



Use of gamma irradiation for inactivation of pathogens inoculated into *Kimbab*, steamed rice rolled by dried laver

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Abstract

The effect of gamma irradiation for inactivating the pathogens inoculated into the ready-to-eat *Kimbab*, steamed rice rolled by dried laver, was investigated. The pathogens used were *Salmonella* Typhimurium, *Escherichia coli*, *Staphylococcus aureus* and *Listeria ivanovii* which are important for public health. The growth of four test organisms inoculated (about 10^6 – 10^7 CFU/g) into the *Kimbab* were sustained by an irradiation treatment during 24 h of storage regardless of the temperature at 10, 20 and 30 °C. Four pathogen inoculated into *Kimbab* decreased 2–3 log CFU/g by 1 kGy treatment and was not detected after 3 kGy. The D_{10} value of pathogens inoculated into the *Kimbab* were 0.31–0.44 kGy among the four organisms. This study indicated that a low dose irradiation can maintain microbial safety for ready-to-eat *Kimbab*, steamed rice rolled by dried laver.

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1. Introduction

A *Kimbab*, steamed rice rolled in a dried laver (dried seaweed), is a popular ready-to-eat food in Korea. Usually, the laver roll is recognized as a lunch menu or a food for picnics. In the center of *Kimbab*, agricultural foods, sea foods and meat products can be placed to improve the organoleptic quality, depending on the maker's preference. With the recent developments in food service industry, the demand of ready-to-eat foods increase dramatically in the food industry (Kwak, Kim, Park, Cho, & Choi, 1996).

However, ready-to-eat foods have been the cause of the major outbreaks of food-borne diseases. In the US, overall incidences of the pathogen in cooked ready-to-eat meat products ranged of 8.1% for a four-year period, 1993–

1996 (Anonymous, 1997). A survey of ready-to-eat cooked meat products in Germany showed an incidence rate of 3.7% for *Listeria monocytogenes* (Noack & Joeckel, 1993). Mytle, Anderson, Doyle, and Smith (2006) reported that illnesses have resulted when supposedly ready-to-eat foods were not reheated before consumption.

The major pathogens caused by the food-borne disease in *Kimbab* were *Salmonella* spp. and *Staphylococcus aureus*. *Salmonella* spp. in *Kimbab* was detected at 623 CFU/g on an average of about 214 samples and the frequency of detection in the summer was higher than in the winter season (Kang et al., 2002). Lee et al. (2005) reported that most contamination of *Kimbab* was caused by food materials such as dried laver, surimi gel, seasoned and blanched spinach, and cucumber. Jo, Lee, Kang, et al. (2004) and Jo et al. (2005) have studied the food materials for *Kimbab* manufacturing and reported that low dose irradiation (less than 3 kGy) can eliminate pathogens inoculated into ready-to-use foods originated from animal and seafood.

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The objective of the present study was to demonstrate the efficacy of irradiation treatment for eliminating the pathogens such as *Salmonella* Typhimurium, *Escherichia coli*, *S. aureus* and *Listeria ivanovii* in a manufactured *Kimbab*, thereby ensuring the microbiological safety of the ready-to-eat *Kimbab*.

2. Materials and methods

2.1. Sample preparation

Kimbab, steamed rice rolled by dried laver, was purchased from local store and sliced to have a 1 cm thickness with aseptic condition. A 10 g sample was packed in oxygen-impermeable nylon bags (2 mL O₂/m²/24 h at 0 °C, 0.09 mm thickness; Sunkyung Co. Ltd., Seoul, Korea). The packed samples were exposed to an irradiation dose of 30 kGy (point source, AECL, IR-79, MDS Nordion, Ontario, Canada) at 12 ± 0.5 °C to inactivate all the indigenous microflora.

2.2. Strains and culture condition

Four pathogens, *Salmonella* Typhimurium (KCTC 1925), *E. coli* (KCTC 1682), *S. aureus* (KCTC 1916), and *L. ivanovii* (KCTC 3444), were used in this research and obtained from a Korean collection for type culture (KCTC, Daejeon, Korea). *E. coli*, *S. aureus*, and *L. ivanovii* were grown in a tryptic soy broth (Difco, Laboratories, Sparks, MD, USA). *S. Typhimurium* was grown in a nutrient broth (Difco, Laboratories, Sparks, MD, USA). The incubation temperature of *E. coli*, *S. aureus* and *S. Typhimurium* was 37 °C. *L. ivanovii* was incubated at 30 °C. The bacterial cultures were prepared for 24 h in a sterilized broth medium from 1 colony from an agar slant, from which 0.1 mL was transferred to a new broth medium, and grown for 18 h. The cultures were centrifuged (2795g for 10 min at 4 °C) in a refrigerated centrifuge (Vs-5500, Vision Scientific, Co., Seoul, Korea). Cultures were washed twice with sterile 0.1% peptone water. The pellet was finally suspended in sterile peptone water to a cell density about 10⁸–10⁹ CFU/mL levels.

