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Gamma Irradiation of Ready-to-Cook *Bulgogi* Improves Safety and Extends Shelf-Life without Compromising Organoleptic Qualities

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Abstract

We investigated the effects of gamma irradiation on the microbiological, chemical and sensory qualities of *bulgogi*, a traditional Korean meat product. Gamma irradiation reduced the number of *coliform* bacteria and bacterial colonies counted in *Salmonella-Shigella* selective agar to a non-detectable level. Thermophillic microorganisms (mainly *Bacillus spp.*) in *bolgogi* refrigerated for 20 days were also significantly reduced by 3 log cycles by 2.5 to 7.5 kGy of irradiation, compared to the control. Electron donating ability and shear force of ready-to-cook *bulgogi* was not significantly affected by gamma irradiation. Irradiation increased the 2-thiobarbituric acid reactive substances (TBARS) value. The irradiated, raw, ready-to-cook *bulgogi* had significantly higher scores in color and appearance than those of non-irradiated controls from sensory evaluation. The overall acceptance of cooked *bulgogi* had higher scores in non-irradiated or 2.5 kGy-irradiated *bulgogi* than the 5.0- or 7.5-kGy irradiated counterparts. In conclusion, irradiation at 2.5 kGy is recommended as a method to improve shelf-life, safety, and to achieve acceptable quality of ready-to-cook *bulgogi* without any adverse changes in the sensory characteristics.

Key words: *bulgogi, ready-to-cook, irradiation, safety, quality*

INTRODUCTION

Bulgogi is one of the most popular traditional Korean dishes with a 1,500 year history, and is gradually gaining international acceptance. *Bulgogi* is prepared by cutting thin slices of beef, usually using brisket or round cut, and marinating them in sauce composed of soy sauce, sesame oil, garlic, onion and other seasonings; and cooking over a hot charcoal grill. Ready-to-cook *bulgogi* is made by local meat packers and sold in a refrigerated state, usually within 3 days. *Bulgogi* sauce and ready-to-cook *bulgogi* is increasing being manufactured by small meat blenders; however, safety of the products during distribution and storage is frequently not monitored and poses a serious risk for possible contamination by pathogens or spoilage bacteria in the fresh vegetables, soy sauce, and raw beef.

It has been reported that raw vegetables may harbor potential foodborne pathogens (1,2). Previously, 890 samples comprised of lettuce, pre-cut salads, growing herbs, parsley, dill, mushrooms and strawberries were screened for bacteriological contamination. Thermo-tolerant *coliform* bacteria (TCB), *Escherichia coli* O157, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus* spp., and *Yersinia enterocolitica* were isolated from the samples (3). *Aeromonas* spp. was also isolated from 41% of the organic vegetables (4). Song et al. (5) reported high counts (in range of 10^5 CFU/g) of *Bacillus* spp. in *Kanjang* (fermented soy sauce, Korean type) and *Shoyu* (fermented soy sauce, Japanese type), which are major components of *bulgogi* sauce.

Lee et al. (6) and Jo et al. (7) recently demonstrated that gamma irradiation can produce higher quality and microbiologically safer commercial *bulgogi* sauce than heat treatment alone. The World Health Organization (WHO) reported that gamma irradiation technology has positive effects in preventing decay by inactivating microorganisms and hence improving the safety and shelf-stability of food products (8). This technology has been officially adopted by international organizations (WHO/IAEA/FAO) and experts for its wholesomeness and economic benefits (8).

Despite the long tradition and frequency of eating *bulgogi* in Korea, the research on *bulgogi* is very limited. The objective of this study was to investigate the effect of irradiation on the microbiological, chemical, and sensory characteristics of ready-to-cook *bulgogi*, a traditional Korean meat product, during chilled storage and to eval-

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uate the potential of irradiation in future industrial applications.

