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Effects of Various Physicochemical Treatments on Volatiles and Sensory Characteristics of Irradiated Beef *Bulgogi*

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Abstract

Off-flavor and lipid oxidation are possible defects of irradiated bulgogi. This study compared the effects of several physico-chemical treatments on microbial safety, volatiles, lipid oxidation, and sensory properties of irradiated beef bulgogi. Samples were separately irradiated with 20 kGy after each treatment such as packaging (aerobic and vacuum), antioxidants (vitamin C + α -tocopherol (0.0 and 1.0%, w/w)), charcoal teabags (0 and 0.5%), or different temperatures (room temperature, -20, and -70°C). No bacterial growth was observed (p<0.05) after irradiation of more than 20 kGy during storage at 35°C. Volatiles created by irradiating bulgogi were toluene, heptane, and 1,3-bis(1,1-dimethylethyl)benzene. Irradiation off-flavor, lipid oxidation, and deterioration of sensory quality induced by irradiation were effectively reduced (p<0.05) by all physico-chemical treatments tested.

Key words: bulgogi, irradiation, volatiles, sensory properties, combination treatments

Introduction

Bulgogi is one of the most popular Korean traditional dishes, and it is gradually popular in international society acceptance. Bulgogi is prepared by marinating thin slices of beef in the sauce formulated with soy sauce, garlic, onion and other seasoning, and grilling them. Commercial bulgogi sauce and ready-to-cook/ready-to-eat bulgogi has been increased. However, potential bacterial contamination of the food by the fresh vegetables, soy sauce, and raw beef has been suggested (Jo et al., 2003). In addition Song et al. (2001) reported that high levels (10⁵ CFU/g) of Bacillus spp. were contaminated in soy sauce, which is a major component of bulgogi sauce.

Use of gamma irradiation on *bulgogi* sauce and *bulgogi* product kept more stable quality, and improved microbiological safety than heat treatment (Jo *et al.*, 2003; Lee *et al.*,

2001). The World Health Organization (WHO) reported that gamma irradiation is a useful technology to inactivate microorganisms related to foodborne disease and food spoilage (WHO, 1999). However, lipid oxidation and off-flavor of meat caused by irradiation make meat industry difficult to use the technology (Ahn *et al.*, 1999; Ahn *et al.*, 2000; Ahn *et al.*, 2001; Kwon *et al.*, 2008; Patterson and Stevenson, 1995). These adverse effects are initiated by the free radicals produced during irradiation, and sulfur volatiles and carbon monoxide are also produced by the reactions between meat components and radiolytic free radicals (Ahn, 2002; Nam and Ahn, 2002).

Brewer (2009), and Kadakal and Nas (2002) reported that the generation of lipid oxidation and off-flavor in irradiated meat and meat product could be reduced by various methods such as modified atmosphere packaging, addition of antioxidants, charcoal teabag and low irradiation temperature. Packaging methods turned out to be a major factor influencing levels of volatiles as well as types of volatiles in irradiated meat (Ahn *et al.*, 2002). Vacuum packaging was more effective than aerobic packaging in preventing lipid oxidation of meat during irradiation (Nam and Ahn, 2003). In addition, the antioxidants

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such as tocopherol, vitamin C, gallate and metal chelators (EDTA) can reduce lipid oxidation and off-flavor formation (Ahn and Nam, 2004; Chen and Ahn, 1998; Xiong et al., 1993). Many researchers also have investigated various absorbants such as activated carbon, silica, alumina, and zeolites to reduce off-flavor compounds (Chu et al., 2002; Diaz et al., 2004; Einaga and Futamura, 2005; Kadakal and Nas, 2002). Among these potential adsorbents, activated carbon is considered as one of the most effective adsorbents for various undesirable substances such as volatile and toxic compounds (Kadakal and Nas, 2002). The product temperature during irradiation is also the factor related to lipid oxidation of food because the initial ionization, excitation events and the reaction of the active oxygen species are dependent on the temperature (Swallow, 1997). Especially, the free radical, which is a major factor for a chemical change such as oxidation reaction, has a limited mobility in the frozen state.

