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Effects of Irradiation Temperature on the Sensory Quality Improvement of Gamma-irradiated *Ganjang-gejang,* Korean Traditional Marinated Raw Crab *Portunus trituberculatus* in Soybean Sauce

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Abstract

This study was conducted to confirm quality properties of sterilized *Ganjang-gejang* (marinated crab *Portunus trituberculatus*) with Korean soy sauce using by gamma irradiation and to improve quality of sterilized *Ganjang-gejang*. The *Ganjang-gejang* was irradiated at dose of 3, 6, 9, 12, and 15 kGy by gamma irradiation and there was evaluated in microbiological, physicochemical, and sensory properties. Total aerobic bacteria and fungi contents of non-irradiated samples were about 6 and 4 log CFU/g level, respectively. Gamma-irradiated samples at above 9 kGy did not contain aerobic bacteria or fungi at detection limit less than 2 log CFU/g, but sensory scores were significantly decreased depending on the irradiation dose. To improve the sensory qualities of gamma-irradiated *Ganjang-gejang*, the temperature was adjusted during sample irradiation. When samples were irradiated under freezing temperatures, especially on dry ice, the TBARS and the deterioration of sensory qualities of *Ganjan-Gejang* were reduced. Different odor patterns were observed among samples, as observed using electronic nose analysis system. The results of this study indicated that treatment with irradiation under low temperatures may help to preparing high-quality *Ganjang-gejang*.

Key words: *Portunus trituberculatus*, *Gejang*, Gamma irradiation, Patient food, Sterilization

Introduction

Traditional Korean cuisine includes many kinds of fermented foods, such as *kimchi* (fermented vegetables), *Jang* (soybean fermented food), and *Jeotgal* (fermented seafood). *Kimchi* is good source of vitamins and minerals, and *Jang* and *Jeotgal* are adequate source of protein. In particular, 54 kinds of *Jeotgal* using fish, crab, and clams with salt or soybean sauce are known in Korea (Seo et al., 2008). *Ganjang-gejang*, a type of *Jeotgal*, is popular because of its racy flavor and taste and, is made of raw crab *Portunus trituberculatus* with Korean soybean sauce. *Ganjang-gejang* is fermented in a refrigerator of 7ºi with a mixture of raw crab, boiled soybean sauce, and vegetables. Crab, which is main ingredient in *Ganjang-gejang*, contains essential amino acid, such as leucine. In addition, crab is beneficial in combatting obesity, liver disorders,

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and hypertension. Korean soybean sauce contains variable bioactive substances resulting from the fermentation process, including peptides, polyphenols, and isoflavone (Cheigh et al., 1993).

Serving to satisfied food to patient who wants their favorite food is a high priority in hospitals in Korea due to the focus on maintaining the patient's nutritional health (Han, 2011). However, serving certain foods like *Ganjang-gejang* to patients, especially seniors and immuno-compromised patients, has limitations due to the presence of bacteria, such as *Escherichia coli* and *Bacillus cereus*, derived from raw crab and soybean sauce; these bacteria can cause food poisoning, which may be detrimental to the health and recovery of patients. Therefore, adequate sterilization procedures are necessary to permit patients to consume their favorite foods while hospitalized.

Energy-efficient non-thermal sterilization is an advanced application of irradiation technology (Byun, 1997). Pasteurization or sterilization of irradiated foods has been shown to effectively control biological hazards associated with foods made from raw materials by non-thermal or low-temperature cooking methods, without compromising nutritional properties (Skala et al., 1987; Thayer et al., 1986). In addition, selective sterilization can be achieved using gamma irradiation technology applied to Korean traditional fermented food; indeed, studies using various fermented foods have reported successful sterilization of *kimchi*, fermented fish paste, red pepper-soybean paste, *ssamjang*, *meju*, and *cheonggukjang* (Kim et al., 2000a; Kim et al., 2000b; Park et al., 2010).

Therefore, the objective of this study was to evaluate the microbiological and sensory characteristics of *Ganjang-gejang* sterilized with gamma irradiation or developed for serving to immuno-compromised patient.