MATERIALS AND METHODS

Sample preparation

Soy sauce, garlic, onion, green onion, sugar, kiwi, sesame oil, and black pepper were purchased from a local store and *bulgogi* sauce was prepared according to Table 1. Sliced meat was marinated in each prepared sauce by a 2 : 1 ratio for 3 hours in a refrigerator (4°C) and then irradiated.

Irradiation

Irradiation was performed in a Co-60 gamma irradiator (point source, AECL, IR-79, MDS Nordion International Co., Ltd., Ottawa, Ontario, Canada) with the source strength of 100 kCi. The dose rate was 83.3 Gy/min at 12 ± 0.5°C and the applied absorbed doses were 0, 2.5, 4.5 and 7.5 kGy. Dosimetry was performed using 5 mm-diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free radical signal was measured using a Bruker EMS 104 EPR Analyzer. The actual dose was within $\pm2\%$ of the target dose. The non-irradiated control was placed outside of the irradiation chamber to maintain consistent temperatures with the irradiated sample. The control and irradiated ready-to-cook *bulgogi* were transferred to a 4°C refrigerator and the analyses were started after 3 hrs.

Microbiological analysis

A 10 g sample was homogenized with 90 mL of deionized distilled water (DDW) using a lab blender (FM 680T, Hanil Co., Ltd., Seoul, Korea) for 60 sec. Each sample was stirred for 30 min at 4°C, and serially diluted with a saline solution (10-fold). Each diluent (1 mL) was pored onto selective agar plates in triplicate. Media for temporary enumeration of thermophillic microorganisms, *coliform* bacteria, and *Salmonella-Shigella* spp. were dextrose tryptone agar (Difco Lab., Detroit, MI, USA), eosine methylene blue agar (Difco Lab.) and *Salmonella-Shigella* selective agar (SS-agar), respectively. The thermophillic microorganisms, *coliform* bacteria and *Salmonella-Shigella* spp. were incubated at 50, 37, and 37°C, respectively, for 72 hrs.

Table 1. Recipe for *bulgogi* sauce

Raw materials	Weight (g)	Ratio (%)
Soy sauce	1,800	48.0
Rice wine	300	8.0
Sugar	600	16.0
Garlic	400	10.5
Kiwi	200	5.3
Green onion	200	5.3
Onion	200	5.3
Sesame oil	50	1.3
Black pepper	10	0.3
Total	3,760	100

Scavenging effects against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

An aliquot of each sample (2 g) was transferred to a 20 mL-test tube and homogenized (DIAX 900, Heidolph Co., Ltd., Germany) for 15 sec at a speed setting of 6 (approximately 23,000 rpm) in 8 mL DDW. The mixture was filtered through filter paper (No. 4, Whatman International Ltd., Springfield Mill, Maidstone, Kent, England) and chloroform (2 mL) was added into the filterate. The filterate was vortexed vigorously to remove any fat in the sample and centrifuged (VS 5500, Vision Scientific Co., Ltd. Bucheon, Korea) at 2,400 rpm for 30 min. The upper layer of the mixture was diluted 5 times with DDW, and free radical scavenging effect was estimated according to the method of Blois (9) with some modification. A 1 mL sample was added to 0.2 mM DPPH radical dissolved in methanol (1 mL). The mixture was shaken and left to stand for 30 min at room temperature and absorbance was measured at 517 nm with a spectrophotometer. The calculation of the scavenging effect was as follows;

DPPH radical scavenging effect (%) = 100 − [(absorbance of sample/absorbance of blank) $\times$ 100]. The blank was prepared by adding 1 mL of ethanol (70%) instead of the sample.

Texture analysis

The total working force for shear value of each sample was measured by a texture analyzer (TA-XT2i, Stable Micro Systems, England). The sample was cut into 3 cm pieces 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 as follows: pretest speed, 2.0 mm/s; test speed, 5.0 mm/s; posttest speed, 2.0 mm/s. The shear value was determined by automatically calculating the highest peak value using texture expert software (Stable Micro Systems, England). Values reported are the mean values of 8 replications.

