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Packaging Effect on Microbial and Physicochemical Changes in Irradiated Cooked Pork Sausage during Frozen Storage at -20°C

Cheorun Jo, Jun Ho Son, Jae Hyun Kim, Kyoung Whan Cho and Myung Woo Byun†

Team for Radiation Food Science and Biotechnology, Korea Atomic Energy Research Institute, Daejeon 305-600, Korea

Abstract

The packaging effect on physicochemical changes in irradiated sausage stored at -20°C was studied. Emulsion-type cooked pork sausage was made with (156 ppm) or without $NaNO_2$ (0 ppm), and packaged in three different conditions such as aerobic, vacuum and CO_2 (100%). The samples irradiated at 0, 5 and 10 kGy absorbed dose, and the total number of microorganisms, lipid oxidation, color and texture were analyzed during frozen storage at -20°C. Irradiation of the sausage at 10 kGy completely controlled microbial growth during storage. An $NaNO_2$ addition to the sausage significantly reduced lipid oxidation, and the TBARS value of the sausage with aerobic packaging was higher than that with the vacuum and CO_2 packaging. The $NaNO_2$ addition increased Hunter color a-value dramatically, but no packaging effect was found ($p > 0.05$). Irradiation influenced shear values resulting in lower shear values in 10 kGy-irradiated sausages with aerobic packaging, and CO_2 packaged sausage showed comparatively lower shear values than other packaging methods. From the results, vacuum or CO_2 (100%) packaging were better than aerobic packaging for frozen stored pork sausage, especially for microbial quality and lipid oxidation.

Key words: packaging, irradiation, lipid oxidation, frozen, sausage

INTRODUCTION

Use of irradiation technology has been gradually expanding worldwide after the approving of raw meat and poultry irradiation by the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) (1). Food irradiation treatment is a useful technique in controlling pathogenic microorganisms such as *E. coli* O157 : H7 and *Salmonella* (2). The technology has been recognized by the health authorities of various countries and utilized in various applications.

By modifying the atmospheric condition with one or more gases such as carbon dioxide (CO_2), nitrogen (N_2) or oxygen (O_2), product life and desired product quality can be maximized (3). The authors reported that 100% CO_2 and/or a CO_2/N_2 combination was desirable over 10% O_2 or vacuum-packed pork loin (3). Sakata et al. (4) reported that there was no significant difference in drip loss between *Longissimus dorsi* muscle frozen at -20°C or -80°C after one-month storage compared to the control (not frozen). They also reported that the water holding capacity was the same as the control, and especially, lipid oxidation did not develop during frozen storage. Much research has been conducted to investigate the effect of irradiation on lipid oxidation and other quality attributes such as color, sensory, and volatile compounds in the production of raw meat or cooked meat products with different packaging (5-7). However, comparative information of irradiated cooked pork sausage using different packaging methods during frozen storage is limited.

The objective of the present study was to determine the effect of different packaging methods on lipid oxidation, color, and texture, as well as microbial quality in irradiated pork sausage during frozen storage at -20°C.

MATERIALS AND METHODS

Sausage manufacture

Vacuum-packaged, refrigerated lean pork and frozen pork backfat were obtained within 48 h of slaughtering from a local meat packer and ground (Model 160, Fatosa, Barcelona, Spain) in a grinder twice through a 9-mm and a 3-mm plate, respectively. An emulsion-type pork product was prepared using ground meat, NaCl (1.5% of meat weight), ice water (20%), pork backfat (20%), trisodium phosphate (0.3%), sugar (0.6%), monosodium glutamate (0.03%), sodium nitrite ($NaNO_2$, 0 or 156 ppm), and spicemix (0.5%). All ingredients were purchased from Sewoo Co. Ltd. (Seoul, Korea), and the spice mix contained coriander, glucose, red pepper, and onion powder. The following procedures were conducted by the same method as in a previous study (8). The produced samples were vacuum-packaged (75 cmHg pulled) in oxygen-impermeable nylon bags (2 mL O_2/m^2/24 h at 0°C; 20 cm × 30 cm; Sunkyung Co. Ltd, Seoul, Korea) by a vacuum packaging machine (Leepack, Hanguk Electronic, Kyungi, Korea), and aerobically-packaged by flushing air into the bag without sealing. The rest of the samples were CO_2-flushed (ultra pure

†Corresponding author. E-mail: mwbyun@kaeri.re.kr
Phone: 82-42-868-8060, Fax: 82-42-868-8043

CO_2, 99.999%) for 9 sec into an oxygen-impermeable nylon bag and sealed. All samples were stored in a -20°C freezer before irradiation.