However, until now, the studies to evaluate the effects of these treatments on reducing adverse effects in *bulgogi* during irradiation have not been conducted. Thus, objective of this study was to determine the effects of each physicochemical treatment such as packaging conditions (aerobic and vacuum), antioxidants (vitamin C + α - tocopherol (0.0 and 1.0%, w/w)), charcoal teabag (0 and 10 g), and different temperature (room temperature, -20 and -70°C) on microbial safety, volatiles, lipid oxidation, and sensory property of beef *bulgogi* during irradiation.

Materials and methods

Materials

Beef (tenderloin), soy sauce, sugar, onion, celery, green onion, garlic, ginger, black pepper, whole dried red pepper and charcoal teabag were purchased from local store. The tenderloin was obtained after 25 h of slaughter, and sliced into $2 \times 2 \times 0.8$ cm. Vitamin-C and α -tocopherol were obtained from a manufacturing food additive company (MSC Co., Korea).

Preparation of bulgogi sauce and bulgogi

Bulgogi sauce was prepared with soy sauce (295 g) and other spices (water: 295 g, sugar: 205 g, onion: 72 g, celery: 29g, green onion: 58 g, garlic: 29 g, ginger: 8 g, black pepper: 1 g, whole dried red pepper: 8 g). All ingredients were mixed in a pot and boiled at 100°C for 30 min, and sauce was then cooled to room temperature. Sliced tenderloin (2,000 g) was marinated in the sauce at 4°C for 2 h. To prepare bulgogi, the marinated beef was

placed on a preheated pan at 170°C, cooked at 78°C of internal temperature for 9 min, and then cooled for 20 min at room temperature.

Preparation of samples treated by each physicochemical condition

Each cooked *bulgogi* was treated by four treatment conditions, and the qualities of samples were evaluated separately by each physicochemical treatment after irradiation. i) Aerobic or vacuum (75 cmHg) package using an aluminium-laminated low-density polyethylene (Al-LDPE, Sunkyung Co. Ltd., Korea) by a packaging machine (Leepack, Hanguk Electronic, Korea). ii) Antioxidants (vitamin C + α -tocopherol (0.0 and 1.0%, w/w)) were added to bulgogi, and vacuum packaged. In our previous result, the optimal addition rates of antioxidants (vitamin C and α-tocopherol) on bulgogi were determined as vitamin C (0.5%, w/w) and α -tocopherol (0.5%, w/w), respectively (date not shown). iii) Bulgogi was vacuum-packaged with a charcoal teabag (0 and 0.5%), iv) Vacuum-packaged bulgogi was stood by different temperature (room temperature, -20 and -70°C) before gamma irradiation.

Gamma irradiation

The samples were irradiated at a cobalt-60 gamma irradiator (point source, AECL, IR-79, Canada) in the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute (Korea) by 0, 5, 10, 15, and 20 kGy. The source strength was approximately 300 kCi with a dose rate of 10 kGy/h and the actual doses were within 2% of the target dose. Dosimetry was performed with 5 mm-diameter alanine dosimeters (Bruker Instruments, Germany). Meanwhile, a temperature of each sample during irradiation was maintained by the method of Park *et al.* (2008). The frozen samples were placed in the container box filled with ice (-20°C) and dry ice (-70°C), respectively.

Total bacterial populations

The bacterial populations in samples were determined on 0, 7, 14, and 30 d. The 10 g portions of samples were aseptically placed in a sterile bag (10×15 cm; Sunkyung Co. Ltd., Korea) containing 90 mL of peptone water (0.1%) and pummeled in a stomacher (Model 400, Tekmar Co., USA) for 1 min. One mL of pummeled sample was appropriately diluted and the diluent was pour-plated using plate count agar (Difco Lab., USA). Plates were incubated at 37° C for 48 h, and colonies on plates were manually counted.