2.3. Inoculation of test organism

Four test organisms were individually inoculated in each sample, respectively. The test culture suspension (200 µL) was aseptically spreaded on the *Kimbab*, respectively. It was kept in a sterile workstation for 1 min to allow it to be absorbed.

2.4. Irradiation

Kimbab inoculated with the test organisms was irradiated in a cobalt-60 irradiator (point source AECL, IR-79, MDS Nordion International Co. Ltd., Ottawa, ON, Canada) at the Korea Atomic Energy Research Institute,

Daejeon, Korea. The source strength was approximately 100 kCi with a dose rate of 10 kGy h⁻¹ at 12 ± 0.5 °C. 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 dosimeters were calibrated against an International standard set by the International Atomic Energy Agency (Vienna, Austria). The applied doses in this study were 0, 1, 2 and 3 kGy. After irradiation, the samples were transferred and microbiological analysis was performed during storage at a commercial storage condition (10 °C), room temperature condition (20 °C), and an abusive temperature storage condition (30 °C).

2.5. Microbiological analysis

A sample (10 g) was aseptically homogenized for 2 min in a sterile stomacher bag containing 90 mL of sterile 0.1% peptone water using a bag mixer[®] (Model 400, Interscience Co, France). Media for enumeration of the *E. coli*, *S. Typhimurium*, *S. aureus* and *L. ivanovii* were prepared by violet red bile lactose agar (Oxoid, Basingstoke, Hampshire, England), xylose lysine desoxycholate agar (Difco Laboratories, Detroit, MI, USA), baird parker agar (Oxoid, England) and palcam agar (Oxoid, England), respectively. Plates were incubated at optimal temperature of bacteria for 48 h and colony forming units (CFU) per gram were counted at a dilution of 30–300 CFU per plate. D₁₀ values (the dose required to inactivate 90% of a population) for each of the organisms tested were determined using the sample immediately after irradiation (0 h) by calculating the reciprocal of the slope. Experiments with each bacterial culture were conducted independently twice.

2.6. Statistical analysis

Each data represents the mean of two different experiments with two measurements in each experiment. Mean values and standard deviation (SD) were calculated using a Statistical Analysis System (SAS Institute, 1990) and reported.

3. Results and discussion

The inhibition effects of an irradiation on four pathogens, *Salmonella* Typhimurium, *E. coli*, *S. aureus* and *L. ivanovii*, inoculated into ready-to-eat *Kimbab* was presented in Tables 1–4.

The inoculated *Salmonella* Typhimurium into the *Kimbab* was 6.22 log CFU/g at the initial stage and 2 kGy of irradiation reduced the level to 2.29 log CFU/g (Table 1). However 3 kGy of irradiation reduced the population of the microorganism to an undetected level (lower than 10² CFU/g). Jo, Lee, Kang, et al. (2004) reported that 3 kGy of irradiation reduced the population of the microorganism in prepared food, seasoned and cooked beef,

Table 1
Effect of irradiation on growth (log CFU/g) of *Salmonella* Typhimurium (KCTC 1925) in *Kimbab* during storage at different storage temperatures^a

Storage temperature (°C)	Irradiation dose (kGy)	<i>Salmonella</i> Typhimurium		
		0 h	8 h	24 h
10	0	6.22 ± 0.25	5.88 ± 0.27	5.85 ± 0.21
	1	3.85 ± 0.15	3.88 ± 0.20	3.52 ± 0.35
	2	2.29 ± 0.16	2.09 ± 0.12	2.20 ± 0.28
	3	ND ^b	ND	ND
20	0	6.22 ± 0.25	5.80 ± 0.14	6.26 ± 0.04
	1	3.85 ± 0.15	3.63 ± 0.04	4.06 ± 0.18
	2	2.29 ± 0.16	2.44 ± 0.06	2.57 ± 0.12
	3	ND	ND	ND
30	0	6.22 ± 0.25	5.98 ± 0.19	7.89 ± 0.08
	1	3.85 ± 0.15	4.93 ± 0.76	4.55 ± 0.02
	2	2.29 ± 0.16	3.00 ± 0.14	3.18 ± 0.11
	3	ND	ND	ND

^a Mean ± standard deviation ($n = 2$).

^b Viable colony was not growth at detection limit $< 10^2$ CFU/g.