Irradiation

Irradiation was performed on the sausage, in the frozen state, the following morning in a Co-60 gamma irradiator (point source, AECL, IR-79, Nordion International Co., Ltd, Ontario, Canada) with a source strength of 100 kCi. The dose rate was 83 Gy/min at 12±0.5°C and the applied absorbed dose was 0, 5 and 10 kGy. 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 actual dose was within ±2% of the target dose. A nonirradiated control was placed outside of irradiation chamber to have the same environmental temperature effect as the irradiated sample. The control and irradiated pork sausages were transferred to a 4°C refrigerator and analyses were started the following day. All samples were thawed 24 h before analysis.

Number of total aerobic bacteria, lipid oxidation, and color measurement

A total aerobic bacterial count was performed by the procedure of Jo et al. (8) and the CFU/g were reported. Lipid oxidation was determined as a 2-thiobarbituric acid reactive substances (TBARS) value by using a spectrophotometer (UV 1600 PC, Shimadzu, Tokyo, Japan) as described by Ahn et al. (6). The lipid oxidation development was reported as mg malondialdehyde/kg meat sample. For color measurement, samples were cut into 3 cm-thick pieces and measured by the previous method (8). The Hunter color L, a, and b-value were reported.

Texture analysis

The sausage was measured for shear force and the total working force for the shear value by a texture analyzer (TA-XT2i, Stable Micro Systems, England). The sausage was cut into pieces of 5 cm thickness and placed on the sample holder. A shear blade (6.8 cm in length) was set on the machine and moved perpendicularly to the sample until 30 mm in depth. The texture analyzer parameters were set; pretest speed, 2.0 mm/s; test speed, 5.0 mm/s; and posttest speed, 2.0 mm/s. The shear value (N/mm) and the total working force for shear (N/mms) were produced by automatically calculating the peak height and the total peak area, using texture expert software (Stable Micro Systems, England).

Statistical analysis

Experimental design used was a 3 (packaging)×3 (irradiation)×2 ($NaNO_2$ level) factorial and the whole experiment was duplicated. Analyses of Variance were performed using SAS software (9) and the Student-Newman-Keuls multiple range test was used to compare differences among mean values. Mean values and pooled standard errors of the mean (SEM) were reported, and the significance was defined at $p<0.05$.

RESULTS AND DISCUSSION

Microbiological study

Irradiation of food, especially meat products, is the most effective method to control pathogenic microorganisms (10). The beneficial microbiological effect of irradiation on food has been well-documented (11-13). The number of total aerobic bacteria was 2.4×10^2, 2.6×10^2, and 3.8×10^2 in non-irradiated sausage with aerobic, vacuum or CO_2 packaging at the beginning of storage without $NaNO_2$ (Table 1). Irradiation at 5 and 10 kGy reduced the number of total aerobic bacteria and no bacterial growth resulted in the sausage irradiated with 10 kGy at up to 3 months storage in vacuum and CO_2 packaging. Especially in aerobic packaging at 0 month, the bacterial growth was detected, but was not seen thereafter. This result can be explained by the postirradiation effect on microorganims. Similarly, the lag phase of the surviving *Listeria monocytogenes* at 6°C was 1 day in non-irradiated poultry meat, 10 days in irradiated to 1 kGy, and 15 days in meat irradiated to 2.5 kGy (14). Compared to aerobic packaging, the sausage with vacuum or CO_2 packaging showed a reduced number of total aerobic bacteria but a difference between vacuum and CO_2 packaging was not seen (Table 1). Hastings et al. reported, however, that some lactobacilli had lower D_{10}-values when irradiated in 100% CO_2 compared to air, vacuum, or N_2 (15).

One of the major reasons for adding $NaNO_2$ in the sausage processing is to control the toxic spores of *Clostridium botulinum* (16). When 156 ppm of $NaNO_2$ was added, the number of total aerobic bacteria in the sausage with aerobic packaging was reduced about 1 and 2 log cycles at 1.5 and 3 months storage, respectively, compared to the sausage without $NaNO_2$ (Table 2).

