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ARTICLE

Development of Ready-to-Eat *Bulgogi* Sauce with No Change of Sensory Properties after High-Dose Irradiation

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Abstract

Viscosity is critically important for *bulgogi* sauce. However, exposure to irradiation may decrease the rheological properties of *bulgogi* sauce. This study was conducted to compare the effects of gamma irradiation (0–9 kGy) on viscosity, molecular weight, pH, reducing end level, and water solubility of xanthan gum, guar gum, and locust bean gum as thickening agents/stabilizers in *bulgogi* sauce. The physicochemical properties of all samples changed ($p<0.05$) from 3965 to 0 cP (viscosity), from 6048 to 28 kDa (molecular weight), from 5.79 to 4.62 (pH), from 0.13 mg/mL to 1.72 mg/mL (reducing end level), and from 6% to 87% (water solubility) following gamma irradiation. Viscosity after irradiation was most stable ($p<0.05$) in xanthan gum (from 1249 to 92 cP) compared with guar gum (from 3965 to 0 cP) and locust bean gum (from 1631 to 0 cP). The sensory properties (texture and taste) of *bulgogi* sauce prepared with xanthan gum (1%, w/w) were highly maintained (about 7.0–5.0) after high-dose irradiation of up to 40 kGy. These results indicate that xanthan gum can be effectively used as a thickening agent/stabilizer in *bulgogi* sauce, which had low viscosity after irradiation.

Key words: *bulgogi* sauce, irradiation, viscosity, thickening agent, sensory property

Introduction

Beef *bulgogi* is a Korean traditional meat product. Thin slices of beef, usually using sirloin or tenderloin, are marinated in sauce composed of soy sauce, onion, garlic, sesame, and other seasonings, and cooked over a hot charcoal grill or fry-pan before consumption. However, the *Bacillus* spp. of fermented soy sauce, which is major components of *bulgogi* sauce, was the range of 5 Log CFU/g initially (Song *et al*., 2001). Therefore, the microbiological control is needed to preserve the quality of *bulgogi* sauce during long storage periods at chilled or severe temperature (2-8°C or 25-35°C).

Chilled storage (2-8°C) is generally used to maintain the quality of *bulgogi* sauce, little studies have been conducted to extend the shelf-life of *bulgogi* sauce by using low-dose irradiation of below 10 kGy (Jo *et al*., 2003; Lee *et al*., 2001). However, in previous results, the *bulgogi* sauce was irradiated under condition of raw material mixture (soy sauce, garlic, onion, green onion, sugar, etc.). The total bacteria in *bulgogi* sauce treated with gamma irradiation up to 10 kGy were not completely removed and injured cells were recovered during storage at 20°C (Lee *et al*., 2001). Some commercial *bulgogi* sauce is produced after high heat treatment in Korea in order to meet the consumers' hygienic quality standard. However, the heat treatment may adversely affect on flavor of the sauce because of losing freshness of such vegetables and fruits (Jo *et al*., 2003).

Meanwhile, the viscosity is critically important for colloidal foods such as sauce, syrup, ice cream and confectionery. However, exposure to irradiation may produce certain changes in the rheological property of colloidal foods (Aliste *et al*., 2000; Dogan *et al*., 2007; Delincee, 2002; Kayacier and Dogan, 2006). Park *et al*. (2010) also reported that the sterilizing treatments such as high-dose irradiation and autoclave on *bulgogi* induced the viscosity decrease of sauce.

Hydrocolloids are polymers or polysaccharides inter-

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acting strongly with water. Xanthan gum, guar gum and locust bean gum are most frequently used hydrocolloids as food additives. In the food industry, they are primarily used for thickening, gelling and stabilizing purposes. However, hydrocolloids are also degraded by ionizing radiation as a result of the free-radical-induced scission of the glycosidic bonds in the polysaccharide chain, and this process is accompanied by other less-specific chemical changes (Grunewald, 1983; Urbain, 1986). Irradiation has an important influence on the viscosity of polysaccharides such as xanthan gum (El-Griany, 2005), guar gum (Dogan *et al*., 2007; Gupta *et al*., 2009; Jumel *et al*., 1996; Sen *et al*., 2007) and locust bean gum (King and Gray, 1993; Sen *et al*., 2007).

