International Journal of Food Microbiology 172 (2014) 125–129

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Inactivation of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in black pepper and red pepper by gamma irradiation

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ARTICLE INFO

Article history:
Received 14 May 2013
Received in revised form 14 November 2013
Accepted 16 November 2013
Available online 22 November 2013

Keywords:
Gamma irradiation
Inactivation
Foodborne pathogen
Black pepper
Red pepper

ABSTRACT

This study evaluated the efficacy of gamma irradiation to inactivate foodborne pathogens in black pepper (*Piper nigrum*) and red pepper (dried *Capsicum annuum*). Black pepper and red pepper inoculated with *Escherichia coli* O157:H7 and *Salmonella* Typhimurium were subjected to gamma irradiation in the range of 0, 1, 2, 3 and 5 kGy, and color change was evaluated after treatment. Pathogen populations decreased with increasing treatment doses. A gamma irradiation dose of 5 kGy decreased *E. coli* O157:H7 and *S.* Typhimurium populations >4.4 to >5.2 log CFU/g in black pepper without causing color change. Similarly, 5 kGy of gamma irradiation yielded reduction of 3.8 to >5.2 log CFU/g for *E. coli* O157:H7 and *S.* Typhimurium in red pepper. During gamma irradiation treatment, L^*, a^* and b^* values of red pepper were not significantly changed except for 297 μm to 420 μm size red pepper treated with 5 kGy of gamma irradiation. Based on the D-value of pathogens in black pepper and red pepper, *S.* Typhimurium showed more resistant to gamma irradiation than did *E. coli* O157:H7. These results show that gamma irradiation has potential as a non-thermal process for inactivating foodborne pathogens in spices with minimal color changes.

1. Introduction

Enterohemorrhagic *Escherichia coli* O157:H7 causes hemorrhagic colitis, and the very serious hemolytic uremic syndrome (Reiss et al., 2006; Tarr et al., 2005). In the USA, 350 outbreaks of *E. coli* O157:H7 and *E. coli* O157:NM infection were reported between 1982 and 2002, and 183 of these cases were of foodborne origin (Rangel et al., 2005). *Salmonella enterica* serovar Typhimurium is the most commonly isolated *Salmonella* serotype and causes a self-limiting gastroenteritis (Boyle et al., 2007). Between 1996 and 2005, there were 68 outbreaks in the USA and 46 outbreaks in EU countries caused by *S.* Typhimurium (Greig and Ravel, 2009). Scallan et al. (2011) estimated that there are over 9 million foodborne illnesses in the United States annually, 63,153 cases of them are caused by Shiga toxin-producing *E. coli* (STEC) O157 and about a million cases of them are caused by *Salmonella* spp.

Black pepper and red pepper are widely used as spices to enhance the flavor of foods. Additionally, they have a positive effect on human health. Capsaicin, a pungent compound found in many red or hot peppers (*Capsicum* spp.), interrupts the consumption of fat and has a hypolipidemic effect (Srinivasan et al., 2004), and black pepper (*Piper nigrum*) seed extracts have antioxidant and radical scavenging activities (Gulcin, 2005). Black pepper and red pepper also have antimicrobial properties (Chaudhry and Tariq, 2006; Cichewicz and Thorpe, 1996). However, many cases of microbial contamination in black pepper and red pepper have been reported. *Clostridium perfringens*, *Bacillus* spp. and *Staphylococcus* spp. have been isolated from black pepper (Banerjee and Sarkar, 2003). *C. perfringens*, *Bacillus cereus*, Enterobacteriaceae and a variety of fungi have been isolated from red pepper (Christensen et al., 1967). In 2010, in the USA, many people who ate sliced salami become ill due to contamination with *Salmonella* Montevideo. The main source of pathogen contamination was traced to black pepper and red pepper added to salami after the pasteurization step. As a result, 272 people in 44 states became ill (CDC, 2010; Gieraltowski et al., 2010).