Lipid oxidation

Lipid oxidation is the primary cause of chemical spoilage in a food system, except for microbial deterioration. The TBARS value of sausage with aerobic packaging increased by a 5 or 10 kGy-irradiation dose at 3 months (Table 3). Sta-

Table 1. Number of total aerobic bacteria in irradiated sausage without $NaNO_2$ in different packaging

Packaging	Irradiation dose (kGy)	Storage (month)		
		0	1.5	3
Aerobic	0	2.4×10^2	3.4×10^4	6.0×10^4
	5	1.7×10^2	5.0×10^2	NG[1)]
	10	1.1×10^2	NG	NG
Vacuum	0	2.6×10^2	1.7×10^3	3.2×10^3
	5	NG	1.1×10^2	NG
	10	NG	NG	NG
CO_2	0	3.8×10^2	3.9×10^3	2.8×10^3
	5	NG	5.0×10^2	NG
	10	NG	NG	NG

[1)]NG: No growth, detection limit at $<10^2$

Table 2. Number of total aerobic bacteria in irradiated sausage with $NaNO_2$ (156 ppm) in different packaging

Packaging	Irradiation dose (kGy)	Storage (month)		
		0	1.5	3
Aerobic	0	3.1×10^2	3.5×10^3	2.0×10^2
	5	1.3×10^2	NG[1)]	NG
	10	NG	NG	NG
Vacuum	0	2.2×10^2	2.9×10^2	1.5×10^3
	5	NG	NG	NG
	10	NG	NG	NG
CO_2	0	2.7×10^2	5.7×10^2	2.6×10^2
	5	1.1×10^2	NG	NG
	10	NG	NG	NG

[1)]NG: No growth, detection limit at $<10^2$.

tistical significance was found in only 5 kGy-irradiated samples at which the lipid oxidation developed by 3 months. In vacuum packaging, the irradiation effect was found in 3-month stored sausage and 10 kGy of irradiation had a higher TBARS value than those with 0 and 5 kGy irradiation. In modified atmosphere packaging with CO_2 (100%), the TBARS value increased with irradiation in 1.5 and 3 month-stored pork sausage but no difference was found at 0 months. Frozen storage for 3 months also increased the TBARS value of the sausage packaged with CO_2 at 5 kGy of irradiation. However, Thakur and Singh (17) reported that malondialdehyde reactive substances have disappeared gradually in modified atmosphere packaging at a high temperature because of the after-effects of irradiation.

When $NaNO_2$ (156 ppm) was added, the TBARS value of the sausage was lower than that of the sausage without $NaNO_2$ ($p<0.05$). The irradiation effect still existed in the aerobically-packaged or CO_2-packaged sausage stored for 3 months in a freezer at -20°C. Generally, the sausage with aerobic packaging resulted in higher TBARS value than those which were vacuum or CO_2 packaged. The effect of irradiation on lipid oxidation in the presence of oxygen is well established and exclusion of oxygen during irradiation should help reduce lipid oxidation (5). Thus, the presence of oxygen influenced the development of lipid oxidation even in frozen storage.

The probability of the main effects on the TBARS value is shown in Table 4. All the main factors including irradiation, packaging methods and $NaNO_2$ addition had significance, while the storage effect did not, suggesting that storage at -20°C may not change the lipid oxidation during a 3-month storage period, if the other main effects were excluded.

Color

Irradiation at 10 kGy reduced the Hunter color L-value of sausage without $NaNO_2$ which was aerobically-packaged (Table 5). In vacuum and CO_2 packaging, the Hunter color L-value was not consistent, but 10 kGy of irradiation reduced the L-value significantly regardless of the packaging methods. When $NaNO_2$ was added to the sausage, the Hunter color L-value was not different in aerobic packaging; however, the sausage with vacuum packaging decreased in L-value at 10 kGy-irradiation compared to the control. In CO_2 packaging, the L-value was increased by 10 kGy of irradiation.

The Hunter color a-value of the sausage without $NaNO_2$ decreased with increase of irradiation dose (Table 5). At 10

Table 4. Probability of main effects on 2-thiobarbituric acid reactive substances (TBARS) values of irradiated cooked pork sausage during storage for 3 months at -20°C

Main effect	DF	F	Pr<F
Irradiation	2	7.72	0.0008
Packaging	2	4.39	0.0149
Storage	2	0.43	0.6532
$NaNO_2$ addition	1	29.37	0.0001

Table 3. TBARS value of differently-packaged irradiated sausage with 0 or 156 ppm of $NaNO_2$ added during a 3-month storage period at -20°C[1),2)]

