korea), respectively, and sealed. All samples were stored in a 4 °C refrigerator before irradiation.

γ Irradiation. The samples were irradiated in a cobalt-60 irradiator (Nordion International, Ottawa, Ontario, Canada). The source strength was ca. 100 kCi with a dose rate of 5 kGy h^{-1} at 12 ± 0.5 °C. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rjeomstettem, Germany), and the free radical signal was measured using a Bruker EMS 104 EPR analyzer. The absorbed doses in this study were 0, 5, and 10 kGy, and the actual doses were within ±2% of the target dose. After irradiation, the analysis was performed and the remainder of the sample was immediately stored at 4 °C for 4 weeks.

Nitrite Determination. Residual sodium nitrite content in emulsion-type cooked pork sausage was determined according to AOAC methods, no. 973.31 (*16*).

Ascorbic Acid Determination. Residual ascorbic acid content was determined by the method of Graham and Annette (*17*). The HPLC system used consisted of the following: separations module (Waters 2690, Waters Co., Milford, MA), photodiode array detector (PDA, Waters 996, Waters Co.), Millennium 32 chromatography manager (system software for the workstation, version 3.0, Waters), and a Bio-Rad Aminex HPX-87H column (7.8 mm × 300 mm i.d.) packed with sulfonated styrene divinylbenzene copolymer resin (8% cross-linked, particle size of 9 μm, Bio-Rad Labs., Watford, U.K.). The mobile phase used was 0.009 N sulfuric acid with a flow rate of 0.5 mL min^{-1}. The injection volume, column temperature, and PDA detection wavelength were 10 μL, ambient, and 245 nm, respectively.

Nitrosoheme Pigments. The content of nitrosoheme pigments in emulsion-type cooked pork sausage was determined according to the Hornsey method (*18*). An acetone/water solution (40:6, v/v) was used as a solvent. This ratio reached 40:10 with the moisture from the sample. Absorbance of the resulting nitrosomyoglobin/acetone complex was measured at 540 nm, and results were found as ppm hematin after standardization.

Color Measurement. For color measurement, sausage was cut into 3 cm thick pieces and measured by the previous method (*19*) using a color difference meter spectrophotometer (CM-3500d, Minolta Co., Ltd. Osaka, Japan). The Hunter color L^* (lightness), a^* (redness), and b^* (yellowness) values were reported.

Statistical Analysis. Experimental was designed as a 3 (irradiation dose) × 3 (packaging) factorial. The experiment including from sausage manufacture to analysis was replicated twice, and the data were then analyzed by SAS software (SAS Institute, Cary, NC). The ANOVA was processed, and Duncan's multiple range test was used to compare mean values. Significance was defined at $P < 0.05$. Pearson's correlation coefficients among experimental results within the same packaging environment were analyzed, and a significance was defined at $P < 0.05$ and 0.01 levels.

Table 1. Residual Nitrite Levels (ppm) of Irradiated Cooked Pork Sausage in Modified Atmosphere Packaging during Storage at 4 °C

packaging	irradiation dose[a] (kGy) 0	5	10	SEM[c]
		0 Week		
CO_2[b]	67.0ay	53.8bz	49.5bz	2.07
N_2	77.3ax	72.8bx	62.5cx	1.39
CO_2/N_2	65.0ay	62.2aby	57.7by	1.13
SEM[c]	2.38	1.16	0.67	
		4 Weeks		
CO_2	45.2a	43.7ax	36.3bx	1.28
N_2	46.2a	45.3ax	40.8bx	0.67
CO_2/N_2	44.6a	38.6ay	24.1by	1.73
SEM[c]	0.86	0.92	1.88	

[a] (a–c) Different letters within the same row differ significantly ($P < 0.05$). (x–z) Different letters within the same column for the same storage differ significantly ($P < 0.05$). [b] Sausage was packaged with 100% CO_2, 100% N_2, or 25% CO_2/75% N_2 gas. [c] SEM: standard error of the mean ($n = 6$).