Materials and Methods

Sample preparation

Commercial *Ganjang-gejnag* [swimming crab *Portunus trituberculatus* 50%, soybean sauce 50% (dark soy sauce 55%, starch syrup 25%, soju 10%, sweet tangle laminaria japonica 2%, red pepper 2%, garlic 2%, onion 2%, green onion 2%) was purchased in frozen form from a local supermarket in Jeongeup-si, Jeollabuk-do, South Korea. All fermented Ganjang-gejang (swimming crab, Portunus trituberculatus, marinated with soybean sauce) samples with crab (50 g) and soybean sauce (50 mL). All samples with crab and soybean sauce (50 mL) were placed into a sterilized package (AL-LDPE, aluminum-laminated low-density polyethylene, Sunkyung Co., Ltd., Seoul, Korea). The packaged samples were kept in refrigerator at 4°C before irradiation.

Gamma irradiation

Samples were irradiated in a cobalt-60 irradiator (IR-7P, MDS Nordion Co., Ottawa, Ontario, Canada) at room temperature. The source strength was approximately 320 kBq, with a dose rate of 10 kGy/h, and the actual doses were within 2% of the target doses of 3, 6, 9, 12, and 15 kGy. To maintain temperature during irradiation, samples were placed in the container box filled with ice or dry ice for refrigeration and freezing conditions, respectively. The absorbed dose was monitored by using a 5-mm diameter alanine dosimeter (Bruker Instruments, Rheinstetten, Germany).

Microbial analysis

Approximately 50 g of swimming crab samples containing the meat and shell were aseptically prepared, put in a sterilized bag with 50 mL of soybean sauce (50 mL) and stomacher (Tekmar Co., Los Angeles, CA, USA) for 5 min. For microbiological analysis of the samples, portions (10 g) of the samples were aseptically transferred into sterile bags containing 90 mL of sterile water diluents and pummeled in a stomacher (Tekmar Co., Los Angeles, CA, USA) for 1 min. The diluted solution (100 μL) was then placed on plate count agar (PCA, Difco Co., Detroit, MI, USA) for determination of total bacteria content, followed by addition to a 1 mL dilution solution in 9 mL thioglycollate medium (Difco Co.) to confirm the sterilization. Yeast and mold contents were determined using potato dextrose agar (PDA, Difco Co.) with a diluted solution (100 μL). PCA plates and thioglycollate medium tubes inoculated with samples were incubated at 35°C aerobically for 48 h and seven days, and PDA inoculated with samples was incubated at 25°C for up to two days. The colonies in all petri dishes were counted, revealing 30-300 colonies in each dish.

Measurement of TBARS

In order to measure lipid oxidation of Ganjang-gejang, 2-Thiobarbituric acid (TBARS) measured as described by Ahn et al. (1999) to determine the lipid oxidation of the samples. Five gram of crap meat was homogenized in a 50 mL centrifuge tube with 50 μL of butylated hydroxyanisol (BHA; 7.2% in ethanol) and 15 mL of distilled water, using a homogenizer (D-91126, Heidolph Instruments, Schwabach, Germany). One-milliliter aliquots of the homogenates were mixed with 3 mL of 2-thiobarbiutric acid (20 mM TBA in 15% trichloroacetic acid), heated in boiling water (100°C) for 20 min, and cooled in ice water for 5 min. The cooled mixture was centrifuged for 10 min at 2,500 *g*, using a UNION 5 KR centrifuge (Hanil Science Industrial, Co., Ltd., Incheon, Korean). The absorbance of the supernatant was measured at 532 nm using a UV 1600 PC spectrophotometer (Shimadzu, Nagoya, Japan), and it was reported as mg of malondialdehyde per kg.