	Storage (month)	None				$NaNO_2$ added			
		0	5	10	SEM[3)]	0	5	10	SEM[3)]
Aerobic	0	0.79	0.89^{xy}	0.94	0.064	0.65	0.76	0.82	0.049
	1.5	0.80	0.63^{y}	0.73	0.091	0.64	0.76	0.86	0.111
	3	0.77^{b}	1.01^{ax}	1.00^{a}	0.071	0.53^{b}	0.65^{ab}	0.73^{a}	0.039
	SEM[4)]	0.023	0.068	0.111		0.055	0.035	0.109	
Vacuum	0	0.58^{z}	0.67	0.71	0.035	0.54	0.60	0.62	0.041
	1.5	0.84^{x}	0.75	0.65	0.044	0.77	0.59	0.71	0.098
	3	0.71^{by}	0.72^{b}	0.88^{a}	0.025	0.62	0.61	0.61	0.014
	SEM[4)]	0.024	0.025	0.051		0.058	0.071	0.057	
CO_2[5)]	0	0.75	0.71^{x}	0.89	0.068	0.55	0.61	0.66	0.030
	1.5	0.56^{b}	0.64^{by}	0.87^{a}	0.021	0.65	0.56	0.63	0.116
	3	0.60^{b}	0.86^{ax}	0.86^{a}	0.065	0.50^{b}	0.64^{a}	0.60^{ab}	0.031
	SEM[4)]	0.089	0.044	0.080		0.097	0.059	0.049	

[1)]Different letters (a, b) within a row with the same $NaNO_2$ addition differ significantly ($p<0.05$).
[2)]Different letters (x~z) within a column with same the packaging differ significantly ($p<0.05$).
[3)]SEM: Pooled standard errors of the mean (n = 6).
[4)]SEM: n = 6.
[5)]The ultra pure (99.999%) CO_2 gas (100%) was used.

kGy of irradiation, the sausage with vacuum packaging has a higher Hunter color a-value than did other packaging methods. Nanke et al. (18) reported that irradiation induced an oxymyoglobin-like pigment in vacuum-packaged beef. Paul et al. (19) also reported that freshly ground mutton irradiated at 2.5 kGy had better color, odor, and microbiological acceptability than nonirradiated or mutton irradiated at 1.0 kGy. When the $NaNO_2$ was added, the vacuum packaged sausage had a higher Hunter color a-value, but at 10 kGy irradiation, no difference in the color a-value of sausage was found. All the a-values, however, were much higher than those of the sausage without $NaNO_2$. Walsh et al. (20) reported that restructured low nitrite (60 ppm) cured turkey products showed significant improvement in color. Without nitrite, however, the desirable color of sausage may not be obtained.

Hunter color b-values were reduced by irradiation but no packaging effect was found except for 10 kGy-irradiated samples, where vacuum packaging had the highest b-value. The b-value was decreased dramatically by $NaNO_2$ addition in all packaging and irradiation treatments. Hunter color b-values were the highest in vacuum packaged sausage (Table 5). Overall probability analysis shows that the packaging effect was not significant in the Hunter color a- and b-value (Table 7).

Table 7. Probability (Pr < F) of main effects on Hunter color L, a, b-values and shear force of irradiated cooked pork sausage during storage for 3 months at -20°C

Main effect	L-value	a-value	b-value	Shear force
Irradiation (IR)	0.0001	0.0722	0.0001	0.0001
Nitrite addition (NI)	0.0001	0.0001	0.0001	0.0001
Package (Pkg)	0.0001	0.5570	0.8851	0.0001
IR × NI[1)]	0.8646	0.0001	0.0001	0.8646
IR × Pkg[1)]	0.0001	0.0001	0.0003	0.0001
NI × Pkg[1)]	0.0042	0.0008	0.0038	0.0042
IR × NI × Pkg[1)]	0.0059	0.0001	0.0001	0.0059

[1)]Interaction among the main effects.

Texture analysis

The sausages prepared without $NaNO_2$ and aerobically-packaged showed a decrease in the shear force value by a 10 kGy of irradiation dose (Table 6). However, a difference with irradiation treatments in the sausage with vacuum and CO_2 packaging was not shown. Lee et al. reported that vacuum packaged and irradiated raw beef had lower shear force than that of nonirradiated beef, resulting in improved tenderness and meat quality (21). They discussed the fact that irradiation may accelerate the breakdown rate of intramus-

Table 5. Hunter color values change of irradiated sausage with different amounts of sodium nitrite addition and packaging during a 3 month storage period at -20°C[1),2)]