Lipid oxidation

Lipid oxidation was determined by measuring the amounts 2-thiobarbituric acid reactive substances (TBARS) using a spectrophotometer (UV 1600 PC, Shimadzu, Tokyo, Japan) as described by Turner et al. (10). Meat samples were homogenized in a Stomach Lab Blender (Model 400, Tekmar Co., Cincinnati, OH, USA) and a 0.5 g sample was added into a 50 mL test tube with 5 mL of 20%

trichloroacetic acid (Jusei Chemical Co. Ltd, Tokyo, Japan) in 2 M phosphoric acid (Allied Chemicals Co., Morristown, NJ, USA) and 10 mL of 0.01 M TBA (Sigma Chemical Co., St. Louis, MO, USA). The sample was vortexed, heated in boiling water for 30 min, and then chilled in ice water for 10 min. A mixture of isoamylalcohol (Yakuri Pure Chemicals Co., Osaka, Japan) and pyridine (2 : 1, v/v, 15 mL, Tedia Company Inc. Fairfield, OH, USA) was added to the test tube and vigorously vortexed for 2 min. Then, the sample was centrifuged (VS-5500, Vision Co. Ltd., Bucheon, Korea) for 15 min at 2,400 rpm. The upper layer of the mixture was used for a spectrophotometric reading at 538 nm.

Sensory analysis

Sensory evaluation (n=28) was used to estimate consumer acceptance and quality of the raw and cooked *bulgogi* after irradiation. For the raw *bulgogi*, the color, odor and appearance were rated for a dish with a 30 g sample. For the cooked *bulgogi*, the marinated beef was placed on a preheated pan at about 170°C and cooked for about 8.5 min to reach the meat temperature of approximately 78°C. After cooling for 2 min at room temperature, about a 30 g sample was served to the panelists individually. The sensory parameters were taste, tenderness and overall acceptance of the cooked *bulgogi*. The sensory scores were evaluated independently by the panelists on each of the 3 parameters with different individuals for each. A 15 cm line scale was provided to the panelists as follows; like extremely (15) and dislike extremely (1).

Statistical analysis

The experimental design used was a 4 (irradiation treatment) × 5 (storage period) factorial and the entire experiment was performed in duplicate. Analyses of variance were performed using SAS software (SAS Institute, Cary, NC USA) (11) and the Student-Newman-Keul's 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 as $p<0.05$.

RESULTS AND DISCUSSION

Microbiological analysis

The growth of thermophillic microorganisms in ready-to-cook *bulgogi* during storage at 4°C is shown in Fig. 1. Song et al. (5) reported that *Bacillus* spp. of Korean type-fermented soy sauce, *Kanjang* and Japanese type-fermented soy sauce, *Shoyu*, were inactivated by 3 log cycles by 10 kGy of irradiation with a D_{10} value of 2.67 kGy. The non-irradiated control showed a gradual growth of thermophillic microorganisms but irradiation effectively reduced the level of growth. Recent reports have indicated that raw vegetables and fruits are heavily contaminated with thermophillic microorganisms initially (7). Soy sauce, which is not a good environment for microbial growth, also had 3.77×10^3 CFU/g of thermophillic microorganisms (7). In the present study, there were initially 1.03 $\times 10^2$ coliform bacteria in the ready-to-cook *bulgogi*, and was sustained during 20 days of storage (Table 2). Irradiation at 2.5, 4.5 and 7.5 kGy reduced the *coliform* counts to non-detectable levels until 20 days of storage.