Table 2
Effect of irradiation on growth (log CFU/g) of *Escherichia coli* (KCTC 1682) in *Kimbab* during storage at different storage temperatures^a

Storage temperature (°C)	Irradiation dose (kGy)	<i>Escherichia coli</i>		
		0 h	8 h	24 h
10	0	7.36 ± 0.17	7.33 ± 0.22	7.10 ± 0.11
	1	5.79 ± 0.12	5.81 ± 0.10	5.76 ± 0.16
	2	3.36 ± 0.37	3.27 ± 0.38	2.85 ± 0.21
	3	ND ^b	ND	ND
20	0	7.36 ± 0.17	7.40 ± 0.10	8.78 ± 0.09
	1	5.79 ± 0.12	6.83 ± 0.11	6.93 ± 0.04
	2	3.36 ± 0.37	5.15 ± 0.20	5.00 ± 0.02
	3	ND	3.40 ± 0.36	3.84 ± 0.01
30	0	7.36 ± 0.17	8.59 ± 0.06	9.14 ± 0.07
	1	5.79 ± 0.12	7.71 ± 0.01	7.80 ± 0.12
	2	3.36 ± 0.37	4.52 ± 0.39	4.47 ± 0.04
	3	ND	3.72 ± 0.18	3.64 ± 0.15

^a Mean ± standard deviation ($n = 2$).

^b Viable colony was not growth at detection limit $< 10^2$ CFU/g.

Table 3
Effect of irradiation on growth (log CFU/g) of *Listeria ivanovii* (KCTC 3444) in *Kimbab* during storage at different storage temperatures^a

Storage temperature (°C)	Irradiation dose (kGy)	<i>Listeria ivanovii</i>		
		0 h	8 h	24 h
10	0	6.04 ± 0.12	6.94 ± 0.34	7.61 ± 0.37
	1	4.64 ± 0.32	4.46 ± 0.31	4.87 ± 0.06
	2	2.09 ± 0.12	2.09 ± 0.12	2.21 ± 0.05
	3	ND ^b	ND	ND
20	0	6.04 ± 0.12	7.94 ± 0.18	8.61 ± 0.03
	1	4.64 ± 0.32	5.21 ± 0.19	5.08 ± 0.03
	2	2.09 ± 0.12	2.27 ± 0.04	2.46 ± 0.03
	3	ND	ND	ND
30	0	6.04 ± 0.12	8.08 ± 0.01	8.06 ± 0.03
	1	4.64 ± 0.32	5.62 ± 0.29	5.82 ± 0.01
	2	2.09 ± 0.12	2.41 ± 0.09	3.51 ± 0.09
	3	ND	ND	ND

^a Mean ± standard deviation ($n = 2$).

^b Viable colony was not growth at detection limit $< 10^2$ CFU/g.

Table 4
Effect of irradiation on growth (log CFU/g) of *Staphylococcus aureus* (KCTC 1916) in *Kimbab* during storage at different storage temperatures^a

Storage temperature (°C)	Irradiation dose (kGy)	<i>Staphylococcus aureus</i>		
		0 h	8 h	24 h
10	0	7.87 ± 0.05	7.63 ± 0.09	7.27 ± 0.37
	1	4.11 ± 0.05	4.87 ± 0.24	3.77 ± 0.32
	2	2.09 ± 0.12	2.59 ± 0.16	2.51 ± 0.05
	3	ND ^b	ND	ND
20	0	7.87 ± 0.05	7.65 ± 0.19	8.79 ± 0.01
	1	4.11 ± 0.05	5.81 ± 0.13	5.74 ± 0.31
	2	2.09 ± 0.12	3.80 ± 0.02	4.40 ± 0.11
	3	ND	2.80 ± 0.14	2.50 ± 0.14
30	0	7.87 ± 0.05	8.88 ± 0.20	8.81 ± 0.56
	1	4.11 ± 0.05	5.85 ± 0.21	6.26 ± 0.43
	2	2.09 ± 0.12	3.95 ± 0.04	4.96 ± 0.02
	3	ND	2.82 ± 0.23	3.09 ± 0.44

^a Mean ± standard deviation ($n = 2$).

^b Viable colony was not growth at detection limit $< 10^2$ CFU/g.

Fried egg and ham, to an undetected level (lower than 10^2 CFU/g). Tessi et al. (2002) reported that the strategy for the prevention of food-borne illness caused by ready-to-eat cooked foods is based on the initial microbial load (nature and quantity), the severity of the heat treatment necessary to destroy pathogens and lower that microbial load, and prevention of growth through temperature controls.

The effect of radiation on *E. coli* inoculated into the ready-to-eat *Kimbab* shown in Table 2. Irradiation at 2 kGy decreased the *E. coli* level in the *Kimbab* to below 3 log CFU/g, and *E. coli* was not detected after 3 kGy. But this level increased to about 3 log CFU/g after 24 h of storage at 20 and 30 °C. Vazgecer, Ulu, and Oztan (2004) reported that *E. coli* was found in 31% of samples of ready-to-eat chicken döner kebab ranged between 2.0×10 and 5.0×10^2 g⁻¹. Lee et al. (2005) reported that combined treatment of irradiation and low temperature storage was effect for microbiological safety of *Kimbab* or ready-to-use materials.