$NaNO_2$	Packaging	L-value			a-value			b-value		
		0	5	10	0	5	10	0	5	10
0 ppm	Aerobic	72.18^{ax}	72.07^{ax}	71.47^{bx}	6.82^{ay}	6.50^{b}	6.11^{cy}	16.68^{a}	15.83^{ab}	15.25^{by}
	Vacuum	71.70^{by}	72.42^{ax}	70.81^{cy}	6.75^{ay}	6.53^{b}	6.91^{ax}	16.58^{a}	15.54	16.42^{ax}
	CO_2	72.40^{ax}	70.64^{cy}	71.82^{bx}	7.63^{ax}	6.70^{b}	6.24^{by}	16.66^{a}	15.50^{b}	15.93^{bxy}
	SEM[3)]	0.130	0.142	0.201	0.098	0.086	0.160	0.266	0.317	0.260
150 ppm	Aerobic	70.37	70.15^{y}	70.57^{y}	10.41^{ay}	10.03^{axy}	9.58^{b}	13.46^{y}	12.82^{y}	13.08
	Vacuum	70.69^{ab}	71.07^{ax}	70.44^{by}	10.81^{ax}	10.24^{bx}	9.63^{c}	14.21^{x}	14.38^{x}	13.78
	CO_2	70.89^{b}	70.24^{by}	71.49^{ax}	10.59^{axy}	9.83^{by}	9.62^{b}	13.68^{axy}	12.57^{by}	13.20^{a}
	SEM[3)]	0.204	0.202	0.296	0.138	0.127	0.164	0.271	0.147	0.292

[1)]Different letters (a, b) within a row with the same Hunter color values differ significantly ($p < 0.05$).
[2)]Different letters (x ~ z) within a column with the same $NaNO_2$ addition differ significantly ($p < 0.05$).
[3)]SEM: Pooled standard errors of the mean (n = 6).

Table 6. Shear force (N/m) and the total working force for shear (N/mms) value of differently-packaged irradiated sausage with 0 or 156 ppm of $NaNO_2$ added during a 3 month storage period at -20°C[1),2)]

	Irradiation dose (kGy)	$NaNO_2$ 0 ppm				$NaNO_2$ 156 ppm			
		Aerobic	Vacuum	CO_2	SEM[3)]	Aerobic	Vacuum	CO_2	SEM[3)]
Shear Force	0	1.45^{ax}	1.30^{ab}	1.14^{b}	0.069	0.95	0.87	0.86	0.026
	5	1.41^{x}	1.43	1.19	0.067	0.95	1.00	0.90	0.027
	10	1.28^{ay}	1.20^{b}	1.20^{b}	0.031	1.00	0.89	0.88	0.035
	SEM[4)]	0.004	0.067	0.032		0.023	0.038	0.065	
Total working force for shear	0	3.95^{x}	3.71	3.42	0.138	2.74	2.90	2.77^{y}	0.051
	5	3.93^{ax}	3.92^{a}	3.46^{b}	0.227	3.09	3.10	3.14^{x}	0.102
	10	3.46^{y}	3.86	3.48	0.162	3.04^{a}	3.01^{a}	2.84^{by}	0.085
	SEM[4)]	0.204	0.200	0.296		0.062	0.100	0.133	

[1)]Different letters (a, b) within a row with the same $NaNO_2$ addition differ significantly ($p < 0.05$).
[2)]Different letters (x ~ z) within a column with same the packaging differ significantly ($p < 0.05$).
[3)]Pooled standard errors of the mean (n = 6).
[4)]n = 6.

cular proteins, titin and nebulin and connective tissues, perimysium and endomysium (22,23), resulting in lower shear force. In the packaging effect, the sausage with CO_2 packaging shows a lower shear value than that with vacuum and aerobic packaging in 0 and 5 kGy-irradiated sausage.

The $NaNO_2$ addition to the sausage seems to have an effect on texture but generally no difference was found among irradiation treatments (Table 6). The CO_2-packaged sausage showed lower total working force for shear values, while aerobically-packaged sausage was the highest in 10 kGy-irradiated sausage.

When statistical probability was calculated, all main effects, irradiation, $NaNO_2$ addition, and different packaging methods, and their interactions had significance except for irradiation $\times$ $NaNO_2$ interaction ($p > 0.05$, Table 7).

In conclusion, no significant effect on lipid oxidation or color in the irradiated cooked pork sausage in a frozen state between vacuum- and CO_2-packaged (100%) sausage was found during a 3-month storage period at -20°C, but both packaging methods were better than aerobic packaging in microbial quality and lipid oxidation development.

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