RESULTS AND DISCUSSION

Residual Nitrite and Ascorbic Acid. Residual nitrite content in emulsion-type cooked pork sausage with modified atmosphere packaging for 4 weeks of storage at 4 °C is shown in **Table 1**. Immediately after γ irradiation, a statistically significant difference at the $P < 0.05$ confidence level was observed between nonirradiated and irradiated samples at 5 and 10 kGy. Residual nitrite in sausage was significantly reduced by irradiation. Simie (*20*) reported that nitrite reduction by irradiation is probably due to its reaction with the hydroxyl radical produced by the radiolysis of water. CO_2 or a mixture of CO_2/N_2 packaging was more effective for reducing the nitrite level compared to N_2 packaging. Even though all packagings were done in an anaerobic environment, the oxidation−reduction potential of each packaging might be different. Ahn et al. (*8*) found that residual nitrite level in a vacuum-packaged sausage was lower than that of aerobic-packaged sausage during storage and explained that the vacuum condition (anaerobic condition) maintains the environment at a reduced state to form a lower redox potential. When the environment is in the reduced state, the nitrite can be easily converted to nitric oxide, resulting in lower residual nitrite levels in samples with anaerobic packaging. After 4 weeks of storage, nitrite levels were decreased in all samples and a significant reduction was observed ($P < 0.05$; data are not shown). The nitrite content was significantly reduced in 10 kGy irradiated samples irrespective of their packaging environment. Nitrite reduction in 10 kGy irradiated samples was 20, 12, or 46% in CO_2, N_2, or CO_2/N_2 packaging, respectively, compared to that of the nonirradiated control in its respective packaging.

Cassens et al. (*12*) reported that residual nitrite level in commercial frankfurters had declined up to 10 ppm, about 80% reduction since the mid-1970s, and also found that a high level of residual ascorbates was present in cured meats. The study also found that the mean value of residual ascorbic acid levels of commercial frankfurters was 209 ppm, or nearly 40% of the maximum allowable addition of 550 ppm (*12*). Fiddler et al. (*21*) found that the reduction of nitrite by sodium ascorbate appeared to be enhanced by the irradiation process. This indicates that the combined effects of irradiation and maximum addition of ascorbic acid will be effective for reducing residual nitrite level in meat products.

Table 2. Residual Ascorbic Acid Levels (ppm) of Irradiated Cooked Pork Sausage in Modified Atmosphere Packaging during Storage at 4 °C

packaging	irradiation dose[a] (kGy) 0	5	10	SEM[c]
		0 Week		
CO_2[b]	93.2x	90.7	86.0	3.28
N_2	83.1y	81.6	78.1	1.56
CO_2/N_2	85.0axy	82.1a	78.1b	0.83
SEM[c]	1.89	2.65	1.82	
		4 Weeks		
CO_2	51.4	46.9	48.1	2.46
N_2	49.8	47.1	48.2	0.76
CO_2/N_2	51.6	48.5	49.4	0.70
SEM[c]	0.54	0.88	2.45	

[a] (a, b) Different letters within the same row differ significantly ($P < 0.05$). (x, y) Different letters within the same column for the same storage differ significantly ($P < 0.05$). [b] Sausage was packaged with 100% CO_2, 100% N_2, or 25% CO_2/75% N_2 gas. [c] SEM: standard error of the mean ($n = 6$).

Table 3. Nitrosoheme Pigments (ppm of Hematin) of Irradiated Cooked Pork Sausage in Modified Atmosphere Packaging during Storage at 4 °C

packaging	irradiation dose[a] (kGy) 0	5	10	SEM[c]
		0 Week		
CO_2[b]	59.4y	46.7y	42.1	5.39
N_2	58.7y	42.8y	43.7	1.59
CO_2/N_2	69.7ax	58.8bx	46.3b	3.80
SEM[c]	4.18	4.07	3.34	
		4 Weeks		
CO_2	49.5x	42.9	43.2	2.22
N_2	44.8y	44.9	42.2	1.08
CO_2/N_2	53.1ax	43.6b	46.6b	1.53
SEM[c]	0.87	2.04	2.47	

[a] (a, b) Different letters within the same row differ significantly ($P < 0.05$). (x, y) Different letters within the same column for the same storage differ significantly ($P < 0.05$). [b] Sausage was packaged with 100% CO_2, 100% N_2, or 25% CO_2/75% N_2 gas. [c] SEM: standard error of the means ($n = 6$).

Residual ascorbic acid of irradiated sausage in modified atmosphere packaging during refrigerating storage is shown in **Table 2**. Generally, ascorbic acid in cured meats plays a role as a reducing agent for a nitrite scavenger, as an enhancer of NO-Mb development, and as an inhibitor of carcinogenic *N*-nitrosamine formation. Thus, residual ascorbic acid allowed the nitrite to be transformed to nitric oxide, resulting in a gradual reduction of residual nitrite in sausage during storage. Ascorbic acid content was reduced by 10 kGy irradiation in only CO_2/N_2 packaged sausage ($P < 0.05$), while the reduction was not observed in other irradiated samples at 0 week. After 4 weeks of storage, residual ascorbic acid levels were significantly decreased (statistical data are not shown), but the levels were not affected by both irradiation and packaging ($P < 0.05$). Stewart (*22*) reported that ionizing radiation can cause only partial conversion of ascorbic acid to dehydroascorbic acid, which has an activity equal to that of ascorbic acid; thus, the loss of ascorbic acid was much lower than that of other cooking methods.