Volatile compounds

Volatile compounds of the samples were identified on 0 d. The samples (4 g) were weighed, placed into a 20 mL vial, and sealed with a silicon/PEFE septum. A SPME fiber (Supelco, USA) coated with carboxen/polydimethylsiloxane (Carbboxen/PDMS, 85 µm thickness) was used for adsorption of headspace volatiles. Before extracting volatiles, the fiber was cleaned at 250°C for 5 min in the gas chromatography (GC) injection port and used immediately to prevent possible contamination. Samples were preheated for 10 min at 40°C in a heating block, and the SPME fiber absorbed the headspace volatiles in a vial for an additional 50 min; the fiber was then injected into the GC and remained for 5 min for desorption. A Varian Star 3400 CX GC with Varian Saturn 2000 MS and HP-5 column (crosslinked 5% diphenyl and 95% dimethylpolysiloxane, 30 m \times 0.32 mm, 0.25 μ m film thickness) was used to quantify volatile compounds. The flow rate of carrier gas (helium) was adjusted to 1 mL/min and the temperature of the injection port was 250°C. The column was held at 40°C for 2 min and raised to 250°C at 3.5°C/ min. The temperatures of the ion source, the manifold and the transfer line into the mass spectrometer (MS) were 180, 50, and 180°C, respectively. The volatile compounds were identified by a mass spectrum database (WILLY Library (Registry of mass spectral data, 6th edition, USA)), and the total ion counts were presented.

2-Thiobarbituric acid (TBA) values

In order to measure lipid oxidation of samples, TBARS values of samples were measured on 0 d according to the method described by Ahn et al. (1999). The sample (5 g) 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 (DIAX 900, Heidolph Co., Ltd., Germany). The 1 mL portions of the homogenates were mixed with 3 mL of 2-thiobarbituric acid (20 mM TBA in 15% trichloroacetic acid), heated in boiling water (100°C) for 20 min, and followed by cooling in ice water for 5 min. The cooled mixture was centrifuged for 10 min at 2,500 g, using a centrifuge (UNION 5 KR, Hanil Science Industrial, Co., Ltd., Korea). The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (UV 1600 PC, Shimadzu, Japan), and it was reported as µg malondialdehyde/g.

Sensory evaluation

Sensory evaluation of samples was conducted by 21 panels who were trained according to the method described

by Ahn *et al.* (2000). Color, texture, taste, flavor, and overall acceptance of the samples were evaluated using a 7 point descriptive scale where 1 = extremely dislike or extremely weak to 7 = extremely like or extremely strong. After irradiation, samples were removed from pouches and reheated in a cooker (NU-VU ES-3 cooker, Menominee, USA) at 130°C for 10 min for sensory evaluation. The samples were cooled to 45-55°C at room temperature, and served to panels. Samples were served randomly to each panel for 15 min after the packages were opened.

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). Turkey's multiple range tests were used to compare least squared means among treatments at $\alpha=0.05$.

Results and Discussion

Doses to decrease below detection limit of total bacterial populations

The bacterial populations of the non-irradiated sample were about 2 log CFU/g, and the bacterial growths in the samples irradiated at more than 5 kGy were below detection limit (1 log CFU/g) on 0 d (Table 1). The bacterial populations of all samples increased (p<0.05) during storage at 35°C, especially the samples which had bacterial populations were below detection limit on 0 day showed gradual increases except for the 20 kGy irradiated samples. Thus, the sterilizing dose below detection limit (1 log CFU/g) of *bulgogi* was determined to 20 kGy. Several studies indicated that irradiation increased the shelf-life of foods (Robert and Weese, 1998; Waje *et al.*, 2008). Park

Table 1. Total bacterial populations (mean±SD; log CFU/g) of bulgogi irradiated with gamma ray during storage at 35°C

Dose	Storage (d)					
(kGy)	0	7	14	30		
0	2.2±0.1 ^{ay}	7.3±0.4 ^{ax}	7.9±0.3 ^{awx}	8.2±0.4 ^{aw}		
5	$< 1 \log^{1)bz}$	3.2 ± 0.2^{by}	4.6 ± 0.3^{bx}	6.1 ± 0.2^{bw}		
10	$< 1 \log^{by}$	$< 1 \log^{cy}$	2.2 ± 0.1^{cx}	$3.4\pm0.2^{\mathrm{cw}}$		
15	$< 1 \log^{bw}$	< 1 log ^{cw}	$< 1 \log^{dw}$	1.4 ± 0.1^{dw}		
20	$< 1 \log^{\text{bw}}$	$< 1 \log^{cw}$	$< 1 \log^{dw}$	$< 1 \log^{ew}$		

^{a-e}Means within the same column with different letters were significantly different (p<0.05).

w-zMeans within the same row with different letters were significantly different (p<0.05).