Sensory evaluation

The sensory properties of *Ganjang-gejang* were evaluated using a descriptive 7-point scale (where 1 = extremely dislike or weak, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, and 7 = extremely like or strong) with 10 taste panelists. Sensory evaluation of the samples was conducted by 10 panels (25 – 40 ages, 8 males and 2 females). A trained ten-member panel consisting of researchers from the department of Advanced Radiation Technology Institute at Korea Atomic Energy Research Institute in Korea evaluated the irradiated Ganjang-gejang. An orientation session was conducted before their participation. To research the consumer perception, the appearance, flavor, taste, texture, and overall acceptance were evaluated. In addition, saltiness, fishy, flavor, and off-flavor were tested to confirm the intensity of sensorial characteristics. Water was provided to the panelists for rinsing the mouth between sample testing.

Flavor pattern analysis

The samples were frozen and stored in the deep freezer at –70°C until electronic nose analysis was performed. Flavor pattern analysis was performed using a fast GC electronic nose (zNose 7100, Electronic Sensor Technology, Newbury Park, CA, USA) with surface acoustic wave (SAW) sensor (Electronic Sensor Technology). A 10 g aliquot of the *Ganjang-gejang* was placed into a 40 mL septa-sealed screw vial. The samples were kept at 20°C for 1 h to analyze flavor pattern. The electronic nose was set to temperature conditions of 30, 60, 120, 150, and 220°C for the SAW sensor, column, valve, inlet, and trap, respectively. The taste results of the flavor pattern with *Ganjang-gejang* were analyzed by using VaporPrint program (Microsense 4.88, Electronic Sensor Technology).

Statistical analysis

The samples were analyzed in triplicate, and all results were expressed as the mean ± standard deviation (SD). The data for TBARS and sensory evaluation were analyzed using general linear models in SAS system version 9.2 software (SAS Institute Inc., Cary, NC, USA). Differences between means were considered significant when $P \leq 0.05$

Results and Discussion

Microbiological evaluation

To evaluate the effects of gamma irradiation on the total amount of aerobic bacteria, the D_{10} value, and sterilization of *Ganjan-Gejang*, *Ganjang-gejang* was gamma irradiated at various dose (Table 1). The total amounts of aerobic bacteria and fungi in untreated control samples were 6.2 and 4.1 log CFU/g, respectively, while no detectable bacteria or fungi were found following gamma irradiation. The D_{10} value of *Ganjang-gejang* by gamma irradiation was 0.51 kGy for the total amount of aerobic bacteria, and 1.07 kGy for the total amount of fungi. In addition, *Ganjang-gejang* was sterilized when it was irradiated at 9, 12, and 15 kGy. Irradiation was considered effective in inhibiting the growth of microorganisms in *Ganjang-gejang*. With increasing fermentation times, fermented seafood contains increased numbers of microorganism, including *Bacillus* sp. and *Staphylococcus saprophyticus*, which can cause significant health problems (Achi et al., 2007). In addition, fermented seafood in Korea, such as *Jeotgal*, has been shown to be highly contaminated with aerobic bacteria at approximately 7 log CFU/g (Park et al., 2002; Jo et al., 2004). For this reason, a number of studies on seafood have researched the sterilization effects of gamma irradiation (Jung et al., 2009; Kanatt et al., 2006; Jo et al., 2004; Kim et al., 2012). In addition, gamma irradiation is effective in controlling food-borne pathogenic microorganisms, such as *Listeria monocytogenes*, *S. aureus*, and V*ibrio parahaemolyticus*, in fermented and seasoned squid (Song et al., 2010). Our result was consistent with these previous studies and indicated that irradiated (9 kGy) *Ganjang-gejang* can be considered microbiologically safety for consumption immuno-compromised patients.

Sensory evaluation

The sensory characteristics of the *Ganjang-gejang* samples are shown in Table 2. The control samples showed the highest scores among all the samples. The appearance, taste, texture, and overall acceptance scores of the samples significantly decreased following irradiation in comparison with control samples. However, there was no significant difference in saltiness, fishy flavor, flavor, and off-flavor between the non-

Table 1. Effect of gamma irradiation on total aerobic bacteria, yeast, molds, and sterilization *Ganjang-gejang*