Results indicated that combinations of irradiation and CO_2 or CO_2/N_2 packaging could be effective for reducing residual nitrite levels in sausage, and residual ascorbic acid levels were rarely affected by irradiation and packaging but by storage time.

Nitrosomyoglobin, Color, and Correlation. The nitrosomyoglobin (NO-Mb) content of irradiated sausage in modified atmosphere packaging is shown in **Table 3**. No significant difference was found in CO_2 or N_2 packaging by irradiation, while the irradiation effect was observed in the sausage with CO_2/N_2 packaging. NO-Mb content in CO_2/N_2 packaging was significantly reduced by 5 kGy irradiation, assuming that denitrosylation of NO-Mb occurred by γ irradiation. Although NO-Mb reduction was observed in sausage with CO_2/N_2 packaging, the content was higher than that of CO_2 or N_2 packaging. CO_2/N_2 packaging showed higher NO-Mb content in 0 or 5 kGy irradiated samples at 0 week. Kamarei and Karel (*23*) previously reported that the bright-red color of NO-Mb changed to brown upon irradiation (0.4−20 kGy) and that NO-Mb became progressively denitrosylated with metmyoglobin as the immediate product. After 4 weeks of storage, the irradiation effect, which was NO-Mb reduction, was maintained in CO_2/N_2 packaged sausage and the packaging effect was observed in only the nonirradiated control. The storage effect, which was NO-Mb reduction, was observed in nonirradiated samples at a confidence level of $P < 0.05$ (data are not shown).

Irradiation significantly reduced the redness (a^* value) of sausage with modified atmosphere packaging (**Table 4**). With the packaging methods, a significant difference was observed in 5 kGy irradiated samples, and CO_2/N_2 packaging may decrease the reduction of the red color compared to the N_2 environment. Decreased redness was more distinct in N_2 packaging at 0 week, while the difference in packaging was not observed after storage. Nam and Ahn (*24*) reported, however, that the redness of precooked turkey breast with vacuum packaging was significantly increased by irradiation while aerobic packaging caused a reduction of the red color. Therefore, changes in the red color in irradiated meat products might be different with packaging environment or species of meats because of different content or types of pigment. According to Ahn et al. (*25*), the change of redness by irradiation in raw meats varied depending on species, muscle type, irradiation dose, and packaging environment. The lightness (L^* value) and yellowness (b^* value) of emulsion-type cooked pork sausage were not different regardless of irradiation and packaging in general. These results indicated that partial reduction of the typical pink color in sausage occurred by irradiation. Giroux et al. (*15*) reported that addition of 1000 ppm of ascorbic acid gives color stabilization to irradiated beef patties. Because ascorbic acid has an affinity for free radicals, residual ascorbic acid could be used as an alternative means to prevent pigment oxidation during storage. After 4 weeks of storage, lightness was significantly reduced in samples irradiated at 5 and 10 kGy ($P < 0.05$).

Pearson's correlation coefficients among irradiation, storage time, residual nitrite, NO-Mb, residual ascorbic acid, and Hunter color values are shown in **Table 5**. In CO_2-packaged pork sausage, irradiation was negatively correlated with redness ($P < 0.01$). Therefore, this result supported the idea that irradiation decreased the redness of cooked pork sausage. Jo et al. (*26*) reported, on the other hand, that electron beam irradiation of a model system sausage, which was made with lean meat, fat, water, and salt and without the nitrite curing process included, induced a significant increase of redness. Therefore, reduction of redness by irradiation might occur in nitrite-cured meat products because of denitrosylation of NO-Mb. Residual nitrite content was positively correlated with NO-Mb content and residual ascorbic acid content, and this correlation can be affected by irradiation. The storage time was negatively correlated with the content of NO_2 and ascorbate and with lightness. Residual nitrite in meat products was reduced by storage time (*3*). In N_2 packaging, NO-Mb and redness were negatively correlated with irradiation dose, and accordingly, the red color with N_2 packaging was lower than that of others. NO-Mb and