Meanwhile, the radiation sensitivities of xanthan gum, guar gum and locust bean gum may be different because of their structural differences. Briefly, it was supposed that rheological property of xanthan gum is the most stable in comparison with guar gum and locust bean gum after irradiation. Generally, xanthan gum has more complex molecular structure than that of guar gum and locust bean gum. It is a water soluble polysaccharide gum having a molecular weight of greater than one million produced by the bacterium *Xanthomonas campestris* and is composed of D-glucosyl, D-mannosyl and D-glucosyluronic acid. Guar gum has a molecular weight of about 220 kDa and is called guaran which consists of linear chains of (1→4)-β-D-mannopyranosyl units with α-D-galactopyranosyl units attached by (1→6) linkages. Locust bean gum has a molecular weight of about 310 kDa.

Therefore, in this study, the gum maintaining stable rheology after irradiation was chosen. In addition, the effects of high-dose irradiation on the sensory properties (color, texture, taste, flavor and overall acceptance) of *bulgogi* sauce containing xanthan gum (1%, w/w) were evaluated during storage at 35°C for 90 days.

Materials and Methods

Preparation of gum solutions (1%, w/w)

Xanthan gum, guar gum and locust bean gum were obtained from a company manufacturing food additives (MSC Co., Korea). Each sample was prepared in 1000 mL water, and vigorously agitated for 30 min at 80°C in a water bath to completely dissolve the gums. Finally, gum solutions (1%, w/w) were prepared, cooled to room temperature. The gum solutions were individually placed into a 50 mL falcon tube (diameter: 30 mm, height: 115 mm).

Gamma irradiation of gum solutions

Gamma irradiation of samples in 50 mL falcon tube was carried out using a cobalt-60 irradiator (point source AECL, IR-79, MDS Nordion International Co. Ltd., Canada) in Korea Atomic Energy Research Institute (Korea). The doses applied in this study were 0, 3, 6 and 9 kGy at room temperature. The source strength was approximately 300 kCi with a dose rate of 10 kGy/h. Dosimetry was carried out using 5 mm diameter alanine dosimeters (Bruker Instruments, Germany). The dosimeters were calibrated against an international standard set by the International Atomic Energy Agency (Austria).

Preparation of *bulgogi* sauce containing xanthan gum (1%, w/w)

The materials (soy sauce, 16%; onion, 8%; green onion, 3%; garlic, 3%; sesame, 1%) of *bulgogi* sauce were put into a pot and boiled at 100°C for 20 min. At this time a pre-made xanthan gum solation (mixture of sugar (8%), xanthan gum (1%) and water (60%)) was added to boiling sauce, and continuously boiled at 100°C for 10 min with gentle agitation. The prepared sauce was cooled to room temperature. The sauce was placed into an aluminium-laminated low-density polyethylene (Al-LDPE, Sunkyung Co., Ltd., Korea), packaged under aerobic condition using an packaging machine (Leepack, Hanguk Electronic, Korea).

Gamma irradiation of *bulgogi* sauce

The sauce samples packaged with Al-LDPE were irradiated in a cobalt-60 gamma irradiator (point source AECL, IR-79, MDS Nordion International Co. Ltd., Canada) for obtaining the absorbed dose of 0, 10, 20 and 40 kGy at room temperature. All samples were stored at 35°C and used in subsequent experiments during storage for 90 days.

Viscosity (cP)

The viscosity of the samples was measured at room temperature (25±1°C) using a Brookfield viscometer (DV-II + pro, Brookfield Engineering Laboratories, MA, USA) equipped with the S21 spindle at 5 rpm.

Molecular weight (kDa) and pH

Gel permeation chromatography (GPC) was used for measurement of molecular weight in sample solution: a separation module (Waters 2690, Waters Co., MA), a refractive index detector (RI, Waters 2410, Waters, Co.), Empower software (System Software, Empower option

GPC, Waters Co.), and PL aquagel-OH-60, -40, and -30 columns (300 × 7.5 mm, 8 μm, Polymer laboratories Ltd., UK). The mobile phase was 0.1 M sodium nitrate at flow rate of 1 mL/min, and the analyses were performed at 40°C. The injection volume was 200 μL, and calibration was carried out using various standard pullulan (Showa Denko K.K., Japan) at a concentration of 0.1% (w/w).