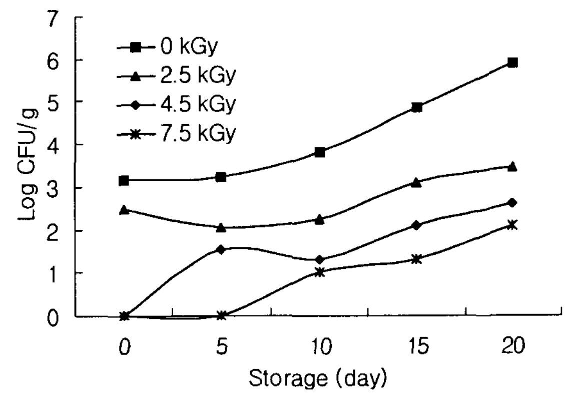

Fig. 1. Growth of thermophillic microorganisms of irradiated *bulgogi* during storage at 4°C.

Table 2. Growth of microorganisms in gamma irradiated ready-to-cook *bulgogi*

Microorganism	Irrdiation dose (kGy)	Storage (day)				
		0	5	10	15	20
Coliform	0	1.03×10^2	9.1×10^2	1.0×10^2	8.0×10^1	1.0×10^1
	2.5	ND[1)]	ND	ND	ND	2.0×10^1
	4.5	ND	ND	ND	ND	4.0×10^1
	7.5	ND	ND	ND	ND	ND
SS-agar[2)]	0	2.1×10^2	1.2×10^1	6.0×10^1	2.0×10^1	ND
	2.5	ND	ND	ND	ND	ND
	4.5	ND	ND	ND	ND	ND
	7.5	ND	ND	ND	ND	ND

[1)]ND: Not detected ($<10^1$).
[2)]SS-agar: *Salmonella-Shigella* selective agar.

SS agar plates also revealed microbial growth in non-irradiated controls, but irradiation eliminated these microbes (Table 2). These results indicate that hygienic quality and safety of commercially available ready-to-cook *bulgogi* products after marinating is questionable because of the possible growth of pathogens.

DPPH radical scavenging activity

The DPPH radical scavenging activity of ready-to-cook *bulgogi* was relatively constant during storage and there were no differences among controls or irradiation treatment groups except on day 10 (Table 3). It has also been demonstrated that electron donating ability is not altered by irradiation treatment in studies using *Chungukjang* and *Doenjang*, the Korean fermented soybean pastes (12), or green tea leaf extract (13).

Lipid oxidation

There were no differences in TBARS values at the initial stage of storage among the treated samples, but irradiation significantly accelerated lipid oxidation during the 20 days of storage (Table 4). Previous reports indicated that irradiation increased lipid oxidation in meats during storage with atmospheric packaging (14,15). Thus, it is recommended that irradiated ready-to-cook *bulgogi* be packed by vacuum or modified atmosphere conditions rather than aerobic packaging before irradiation to inhibit lipid oxidation during distribution and storage. The addition of natural antioxidants may also help prevent oxidative damage (16).

Texture and sensory analysis

Shear force was not significantly different in ready-to-cook *bulgogi* among irradiation treatment groups (Table 5), but exhibited a gradual decreasing trend during storage. Lee et al. (17) reported that vacuum packaged and irradiated beef had a lower shear force than that of non-irradiated beef, resulting in improved tenderness and meat quality. Similar results were obtained using a sausage with irradiated natural pork or lamb casing, which indicated that irradiation reduced the value of total working force for shear (18).

Raw *bulgogi* was evaluated by sensory panelists for color, odor and appearance (Table 6). Significant differences were observed in color evaluations; samples irradiated at 4.5 or 7.5 kGy had the higher score while the non-irradiated control had the lowest. Byun et al. (19) reported that irradiation of raw meat at 5 kGy did not affect the organoleptic quality of the ham after cooking. Ahn et al. (20) also reported that irradiated muscle strips produced stronger irradiation odor than non-irradiated counterparts, but the preference was not significant.