Table 4. Hunter Color Values of Irradiated Cooked Pork Sausage in Modified Atmosphere Packaging during Storage at 4 °C

packaging	0 week[a] 0 kGy	5 kGy	10 kGy	SEM[2]	4 weeks[a] 0 kGy	5 kGy	10 kGy	SEM[c]
				L Value				
CO_2[b]	65.6	66.3	67.3x	0.65	62.8b	63.8ab	64.8a	0.90
N_2	65.3	65.1	64.0y	0.70	63.0	63.9	62.5	0.91
CO_2/N_2	64.6b	66.3a	66.0ax	0.55	63.2	63.1	63.4	1.02
SEM[c]	0.63	0.71	0.56		0.99	0.93	0.91	
				a Value				
CO_2	3.1a	2.7bxy	2.4c	0.07	3.3a	2.8b	2.3c	0.05
N_2	3.3a	2.6by	2.3b	0.11	3.0a	2.7b	2.4b	0.11
CO_2/N_2	3.2a	2.8bx	2.6c	0.07	3.2a	2.7b	2.4c	0.07
SEM[c]	0.06	0.07	0.12		0.10	0.08	0.06	
				b Value				
CO_2	7.1x	7.0	7.0x	0.11	6.7	7.2	7.3	0.17
N_2	7.0ax	6.6ab	6.2by	0.17	6.5b	7.4a	6.9a	0.24
CO_2/N_2	6.7y	7.0	7.0x	0.13	6.7	6.9	7.1	0.21
SEM[c]	0.11	0.14	0.17		0.24	0.19	0.19	

[a] (a–c) Different letters within the same row differ significantly ($P < 0.05$). (x, y) Different letters within the same column for the same color value differ significantly ($P < 0.05$). [b] Sausage was packaged with 100% CO_2, 100% N_2, or 25% CO_2/75% N_2 gas. [c] SEM: standard error of the mean ($n = 6$).

Table 5. Pearson's Correlation Coefficients for Irradiation, Storage Time, Residual Nitrite, NO-Mb, Residual Ascorbic Acid, and Color Values of Irradiated Cooked Pork Sausage in Modified Atmosphere Packaging

	Pearson's correleation coefficient[a] irradiation	storage time	NO_2	NO-Mb	ascorbate	*L**	*a**
			CO_2 Packaging				
NO_2	−0.56	−0.77**					
NO-Mb	−0.66	−0.29	0.64*				
ascorbate	−0.10	−0.98**	0.81**	0.44			
*L**	0.36	−0.83**	0.49	0.06	0.79**		
*a**	−0.96**	−0.11	0.45	0.56	−0.02	−0.53	
*b**	0.49	−0.31	0.14	−0.09	0.28	0.69*	−0.62*
			N_2 Packaging				
NO_2	−0.35	−0.91**					
NO-Mb	−0.62*	−0.22	0.48				
ascorbate	−0.08	−0.99**	0.94**	0.26			
*L**	−0.23	−0.59*	0.58*	0.14	0.61		
*a**	−0.72**	0.28	0.02	0.47	−0.22	−0.11	
*b**	−0.41	0.23	−0.01	0.29	−0.18	0.30	0.44
			CO_2/N_2 Packaging				
NO_2	−0.39	0.89**					
NO-Mb	−0.64*	−0.57	0.64*				
ascorbate	−0.12	−0.99**	0.91**	0.66*			
*L**	0.59*	−0.57	0.33	−0.12	0.48		
*a**	−0.69*	0.01	0.29	0.44	0.03	0.34	
*b**	0.75**	0.02	−0.28	−0.47	−0.14	0.64*	−0.16

[a] (*) Value with significant correlation with $P < 0.05$. (**) Value with significant correlation with $P < 0.01$.

the Hunter color values were negatively correlated with irradiation in CO_2/N_2 packaged sausage. Although NO-Mb and color reductions in CO_2/N_2 packaging were significant, a significantly high NO-Mb content and redness were observed in this packaging. Nam and Ahn (*27*) reported that the redness of turkey breast was positively correlated with the irradiation dose and with the CO gas produced and that irradiation caused the CO-Mb formation. However, in this study, irradiation can cause reduction of the red color in emulsion-type cooked pork sausage with modified atmosphere packaging.

In conclusion, γ irradiation can reduce the residual nitrite in emulsion-type cooked pork sausage, while residual ascorbic acid content was nearly affected by irradiation. Reduction of redness in sausage might be due to NO-Mb being denitrosylated by irradiation. In modified atmosphere conditions, CO_2 or CO_2/N_2 packaging was effective for reducing residual nitrite and for inhibiting loss of red color of sausage compared to N_2 packaging.

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