A number of methods to reduce foodborne pathogens and microorganisms in black pepper and red pepper have been developed, such as ozone treatment, ethylene oxide and steaming. Zhao and Cranston (1995) reported that ozone treatment (40 mg/min for 60 min) of black pepper produced 2–3 log CFU/g reductions of *E. coli* and *Salmonella*, and Akbas and Ozdemir (2008) reported that ozone treatment (1 ppm for 6 h) of red pepper led to 1.5–2 log CFU/g reductions of *E. coli* and *B. cereus*. These results show that ozone treatment is time consuming and an additional decontamination step is required to achieve a 5 log reduction, which is recommended by the FDA for fruits and vegetables (Han et al., 2001). Using ethylene oxide to control pathogens in spices is banned in the EU. Ethylene oxide is a potentially carcinogenic substance which can leave toxic residues (Uijl, 1992). Waje et al. (2008) reported that steam treatment (at 1020 mbar 100 °C for 16 min) of black

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http://dx.doi.org/10.1016/j.ijfoodmicro.2013.11.017

pepper led to less than 3 log CFU/g reduction of total microorganisms and Rico et al. (2010) reported that steam treatment (at 1020 mbar 100 °C for 16 min) resulted in 1 log CFU/g reduction of total microorganisms. However, steam treatment negatively affects the color of black pepper and red pepper causing significant degradation of color values. Therefore, new technologies are needed for decontamination of black pepper and red pepper while maintaining product quality.

Gamma irradiation has been widely investigated for inactivating microorganisms in foods with this non-thermal processing strategy being less detrimental to food quality than thermal pasteurization. Park et al. (2010) reported that 5 kGy gamma irradiation treatment did not significantly affect hardness, color, chewiness or taste of beef sausage patties. Chervin and Boisseau (1994) reported that irradiation-treated shredded carrots showed better sensory and nutritional quality than conventional treatment (rinsing with chlorinated water). Prakash et al. (2000) reported that irradiated diced celery retained its color, texture and aroma longer than acidified, blanched and chlorinated samples. In 1997, WHO informed that foods treated with doses of about 10 kGy are safe and the exposure level does not affect the nutritional values of foods (Farkas, 2006).

Oularbi and Mansouri (1996) reported that gamma irradiation greatly reduces levels of microorganisms in black pepper and red pepper. To date, there have been no studies documenting inactivation of *E. coli* O157:H7 and *S.* Typhimurium in black pepper and red pepper. Therefore the objectives of this study were to evaluate efficacy of gamma irradiation to inactivate *E. coli* O157:H7 and *S.* Typhimurium in black pepper and red pepper of three different sizes and confirm quality changes during gamma irradiation processing by color value measurement.

2. Materials and methods

2.1. Bacterial strains and cell suspension

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890) and *S.* Typhimurium (ATCC 19586, ATCC 43174, ATCC 700408) were used. These strains were used in several studies which investigated black pepper and red pepper or foods containing black or red pepper (Calicioglu et al., 2003; Cho et al., 2011; Choi et al., 2009; Kim et al., 2012; Yang et al., 2013). Each strain was obtained from the bacteria culture collection of Seoul National University (Seoul, Korea) for this study. Stock cultures were prepared by mixing 0.7 ml of a tryptic soy broth (TSB; Difco, BD, Sparks, MD) 24 h, 37 °C culture with 0.3 ml of sterile 50% glycerol and then stored at −80 °C. Working cultures were streaked onto tryptic soy agar (TSA; Difco, BD), incubated at 37 °C for 24 h and stored at 4 °C. Each strain of *E. coli* O157:H7 and *S.* Typhimurium was cultured in 5 ml TSB at 37 °C for 24 h, harvested by centrifugation at 4000 ×*g* for 20 min at 4 °C and washed three times with buffered peptone water (BPW; Difco, Sparks, MD). The final pellets were resuspended in BPW, corresponding to approximately 10^8–10^9 CFU/ml. Subsequently, suspended pellets of each strain of the two pathogen species were combined to produce culture cocktails.