In cooked *bulgogi*, there was no statistically significant difference found in taste and tenderness but overall acceptance in the 2.5 kGy-irradiated and non-irradiated sample was higher than that of the samples irradiated at 7.5 kGy (Table 7). Hashim et al. (21) reported that irradiated

Table 3. Scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in irradiated ready-to-cook *bulgogi* during storage at 4°C

Irradiation dose (kGy)	Storage (day)				
	0	5	10	15	20
0	78.3	60.2	73.1^{a}[1)]	77.5	73.4
2.5	64.2	65.5	70.6^{ab}	71.9	72.3
4.5	76.4	68.7	70.8^{ab}	65.4	74.0
7.5	72.8	67.2	67.5^{b}	69.5	71.3
SEM	2.56	3.17	1.30	2.10	2.62

[1)]Different letters within the same column differ significantly ($p<0.05$).

Table 4. TBARS values in irradiated ready-to-cook *bulgogi* during storage at 4°C

Irradiation dose (kGy)	Storage (day)				
	0	5	10	15	20
0	0.62^{b}[1)]	0.57^{b}	0.51^{b}	0.46^{b}	0.62^{b}
2.5	0.71^{a}	0.70^{a}	0.82^{a}	0.86^{a}	0.96^{a}
4.5	0.75^{a}	0.76^{a}	0.76^{a}	0.93^{a}	1.04^{a}
7.5	0.70^{a}	0.73^{a}	0.89^{a}	0.83^{a}	1.12^{a}
SEM	0.011	0.030	0.028	0.034	0.050

[1)]Different letters within the same column differ significantly ($p<0.05$).

Table 5. Shear force (Nmm) of irradiated ready-to-cook *bulgogi* during storage

Irradiation dose (kGy)	Storage (day)				
	0	5	10	15	20
0	1220	1107	856	834	801
2.5	836	1279	1059	705	724
4.5	762	995	1165	855	828
7.5	1177	855	981	764	821
SEM	186.8	227.1	159.7	174.1	158.8

Table 6. Sensory evaluation of raw *bulgogi* after gamma irradiation[1)]

Irradiation dose (kGy)	Sensory parameter		
	Color	Odor	Appearance
0	3.5^{c}[3)]	8.2	4.2^{b}
2.5	9.3^{b}	7.0	8.7^{a}
4.5	11.2^{a}	6.7	9.3^{a}
7.5	11.1^{a}	8.3	11.1^{a}
SEM[2)]	0.68	1.03	0.93

[1)]Color, odor and appearance were evaluated using a dish with 30 g of raw marinated beef (*bulgogi*). A 15 cm line scale was used; like extremely (15) to dislike extremely (0).
[2)]Pooled standard errors of the mean (n=28).
[3)]Different letters within the same column differ significantly ($p<0.05$).

Table 7. Sensory evaluation of gamma irradiated and cooked *bulgogi*[1)]

Irradiation dose (kGy)	Sensory parameter		
	Taste	Tenderness	Acceptance
0	8.4	8.6	10.1[a3)]
2.5	9.4	8.6	10.7[a]
4.5	6.6	9.6	9.6[ab]
7.5	7.0	6.6	6.3[b]
SEM[2)]	1.09	0.95	0.99

[1)]Raw *bulgogi* was placed on a preheated pan at about 170°C and cooked for about 8.5 min to reach the meat temperature of approximately 78°C. After cooling for 2 min at room temperature, about 30 g of sample were served to the panelists (n=28) individually. A 15 cm line scale was used; like extremely (15) to dislike extremely (0).
[2)]Pooled standard errors of the mean (n=28).
[3)]Different letters within the same column differ significantly ($p<0.05$).

uncooked chicken breast and thigh produced a characteristic bloody and sweet aroma that remained after the thighs were cooked, but was not detectable after the breasts were cooked.

CONCLUSION

Irradiation of ready-to-cook *bulgogi*, a Korean traditional marinated beef, improved hygienic and sensory qualities following refrigerated storage. Our results indicate that 2.5 kGy of gamma irradiation is the optimum dose in terms of sensory quality and economical practice.

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