Irradiation dose (kGy)	Microbes (log CFU/g)		Sterilization
	Total aerobic bacteria	Yeast and molds	
0	6.2 ± 0.2*	4.1 ± 0.4	+†
3	4.8 ± 0.6	3.7 ± 0.2	+
6	3.5 ± 0.2	2.4 ± 0.3	+
9	ND	ND	-‡
12	ND	ND	-
15	ND	ND	-
D_{10} value (kGy)	0.51	1.07	

Values are mean ± standard deviation (n=5).
*Not detected within the detection limit < 2 log CFU/g.
†Bacterial growth was detected (positive).
‡Bacterial growth was not detected (negative).

irradiated and irradiated samples. While *Ganjang-gejang* was considered adequately sterilized when it was irradiated at 9 kGy (Table 1), irradiation doses over 9 kGy induced a taste off-flavor and deteriorated sensory qualities of *Ganjang-gejang*. The deterioration of flavor and taste is related mainly to lipid oxidation, but can also be attribute to amino acids and/or carbohydrate breakdown (Diehl. 1981; Murano, 1995). We also found that the intensities of fishy flavor and off-flavor were increased with rising doses by gamma irradiation. Overall acceptance of sterilized *Ganjang-gejang* (9 kGy gamma irradiation) scored 4.0 (acceptable) in the 7 point scale. However, gamma irradiation-sterilized *Ganjang-gejang* had lower sensory qualities than non-irradiated samples. Therefore, it is necessary to improve sensory qualities of gamma irradiation-sterilized *Ganjang-gejang*.

Table 2. Effect of gamma irradiation on sensory properties for *Ganjang-gejang*

Attributes	Irradiation dose (kGy)					
	0	3	6	9	12	15
Appearance	6.0 ± 0.89^{a}	5.5 ± 0.83^{ab}	5.3 ± 1.21^{abc}	4.5 ± 1.76^{abc}	3.8 ± 1.83^{bc}	3.5 ± 1.97^{c}
Flavor	$5.5 \pm 0.54^{NS*}$	5.1 ± 0.75	5.0 ± 1.41	4.6 ± 1.36	4.5 ± 1.37	3.8 ± 1.72
Taste	6.1 ± 0.75^{a}	5.0 ± 1.09^{b}	4.6 ± 1.36^{b}	4.0 ± 0.89^{bc}	3.5 ± 0.54^{c}	3.0 ± 0.00^{c}
Texture	6.0 ± 0.63^{a}	5.5 ± 0.54^{a}	4.8 ± 0.98^{ab}	4.1 ± 0.98^{bc}	3.6 ± 1.21^{bc}	3.3 ± 1.36^{c}
Overall acceptance	6.3 ± 0.51^{a}	5.3 ± 0.51^{b}	4.6 ± 0.81^{bc}	4.0 ± 0.89^{cd}	3.3 ± 1.03^{de}	2.8 ± 0.98^{e}
Saltiness	4.1 ± 1.47^{NS}	4.0 ± 1.41	3.8 ± 0.98	3.5 ± 0.54	3.3 ± 1.21	3.0 ± 1.54
Fishy	3.3 ± 0.81^{NS}	3.3 ± 0.81	3.6 ± 0.81	4.1 ± 0.98	4.5 ± 1.37	4.3 ± 1.63
Off-flavor	2.0 ± 2.00^{NS}	2.3 ± 1.50	2.6 ± 0.81	3.0 ± 0.89	3.3 ± 1.21	3.8 ± 1.32

Values are mean ± standard deviation (n=10).
*NS, Non-significantly.
[a-e]Mean values within a row follow by the different letter are significantly different ($P < 0.05$).

Table 3. Effect of gamma-irradiated 9 kGy on total aerobic bacteria, yeast, molds, sterilization, and TBARS for *Ganjang-gejang* in different temperature

Treatments		Microbes (log CFU/g)[1]		Sterilization	TBARS (mg malondialdehyde/kg)
		Total aerobic bacteria	Yeast and molds		
Non-irradiated		6.2 ± 0.07	4.1 ± 0.04	+‡	0.84 ± 0.05^{d}
Irradiated	Room temperature	ND†	ND	-§	5.22 ± 0.04^{a}
	In ice	ND	ND	-	1.46 ± 0.07^{b}
	In dry ice	ND	ND	-	1.09 ± 0.01^{c}

Values are mean ± standard deviation (n=5).
*Irradiated 9 kGy for sterilization by gamma ray.
†Not detected within the detection limit < 2 log CFU/g.
‡Bacterial growth was detected (positive).
§Bacterial growth was not detected (negative).
[a-d]Mean values within a column follow by the different letter are significantly different ($P < 0.05$).