Table 2. Volatiles (mean \pm SD; total ion counts \times 10°) of irradiated <i>bulgogi</i> under	each treatment condition
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		20 kGy					
Volatile compounds	0 kGy	Aerobic packaging	Vacuum packaging	Antioxidants (1.0%)	Charcoal teabag (0.5%)	-20°C	-70°C
Methylallyl sulfide	42±3 ^x	231±19°	282±13 ^u	154±13 ^w	293±12 ^u	355±21 ^t	150±11 ^w
2-Propionic acid	116±5 ^x	126 ± 9^{wx}	137 ± 12^{w}	140±5 ^w	160 ± 7^{v}	229 ± 9^t	$194{\pm}13^u$
Toluene	0 ± 0^{z}	91 ± 6^{t}	$78\pm3^{\mathrm{u}}$	69±1°	46 ± 2^{y}	$63\pm2^{\mathrm{w}}$	52 ± 1^x
Hexanal	47 ± 1^{y}	$149{\pm}12^t$	101 ± 8^{C}	$89\pm4^{\mathrm{w}}$	68 ± 2^x	112 ± 9^u	110 ± 6^{uv}
α-Pinene	$128{\pm}16^{uv}$	110 ± 18^{v}	134 ± 9^u	$141{\pm}16^u$	167 ± 18^{tu}	188 ± 5^{t}	175 ± 9^{t}
Heptane	277 ± 12^{x}	459 ± 10^{t}	$396{\pm}14^u$	372 ± 6^{v}	329±11 ^w	$359{\pm}8^u$	$348{\pm}16^{uv}$
3-Carene	2011 ± 87^{x}	1914 ± 152^{x}	2379 ± 106^{w}	2690 ± 79^{v}	2404 ± 136^{w}	3784 ± 117^{t}	$3053{\pm}109^u$
p-Cymene	62 ± 1^{y}	$76\pm1^{\mathrm{w}}$	$84\pm3^{\mathrm{v}}$	$109\pm7^{\mathrm{u}}$	69 ± 2^x	159±21 ^t	$121{\pm}14^u$
dl-Limonene	1295 ± 42^{x}	1324 ± 83^{x}	1519 ± 67^{w}	1877 ± 109^{v}	1373 ± 109^x	2580 ± 97^t	$2025{\pm}76^u$
1,3-bis(1,1-dimethylethyl)-benzene	0 ± 0^{z}	100 ± 8^t	81 ± 5^u	63±2°	14 ± 1^{y}	$48{\pm}1^{\rm w}$	27 ± 1^x
Caryophyllene	238±9 ^u	186±7 ^w	235±11 ^u	207±8°	$210{\pm}13^{uv}$	$267{\pm}18^t$	228±13 ^u

^{t-z}Means within the same row with different letters were significantly different (p<0.05).

et al. (2010) also reported that use of gamma or electron beam irradiation at more than 15 kGy can ensure the microbial safety of bulgogi.

Volatiles

Irradiation of the *bulgogi* produced volatile compounds such as toluene and 1,3-bis(1,1-dimethylethyl)benzene which were not found in non-irradiated meat (Table 2). Methylallyl sulfide, 2-propionic acid, hexanal, and heptane were detected in non-irradiated samples, and the amount of these volatile compounds significantly increased (p<0.05) with gamma irradiation (Table 2). However, the irradiation off-flavor compounds (toluene and 1,3-bis (1,1-dimethylethyl)benzene) reduced (p<0.05) by physicochemical treatments such as vacuum condition, antioxidants (1.0%), charcoal teabag (0.5%), and frozen temperature (-70°C) during irradiation process.