2.2. Sample preparation, inoculation

Black pepper (*P. nigrum*) and red pepper (dried *Capsicum annuum*) used for this study were purchased at a local grocery store (Seoul, Korea). Three types of black pepper, whole (5 mm) and ground (707 μm to 1.19 mm, and 297 μm to 420 μm) and three types of red pepper (over 1.19 mm, 707 μm to 1.19 mm, and 297 μm to 420 μm) were used. Using a series of sieve shakers (Chung Gye Industrial Mfg., Co., Gyeonggi, Korea), black pepper and red pepper were divided by particle size differences. Four sieve shakers of the following mesh sizes were used; 16, 25, 40 and 50 mesh, corresponding to 1.19 mm, 707 μm, 420 μm and 297 μm particle sizes, respectively. For inoculation, 0.3 ml of culture cocktail was applied to each 25 g sample inside a sterile stomacher bag (Labplas Inc., Sainte-Julie, Quebec, Canada). The inoculated samples were mixed by hand massaging for 2 min to ensure even distribution of the pathogens and dried for 1 h in a biosafety hood (Akbas and Ozdemir, 2008; Emer et al., 2008). Uniform distribution of inoculum was confirmed by similar CFU counts (10^5–10^6 CFU/g) on selective agar that were obtained from 1 g of inoculated pepper taken from different locations after the drying step. After drying, all samples were stored in a 4 °C incubator, and transferred to an insulated cooler with ice during transportation.

2.3. Gamma irradiation treatment

All inoculated black pepper and red pepper samples in stomacher bags were irradiated in a cobalt-60 gamma irradiator (point source AECL, IR-79, MDS Nordion International Co., Ltd., Ottawa, Ont., Canada) at the Advanced Radiation Technology Institute, Jeongeup, Korea. The source strength was approximately 100 kCi with a dose rate of 10 kGy/h at room temperature (25 °C). The doses applied in this study were 0.0 (control), 1.0, 2.0, 3.0 and 5.0 kGy. Doses were applied by controlling the treatment time (0, 6, 12, 18 and 30 min). An absorbed dose was confirmed by using a ceric/cerous dosimeter (Bruker Instruments, Rheinstetten, Germany). Non-irradiated controls were also maintained under the same storage and transport conditions as the irradiated samples.

2.4. Bacterial enumeration

Twenty-five gram samples were diluted in 225 ml of BPW and homogenized for 2 min in a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, 1 ml aliquots of homogenized samples were tenfold serially diluted in 9 ml of BPW, and 0.1 ml of sample or diluent was spread-plated onto each selective medium. Sorbitol MacConkey agar (Difco) and Xylose Lysine Desoxycholate agar (Difco) were used as selective media for the enumeration of *E. coli* O157:H7 and *S.* Typhimurium, respectively. Where low populations of surviving cells were anticipated, 1 ml aliquots of the original homogenate were equally distributed onto four plates of each respective medium and spread-plated. All plates were incubated at 37 °C for 24 h and colonies were counted. Uninoculated samples were also processed as described above to detect any possible background contamination with *E. coli* O157:H7 or *S.* Typhimurium in black pepper and red pepper.

2.5. D-value calculation

Number of surviving pathogens was plotted on a logarithmic scale as a function of dose (kGy), resulting in a survivor curve. D-value, the dose in kGy needed to decrease population of pathogen by 90% (1 log), was calculated by the following equation.

$$\log\left(\frac{N}{N_0}\right) = -\frac{1}{D}\cdot d$$

where N = the number of survivors at gamma irradiation dose, N_0 = initial population of pathogen, D = D-value (decimal reduction dose) and d = gamma irradiation dose (Hvizdzak et al., 2010).