The pH of gum solution was measured with a pH meter (Orion 520A, Boston, USA).

Reducing end level (mg/mL)

Reducing end level was determined by the 3,5-dinitrosalicylic acid (DNSA) method (Miller, 1959). One milliliter of the gum solution (1 mg/mL) was transferred into 15-mL glass tubes, and 2 mL of the modified DNSA reagent (0.5 g of dinitrosalicylic acid (Sigma-Aldrich Co., USA), 8 g of sodium hydrate (Sigma-Aldrich Co., USA), and 150 g of Rochell salt (Sigma-Aldrich Co., USA) in 500 mL of distilled water) was added to the tube. The mixture was stirred for 5 sec and boiled at 90°C for 10 min, and then cooled in ice. Absorbance of samples was measured at 550 nm by a spectrophotometer (UV-1601 PC, Shimadze CO., Japan), and the absorbance were converted to levels of reducing end of gum solutions using a standard curve prepared with different concentrations (0.078, 0.156, 0.313, 0.625, 1.25, 2.5, and 5 mg/mL) of glucose

Water solubility (%)

Water solubility of each lyophilized gum sample was evaluated by modified method of Dakia *et al*. (2008). Sample powders (0.5 g) were placed into a 50-mL glass tube, stirred with 10-mL deionized water for 20 min, and centrifuged at 6000 × *g* for 20 min at 4°C. The supernatant was then dried at 100°C for 1 hr in oven (MOV-112F, Sanyo Co., Japan), the weight of the dried products was determined. The solubility was calculated as follow:

$$\%\ \text{Solubility} = \frac{\text{Weight of dried supernatant}}{\text{Weight of initial sample powder}} \times 100$$

Sensory evaluation of *bulgogi* sauce

Sensory evaluation was conducted by 21 panelists. Panelists were trained in three 1-h sessions in which *bulgogi* sauce were served to the panelists from a wide variety of treatments to familiarize them with a wide range of color, texture, taste, flavor and overall acceptance of *bulgogi* sauce. Sensory scores of *bulgogi* sauce were referred to using a 7 point descriptive scale where 1 = extremely dislike to 7 = extremely like.

After irradiation treatment, *bulgogi* sauce was removed from pouch, served randomly to each panelist for 15 min. Water and unsalted crackers were provided, and panelists were asked to expectorate and rinse their mouths between each sample. Results were expressed as the predominant score given by panelists.

Statistical analysis

Samples were analyzed in triplicate. All data were analyzed by the general linear model procedures of the SAS® 9.2 (SAS Institute, USA). Tukey's multiple range tests were used to compare least squared means among treatments at α = 0.05.

Results and Discussion

Viscosity, molecular weight and pH

The viscosities of xanthan gum, guar gum and locust bean gum solutions were decreased ($p<0.05$) by gamma irradiation, especially the decrement of guar gum and locust bean gum solutions was greater ($p<0.05$) than that of xanthan gum solution by irradiation at more than 3 kGy (Table 1). Generally, the gamma irradiation decreased the values of density, intrinsic viscosities and molar mass in the aqueous salt solutions of xanthan gum (El-Griany, 2005), guar gum (Dogan *et al*., 2007; Gupta *et al*., 2009; Jumel *et al*., 1996; King and Gray, 1993; Sen *et al*., 2007) and locust bean gum (King and Gray, 1993; Sen *et al*., 2007). Even though the viscosity of xanthan gum, guar