Effective methods for killing *L. monocytogenes* in foods would reduce the likelihood of food-borne outbreaks of listeriosis, and decrease economic losses to the food industry (Nair, Vasudevan, & Venkitanarayanan, 2005). *L. ivanovii* inoculated into *Kimbab* decreased about 4 log CFU/g with 2 kGy (Table 3). *L. ivanovii* inoculated into *Kimbab* was not detected after 3 kGy of irradiation regardless of the storage temperatures. The dosages for 3 log reduction were 1.5 kGy for bologna, roast beef, and both types of turkey and 2.0 kGy for frankfurters and ham on the basis of use of selective medium (Foong, Gonzalez, & Dickson, 2004). Sommers, Fan, Niemira, and Rajkowski (2004) reported that anti-microbial compounds, sodium diacetate and potassium lactate, both of which are approved for use in ready-to-eat meat, can inhibit the growth of *L. monocytogenes* during long-term refrigerated storage.

The inhibition effects of irradiation on *S. aureus* inoculated into the *Kimbab* was presented in Table 4. *S. aureus* inoculated into the *Kimbab* showed about a 5 decimal reduction by 2 kGy. Coagulase positive staphylococci was isolated in 15 of 20 (75%) meatball samples, 20 of 20 (100%) cooked liver samples, and 20 of 20 (100%) Russian salad samples, at the level of 3.5, 4.9 and 5.1 log CFU/g, respectively (Öner, 1997). Jo et al. (2005) reported that *S. aureus* inoculated into prepared seafood products including imitation crab leg, surimi gel and dried laver was not detected after 3 kGy of a gamma irradiation.

Table 5 shows the calculated D_{10} value of the irradiation treatment of the *Kimbab* tested. The range of the D_{10} value was from 0.31 to 0.44 kGy among the four strains. Inactivation of food-borne microorganisms by ionizing radiation is influenced by food composition, temperature, preservation method, atmospheric gas composition, numbers and types of microorganisms and irradiation dose (Juneja & Sofos, 2002). Also it should be pointed out that the D_{10} values were estimated using selective media after sterilization of samples, which may overestimate the effect of irradiation. Rajkowsdi, Boyd, and Thayer (2003) found the D_{10} value of *E. coli* O157:H7 ranged from 1.11 to 1.43 kGy on broccoli seeds. Clavero, Monk, Beuchat, Doyle, and Brackett (1994) reported that D_{10} value of *Salmonella* spp. was 0.621–0.624 and 0.618–0.661 on ground beef (low fat) and ground beef (high fat), respectively. The radiation D -value of *L. monocytogenes* on beef was 0.45 kGy at 0 °C and 1.21 kGy at –20 °C (Thayer & Boyd, 1995). Lamb, Gegley, Thompson, Solis, and Sen (2002) reported that two experiments on *S. aureus* in ready-to-eat ham and cheese sandwiches yielded D_{10} value of 0.62 and 0.63. Thayer and Boyd (1992) also reported that D value for stationary phase *S. aureus* in mechanically deboned chicken meat was 0.36 ± 0.01 kGy. The major extracellular environmental factors that influence the survival of irradiated cells are temperature, phase, the nature of the gaseous environment, water activity, pH and chemical composition of the foods (WHO, 1999). A low dose gamma irradiation for the extension of the shelf-life, microbiological safety and a retention of the quality in minimally processed vegetables and fruits has been gaining importance in industry including restaurants, institutional and airline catering (Farkas, 1998).

Jo, Lee, Hong, Kim, and Byun (2004) reported that the sensory parameters including appearance, texture, flavor, and overall acceptance were negatively correlated with the irradiation dose of 0, 0.5, 1, 2, and 3 kGy, and 2 and

3 kGy was not acceptable. Therefore, the authors suggested that the optimum conditions for preserving sensory quality should be established before application.

The results of microbiological analysis indicated that the irradiation treatment can minimized the risk of the harmful pathogens for public health in the favorite ready-to-eat foods in Korea.

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Table 5
Radio-sensitivity (D_{10} -value) of pathogens inoculated *Kimbab*

Pathogens	D_{10} value
<i>Salmonella</i> Typhimurium KCTC 1925	0.44 ± 0.01^a
<i>Escherichia coli</i> KCTC 1682	0.42 ± 0.04
<i>Staphylococcus aureus</i> KCTC 1916	0.31 ± 0.01
<i>Listeria ivanovii</i> KCTC 3444	0.43 ± 0.01

^a Mean \pm standard deviation ($n = 2$).

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