2.6. Color measurement

Color values of L^*, a^*, and b^* were used to measure the color of samples. Hunter's color values (L^*, a^*, b^*) were measured using a Minolta colorimeter (model CR300, Minolta Co., Osaka, Japan). Two g of sample was put into the bottom half of the measurement device. The measuring head of the colorimeter was placed on top of the measurement device. Color was measured from three random locations.

Table 1
Effect of gamma irradiation treatment on inactivation of *Escherichia* O157:H7 and *Salmonella* Typhimurium in black pepper.

Irradiation dose (kGy)	Reduction of *E. coli* O157:H7 (log CFU/g)[a]			Irradiation dose (kGy)	Reduction of *Salmonella* Typhimurium (log CFU/g)		
	Whole	707 μm to 1.19 mm	297 μm to 420 μm		Whole	707 μm to 1.19 mm	297 μm to 420 μm
1	2.5 ± 0.16Aa	1.4 ± 0.31Ba	1.5 ± 0.20Ba	1	1.9 ± 0.11Aa	1.3 ± 0.53ABa	1.2 ± 0.17Ba
2	>5.2 ± 0.08Ab	4.1 ± 0.41Bb	4.4 ± 0.48Bb	2	4.0 ± 0.29Ab	2.7 ± 0.06Bb	2.3 ± 0.25Bb
3	>5.2 ± 0.08Ab	>4.6 ± 0.05Bc	4.3 ± 0.41Cb	3	4.9 ± 0.44Ac	4.0 ± 0.36Bc	4.0 ± 0.23Bc
5	>5.2 ± 0.08Ab	>4.6 ± 0.05Bc	>4.6 ± 0.14Bb	5	>5.1 ± 0.25Ac	>4.6 ± 0.17Bc	>4.4 ± 0.10Bd

[a] Mean of three replications ± standard deviation. Values followed by the same upper case letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).

L*, a*, and b* values indicate color lightness, redness, and yellowness of the sample, respectively.

2.7. Statistical analysis

All data were analyzed with one-way ANOVA using Statistical Analysis System (SAS Institute, Cary, NC, USA) and Duncan's multiple range test to determine if there were significant differences ($P < 0.05$) in mean values of microorganism populations. Microbial counts were transformed to $\log_{10}$ values for analysis. One log was used for calculations in the case of populations below the detection limit.

3. Results

Initial populations of *E. coli* O157:H7 and *S.* Typhimurium in inoculated black pepper and red pepper were approximately 10^5–10^6 CFU/g. In uninoculated samples, populations of the pathogens were below the detection limit (1 log CFU/g). Gamma irradiation greatly reduced levels of *E. coli* O157:H7 and *S.* Typhimurium in black pepper and red pepper.

The inactivation effect of gamma irradiation (1, 2, 3, 5 kGy) on *E. coli* O157:H7 and *S.* Typhimurium in whole and ground black pepper is shown in Table 1. Reduction of pathogens increased with an increasing irradiation dose from 1 to 5 kGy. The initial populations of *E. coli* O157:H7 in black pepper (whole, 707 μm to 1.19 mm, and 297 μm to 420 μm particle size) were 6.2, 5.6 and 5.6 log CFU/g, respectively. After 5 kGy gamma irradiation exposure, populations were decreased to under the detection limit in all cases. In 1 and 2 kGy treated samples, whole (5 mm) black pepper showed greater reduction of *E. coli* O157:H7 than the ground type.

The initial populations of *S.* Typhimurium in black pepper (whole, 707 μm to 1.19 mm, and 297 μm to 420 μm) were 6.1, 5.6 and 5.4 log CFU/g, respectively. After 5 kGy gamma irradiation exposure, populations were decreased to under the detection limit in all cases. In 2 and 3 kGy treated samples, whole (5 mm) black pepper showed greater reduction of *S.* Typhimurium than the ground type.