Table 1. Values (mean±SD) of viscosity (cP), molecular weight (kDa) and pH of gamma-irradiated gum solutions at 0, 3, 6, and 9 kGy

	kGy	Xanthan gum	Guar gum	Locust bean gum
Viscosity	0	1248±91^{aC}	3965±174^{aA}	1631±118^{aB}
	3	927±62^{bA}	0±0^{bB}	0±0^{bB}
	6	381±29^{cA}	0±0^{bB}	0±0^{bB}
	9	92±8^{dA}	0±0^{bB}	0±0^{bB}
Molecular weight	0	6048±412^{aA}	4897±209^{aB}	3513±148^{aC}
	3	5317±194^{bA}	96±5^{bB}	83±4^{bB}
	6	1481±76^{cA}	49±3^{bB}	42±3^{bB}
	9	575±23^{dA}	31±2^{bB}	28±1^{bB}
pH	0	4.69±0.03^{aC}	5.79±0.04^{aA}	5.68±0.03^{aB}
	3	4.65±0.02^{aB}	5.31±0.04^{bA}	5.27±0.05^{bA}
	6	4.67±0.02^{aB}	5.12±0.03^{cA}	5.08±0.03^{cA}
	9	4.62±0.04^{aB}	5.02±0.04^{dA}	5.01±0.04^{cA}

$^{a-d}$Means within the same column with different letters were significantly different ($p<0.05$).
$^{A-C}$Means within the same row with different letters were significantly different ($p<0.05$).

gum and locust bean gum solutions was also decreased by irradiation, the viscosity of xanthan gum solution was the highest among irradiated sample solutions.

In the non-irradiated sample solutions, the molecular weights of xanthan gum, guar gum and locust bean gum in each solution have been found to be 6048, 4879 and 3513 kDa, respectively (Table 1). Meanwhile, the considerable decrease of the molecular weight is observed (p<0.05) in the gamma-irradiated samples. The molecular weights of the each sample solution irradiated at 3, 6 and 9 kGy were 5317, 1481 and 575 kDa in xanthan gum solution, 96, 49 and 31 kDa in guar gum solution, and 83, 42 and 28 kDa in locust bean gum solution, respectively. The changes that occur after irradiation might be due to the breakage of the glycosidic bond of the polysaccharide (Sokhey and Hanna, 1993). In this result, the molecular weight of xanthan gum solution was the highest in all samples after gamma irradiation up to 9 kGy.

Gamma irradiation significantly decreased (p<0.05) pH of guar gum and locust bean gum solutions, the change of pH in xanthan gum solution was not significant after irradiation (Table 1). Raffi *et al*. (1981), and Sokhey and Chinnaswamy (1993) reported that irradiation dosage and level of acidity had shown inter-relationship because irradiation produced COOH groups in polysaccharides. Increases in acidity of irradiated starches could have been due to breakdown of starch molecules which perhaps induced COOH formation (Sokhey and Chinnaswamy, 1993). A breakdown of glycosidic linkages due to the action of free radicals is perhaps the cause for an increase in starch acidity (Radley, 1960). In this result, decreased pH of irradiated guar gum and locust bean gum solutions could be due to a breakdown of guar gum and locust bean gum molecules, and it might be due to the formation of carboxylic groups (COOH). Kim *et al*., (2008) also indicated that gamma irradiation decreased molecular weight size, viscosity and pH of the hyaluronic acid. Meanwhile, the reason of no differences of pH in irradiated xanthan gum solutions is supposed that initial pH value (about 4.6) of xanthan gum solution is low compared with guar gum (5.7) and locust bean gum (5.7). Therefore, the change of pH in xanthan gum was not observed after irradiation.

Reducing end level and water solubility of gum solutions

The reducing end level of gum solutions was increased (p<0.05) by irradiation (Table 2). The increase rates of reducing end level after irradiation at 0, 3, 6, and 9 kGy were ranked as follows: locust bean gum solution (0.15, 0.84, 1.36, and 1.72 mg/mL) ≥ guar gum solution (0.13, 0.76, 1.24, and 1.61 mg/mL) > xanthan gum (0.13, 0.33, 0.70, and 1.26 mg/mL) with significant differences of p<0.05. It was also reported that the reducing end level of wheat starch was increased depending on irradiation dose (Ananthaswamy *et al*., 1970; Raffi *et al*., 1981). Irradiation may induce scission of the glycosidic linkage by the generation of a radical (Molins, 2001). For this reason, reducing end level of xanthan gum, guar gum and locust bean gum solutions may be increased.