Table 4. Effect of 9 kGy gamma-irradiated on relative sensory properties for *Ganjang-gejang* in different temperature

Attributes	Non-irradiated	Irradiated		
		Room temperature	In ice	In dry ice
Appearance	$7.0 \pm 0.01^{*a}$	6.3 ± 0.50^{b}	6.3 ± 0.50^{b}	6.3 ± 0.50^{b}
Flavor	7.0 ± 0.01^{a}	4.3 ± 0.50^{c}	5.0 ± 0.01^{b}	5.0 ± 0.01^{b}
Taste	7.0 ± 0.01^{a}	4.0 ± 0.01^{c}	5.0 ± 0.01^{b}	5.3 ± 0.50^{b}
Texture	7.0 ± 0.01^{a}	4.5 ± 0.58^{b}	5.3 ± 0.96^{b}	5.5 ± 0.58^{b}
Overall acceptance	7.0 ± 0.01^{a}	4.3 ± 0.50^{d}	5.0 ± 0.01^{c}	5.8 ± 0.50^{b}
Saltiness	1.0 ± 0.01^{c}	2.5 ± 0.58^{a}	2.0 ± 0.01^{ab}	1.5 ± 0.58^{bc}
Fishy	1.0 ± 0.01^{c}	3.0 ± 0.01^{a}	2.3 ± 0.50^{ab}	1.8 ± 0.96^{bc}
Off-flavor	1.0 ± 0.01^{b}	2.5 ± 0.58^{a}	1.5 ± 0.58^{a}	1.5 ± 0.58^{b}

*Values are mean ± standard deviation (n=10).
[a-c]Mean values within a row follow by the different letter are significantly different ($P < 0.05$).

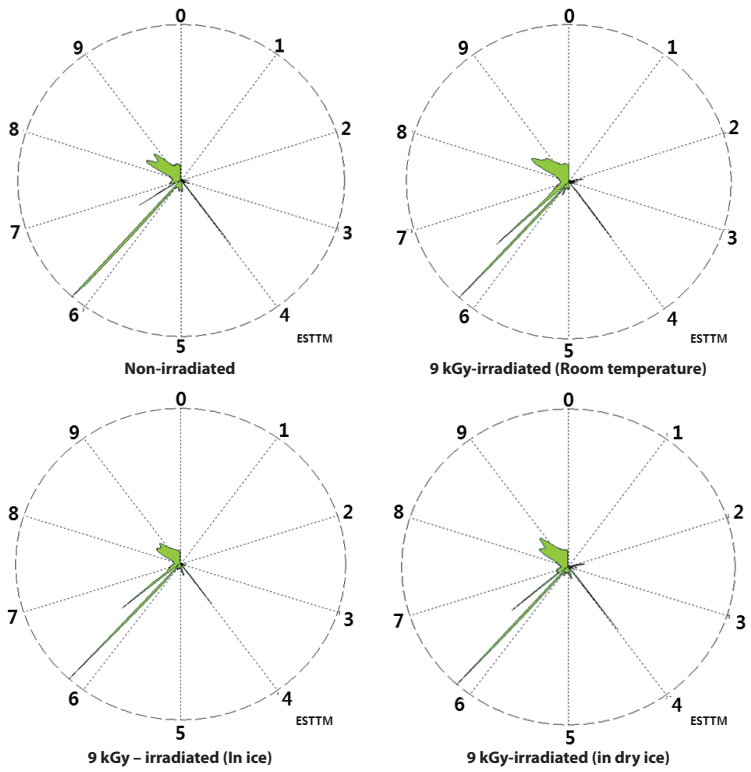

Fig. 1. Polar graphs of frequency for gamma-irradiated *Ganjang-gejang* in different temperature.