Irradiation is shown to have a high correlation with lipid oxidation because free radical produced by irradiation induces lipid oxidation (Jo and Ahn, 2000). The volatile compounds can be associated with radiolytic degradation of lipids, amino acids, and proteins (Jo and Ahn, 1999). Kim *et al.* (2005) reported that 1,2-bis(1,1-dimethylethyl)benzene could be as a volatile compound by SPME and P&T methods to determine irradiated beef extract powders. Kwon *et al.* (2008) indicated that major volatiles found in irradiated meats were 2-butanone, heptane, dimethyl disulfide, toluene, and 1-octene for beef. Kim *et al.* (2002) also reported that on 0 day, major volatiles found in irradiated beef were butane, 2-butene, pentane, hexane, heptane, octane, toluene, and dimethyl sulfide. Meanwhile, irradiation off-flavor compounds of fresh

meat could be reduced by physicochemical treatments such as vacuum packaging, antioxidant, charcoal teabag, and frozen temperature (Brewer, 2009; Kim *et al.*, 2008; Shon *et al.*, 2009).

Lipid oxidation

TBARS values of all samples were increased (p<0.05) by gamma irradiation (Table 3). Meanwhile, lipid oxidation could be inhibited through each treatment condition. Vacuum-packaged sample had low TBARS value compared with aerobic packaged sample. The 1% of antioxidants (vitamin C (0.5%, w/w) + α -tocopherol (0.5%, w/w)) effectively inhibited (p<0.05) an increment of TBARS value of samples during irradiation process. The effect of charcoal teabag on lipid oxidation of irradiated sample was not observed (p>0.05). Meanwhile, the lipid oxidation of irradiated samples under the different temperatures was ranked as follows: irradiation at room temperature > irradiation at -20°C > irradiation at -70°C with significant differences of p<0.05.

The lipid oxidation is generally increased during irradiation process, and different physicochemical treatments can also retard lipid oxidation caused by irradiation. Many researchers have noted that packaging (aerobic vs. vacuum) appears to have a greater effect on quality of irradiated meat (Ahn *et al.*, 2002; Kim *et al.*, 2002; Murano *et al.*, 1998). Ahn *et al.* (2000) reported that TBARS of irradiated pork patties after aerobic packaging was higher than those of vacuum packaged samples. Antioxidants are also regarded as compounds that are able to delay, retard or prevent oxidation processes. The free radical scavenging activities of antioxidant may help

Dose (kGy)	TBARS	Sensory property			
		Taste	Flavor	Overall acceptance	
0	0.995±0.012°	6.6 ± 0.8^{a}	6.7 ± 0.6^{a}	6.9±0.7 ^a	
20	1.764 ± 0.021^a	3.1 ± 0.2^{c}	4.3 ± 0.3^{b}	$3.2 \pm 0.3^{\circ}$	
20	1.613 ± 0.024^{b}	4.6 ± 0.4^{b}	5.1 ± 0.5^{b}	4.1 ± 0.3^{b}	
0	0.753±0.008°	6.9±0.6ª	6.8±0.6 ^a	6.9±0.7 ^a	
20	1.525 ± 0.017^a	3.8 ± 0.4^{c}	3.2 ± 0.3^{c}	3.7 ± 0.4^{c}	
20	1.359 ± 0.011^{b}	4.9 ± 0.5^{b}	4.2 ± 0.4^{b}	4.8 ± 0.5^{b}	
0	0.673±0.009 ^b	6.7±0.4ª	6.9±0.6a	6.7±0.5 ^a	
20	0.981 ± 0.019^a	3.9±0.1°	3.4 ± 0.2^{c}	$3.8 \pm 0.3^{\circ}$	
20	0.974 ± 0.007^a	4.5±0.3 ^b	4.7 ± 0.4^{b}	4.6 ± 0.3^{b}	
0	0.982 ± 0.009^{d}	6.8±0.7ª	6.9±0.7 ^a	6.9±0.5a	
	$1.871{\pm}0.025^a$	$3.2{\pm}0.4^{c}$	4.6 ± 0.3^{c}	3.1 ± 0.2^{d}	
20	1.796 ± 0.011^{b}	3.7 ± 0.2^{c}	5.2 ± 0.4^{bc}	$3.8 \pm 0.2^{\circ}$	
	1.683 ± 0.006^{c}	$4.4{\pm}0.4^{b}$	5.6 ± 0.5^{b}	4.5 ± 0.4^{b}	
	0 20 0 20 0 20 20	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Taste 0 0.995 \pm 0.012° 6.6 \pm 0.8° 1.764 \pm 0.021° 3.1 \pm 0.2° 1.613 \pm 0.024° 4.6 \pm 0.4° 0 0.753 \pm 0.008° 6.9 \pm 0.6° 1.525 \pm 0.017° 3.8 \pm 0.4° 1.359 \pm 0.011° 4.9 \pm 0.5° 0 0.673 \pm 0.009° 6.7 \pm 0.4° 20 0.981 \pm 0.019° 3.9 \pm 0.1° 0.974 \pm 0.007° 4.5 \pm 0.3° 0 0.982 \pm 0.009° 6.8 \pm 0.7° 1.871 \pm 0.025° 3.2 \pm 0.4° 20 1.796 \pm 0.011° 3.7 \pm 0.2°	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	