Table 2. Values (mean±SD) of reducing end level (mg/mL) and water solubility (%) of gamma-irradiated gum solutions at 0, 3, 6, and 9 kGy

	kGy	Xanthan gum	Guar gum	Locust bean gum
Reducing end level	0	0.13±0.02^{dA}	0.13±0.01^{dA}	0.15±0.03^{dA}
	3	0.33±0.01^{cB}	0.76±0.05^{cA}	0.84±0.05^{cA}
	6	0.70±0.04^{bC}	1.24±0.03^{bB}	1.36±0.06^{bA}
	9	1.26±0.05^{aB}	1.61±0.07^{aA}	1.72±0.08^{aA}
Water solubility	0	6±2^{cC}	20±3^{bB}	32±4^{bA}
	3	34±4^{bB}	78±6^{aA}	82±7^{aA}
	6	48±5^{aB}	82±8^{aA}	83±5^{aA}
	9	54±3^{aB}	85±6^{aA}	87±8^{aA}

[a-d]Means within the same column with different letters were significantly different (p<0.05).
[A-C]Means within the same row with different letters were significantly different (p<0.05).

The water solubilities of lyophilized sample powders were increases (p<0.05) by gamma irradiation at 0, 3, 6, and 9 kGy (Table 2). Especially, the increase rates of guar gum and locust bean gum were faster than that of xanthan gum. This result indicates that free radicals were generated by irradiation brought about fragmentation of gum molecules, and xanthan gum had the stable rheological property and durability in comparison to guar gum and locust bean gum. MacArthur and D'Appolonia (1984) also indicated that the breakdown and depolymerization of polysaccharide by radiolysis with high energy induce the production of the smaller fragments and the low molecular fraction to be dissolved in water more easily.

Sensory properties

On day 0, the sensory quality of sauce was decreased by irradiation (Table 3). However, the color, texture, taste and flavor of sample irradiated at 40 kGy were 5.2, 5.4, 5.5 and 5.2, respectively, and generally maintain the acceptable quality during storage. The color of sauce was also changed darkly depending on irradiation dose during

Table 3. Sensory properties of *bulgogi* sauce containing xanthan gum 1% (w/w) treated with gamma ray during storage at 35°C

Days	Dose (kGy)	Color	Texture	Taste	Flavor	Overall acceptance
0	0	6.9±0.5[a]	6.8±0.6[a]	6.9±0.8[a]	6.8±0.5[a]	6.9±0.7[a]
	10	6.4±0.4[a]	6.3±0.7[a]	6.3±0.5[a]	6.1±0.4[a]	6.4±0.6[a]
	20	5.9±0.5[ab]	5.9±0.4[ab]	5.8±0.5[ab]	5.9±0.7[a]	6.1±0.8[a]
	40	5.2±0.6[b]	5.4±0.6[b]	5.5±0.3[b]	5.2±0.5[b]	5.3±0.4[b]
90	10	6.1±0.5[a]	6.2±0.6[a]	5.9±0.4[a]	5.6±0.6[ab]	5.8±0.5[ab]
	20	6.5±0.4[a]	5.7±0.3[b]	5.2±0.4[bc]	5.1±0.5[b]	5.5±0.3[b]
	40	6.7±0.5[a]	5.2±0.5[b]	4.8±0.3[c]	4.9±0.5[b]	4.8±0.5[b]

[a-c]Means within the same column with different letters were significantly different ($p<0.05$).

storage periods. The flavor acceptability of each irradiated sample was generally high because the generations of irradiation off-flavor were not observed. Jo *et al*. (2003) and Lee *et al*. (2001) reported that the sensory qualities (odor, taste and color) of *bulgogi* sauce irradiated up to 10 kGy were excellently maintained. However, this result indicated that the sauce irradiated up to 40 kGy was able to maintain high qualities (color, texture, taste and flavor) during storage periods.

It was confirmed that xanthan gum had stable rheology compared with guar gum and locust bean gum after radiation treatment. The sensory properties (texture and taste) of *bulgogi* sauce contained xanthan gum (1%, w/v) could be excellently maintained after irradiation up to 40 kGy.

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