Microbiological and sensory evaluation of gamma-irradiated Ganjang-gejang in different irradiating temperature

Table 3 and 4 show the microorganism, lipid oxidation (TBARS) and sensory properties of *Ganjang-gejang* irradiated under different temperature conditions. The TBARS values of all samples were increased by gamma irradiation (Table 3). However, the dry ice sample had a low TBARS value compared with ice and room temperature samples, indicating that lipid oxidation could be inhibited through low temperature conditions. As supported by our data, lipid oxidation is generally increased during irradiation process, and different physicochemical treatments can also retard irradiation-dependent lipid oxidation. In our result of sensory evaluation, non-irradiated *Ganjang-gejang* scored 7.0 on a 7.0 point scale. With a decreasing in irradiation temperature, all of the preferred attributes increased with irradiated *Ganjang-gejang*. In particular, the overall acceptance of samples irradiated in dry ice had similar scores as non-irradiated samples at 5.8 (in dry ice) and 7.0 point (control). In addition, the intensities of saltiness, fishy flavor, and off-flavor were reduced among lower temperature samples. However, the saltiness, fishy flavor, and off-flavor score of the samples no significantly following irradiation (in dry ice) in comparison with control samples. The temperature during irradiation is important because the initial ionization, excitation event, and reactions of the active species are dependent on the temperature (Swallow, 1997). In particular, the free radical, which is a major factor of chemical change, have a limited mobility in a frozen state and the production of more free radicals and radiolysis products is inhibited during gamma irradiation under a frozen state (Furuta et al., 1992; Raffi and Agnel, 1983; Shultz et al., 1977). Thus, our results indicated that the irradiation of *Ganjang-gejang* under frozen conditions is able to control the growth of microorganisms and maintain sensory properties.

Flavor patterns of gamma-irradiated *Ganjang-gejang* at different irradiating temperature

Fig. 1 show the polar graphs of frequency of *Ganjang-gejang* irradiated under different temperature conditions. Z-nose analysis based on SAW sensor has advantage of a rapid analysis and a simple sample preprocessing, although the measured data are greatly dependent on the retention time (Cho and Noh, 2002). To detect and evaluate changes in flavor patterns of various foods from irradiation, most studies have used a metal oxide sensor (MOS), which respond to specific substances, and a conducting polymer sensor (Cho and Noh, 2002; Kim and Noh, 1999). According to Han et al. (2009), an electronic nose equipped with a SAW sensor can measure the flavor patterns of gamma-irradiated raw oyster rapidly. In this

study, we investigated the effects of different temperature conditions on flavor patterns of irradiated *Ganjang-gejang*. Different flavor patterns were found in irradiated samples (room temperature) compared to the control. However, for samples irradiated at low temperature (in ice or in dry ice), flavor patterns were similar to those obtained for the control, indication that the odor of samples subjected to gamma irradiation on ice or dry ice was more acceptable in terms of sensory evaluation than sample subjected to gamma irradiation at room temperature (Table 3 and 4). Flavor pattern analysis using an electronic nose with a SAW (surface acoustic wave) sensor determined that the main peak at retention time 6.4 sec was related with flavor induced by irradiated sample (room temperature). According to Han *et al*., the irradiated oyster flavors were influenced by the both of lipid oxidation and amino acid breakdown. Thus, different flavor patterns were found in irradiated samples (room temperature) compared to the control.

Ganjang-gejang is a popular Korean fermented foods, made of raw crab and soy sauce. However, many cases of food poisoning due to consumption of *Ganjang-gejang* have been recently reported, and hygienic measures for fermented foods are needed. This study was conducted to evaluate the microbiological and sensory qualities of gamma-irradiated *Ganjang-gejang* and to improve its sensory qualities by adjusting the temperature during sample irradiation. The results of this study indicated that treatment with irradiation under low temperatures may help to prepare higher quality *Ganjang-gejang*.

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