Table 3. TBARS (mean ± SD; μg malondialdehyde/g sample) and sensory properties of irradiated *bulgogi* under each treatment condition

to protect the irradiated meat and meat products from chemical changes such as lipid oxidation (Ahn *et al.*, 2007; Badr, 2007; Nam *et al.*, 2006). Meanwhile, the minimal lipid oxidation detected in frozen meat during irradiation should be due to the limited mobility of free radicals in frozen states (Shultz *et al.*, 1977).

Sensory quality

The sensory properties (taste and flavor) of aerobic-packaged bulgogi were generally decreased (p<0.05) by irradiation (Table 3). Meanwhile, the vacuum-packaged samples had better (p<0.05) sensory quality compared with the aerobic-packaged samples. The bulgogi irradiated after addition of antioxidants (1.0%, w/w) had (p<0.05) a high sensory acceptability (Table 3). The granular-activated carbon (charcoal) effectively absorbed (p<0.05) the off-flavor compounds of irradiated bulgogi. Meanwhile, the gamma irradiation of bulgogi under the frozen state (-70°C) was able to effectively maintain (p<0.05) their sensory properties when compared with samples irradiated under the room temperature and -20°C.

Nam and Ahn (2003) reported that sensory quality and lipid oxidation in irradiated meats showed a close high correlation, and quality deterioration of meats could be inhibited by different physicochemical treatments. Brewer (2009) indicated that irradiation of fresh meat under the nitrogen or under vacuum environments was able to inhibit lipid oxidation and deterioration of sensory quality compared with irradiation under the aerobic condition. Antioxidants (vitamin C, tocopherol, sesamol, carnosin

and so on) can maintain sensory quality of meat during irradiation because antioxidants are able to reduce lipid oxidation and formation of off-flavor compounds in irradiated meat (Chen and Ahn, 1998; Nam *et al.*, 2006). Kim *et al.* (2008) and Sohn *et al.* (2009) also indicated that package with a charcoal teabag during the irradiation process is a good method to eliminate an off-flavor effectively in ground beef or pork caused by irradiation. Meanwhile, Park *et al.* (2008) indicated that when kimchi was irradiated under frozen temperature, especially dry ice (-70°C), the deterioration of sensory qualities (taste and flavor) of kimchi was reduced. In the present result, the irradiation under the frozen temperature (-70°C) was the most effective to maintain satisfied quality of *bulgogi*.

Irradiated *bulgogi* samples produced more off-flavors (toluene, heptane, and 1,3-bis(1,1-dimethylethyl)benzene) and higher TBARS than non-irradiated samples. Each physicochemical treatment such as vacuum packaging (75 cmHg), antioxidants (vitamin C + α -tocopherol (1.0%, w/w)), charcoal teabag (0.5%), and frozen temperature (-70°C) were effective to inhibit generation of irradiation off-flavors, lipid oxidation and deterioration of sensory characteristics (taste and flavor) during irradiation of *bulgogi*.

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

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