Table 2 shows the bactericidal effect of gamma irradiation (1, 2, 3, 5 kGy) against *E. coli* O157:H7 and *S.* Typhimurium in red pepper (over 1.19 mm, 707 μm to 1.19 mm, and 297 μm to 420 μm). The populations of surviving pathogens decreased in all samples as irradiation dose increased from 1 to 5 kGy. The initial populations of *E. coli* O157:H7 in red pepper (over 1.19 mm, 707 μm to 1.19 mm, and 297 μm to 420 μm) were 6.2, 6.2 and 5.4 log CFU/g respectively. After 5 kGy gamma irradiation exposure, populations were decreased under the detection limit in particles of the over 1.19 mm and 707 μm to 1.19 mm size. In 3 kGy gamma irradiation treatment, the reduction of over 1.19 mm size red pepper is significantly larger than that of 707 μm to 1.19 mm and 297 μm to 420 μm size red peppers.

The initial populations of *S.* Typhimurium in red pepper (over 1.19 mm, 707 μm to 1.19 mm, and 297 μm to 420 μm) treatment were 5.9, 5.8 and 5.3 log CFU/g, respectively. After 5 kGy gamma irradiation exposure, populations were decreased to under the detection limit in samples of 707 μm to 1.19 mm size. In 3 kGy gamma irradiation treatment, pathogen reduction in particles of over 1.19 mm in red pepper was significantly greater than those of 297 μm to 420 μm particle sizes.

Table 3 shows the decimal reduction dose (D-value) of pathogens in black pepper and red pepper. The D-value of *E. coli* O157:H7 in whole black pepper was smaller than that of ground black pepper. A similar tendency was observed in *S.* Typhimurium. In the case of red pepper, the D-value of *E. coli* O157:H7 in particles over 1.19 mm was smaller than that in 707 μm to 1.19 mm and 297 μm to 420 μm size particles. But *S.* Typhimurium showed no significant difference in red pepper of these size ranges. Under all treatment conditions, *S.* Typhimurium showed larger D-values than those of *E. coli* O157:H7.

The Hunter color values of black pepper and red pepper after gamma irradiation treatment are shown in Table 4. L*, a*, and b* values of irradiated samples were not significantly different ($P > 0.05$) from those of non-treated samples except for the a* value of 297 μm to 420 μm size red pepper treated with 5 kGy of gamma irradiation. But color differences could not be detected with an unaided eye.

4. Discussion

A number of studies have reported the effect of gamma irradiation for reducing microorganisms in black pepper and red pepper (Oularbi and Mansouri, 1996; Rico et al., 2010; Waje et al., 2008). However, to date, no studies have evaluated the effect of gamma irradiation on *E. coli* O157:H7 and *S.* Typhimurium in black pepper and red pepper. This study confirmed that gamma irradiation is effective on reducing

Table 2
Effect of gamma irradiation treatment on inactivation of *Escherichia* O157:H7 and *Salmonella* Typhimurium in red pepper.

Irradiation dose (kGy)	Reduction of *E. coli* O157:H7 (log CFU/g)[a]			Irradiation dose (kGy)	Reduction of *Salmonella* Typhimurium (log CFU/g)		
	Over 1.19 mm	707 μm to 1.19 mm	297 μm to 420 μm		Over 1.19 mm	707 μm to 1.19 mm	297 μm to 420 μm
1	1.1 ± 0.21Aa	0.9 ± 0.35Aa	1.4 ± 0.12Aa	1	1.4 ± 0.12Aa	1.3 ± 0.23Aa	1.4 ± 0.47Aa
2	2.7 ± 0.58Ab	2.4 ± 0.17Ab	2.7 ± 0.29Ab	2	2.1 ± .041Ab	1.9 ± 0.23Ab	2.1 ± 0.41Aab
3	4.3 ± 0.59Ac	3.2 ± 0.25Bc	3.1 ± 0.22Bc	3	2.9 ± 0.08Ac	2.7 ± 0.21ABc	2.4 ± 0.18Bb
5	>5.2 ± 0.35Ad	>5.2 ± 0.50Ad	4.3 ± 0.19Bd	5	4.5 ± 0.43ABd	>4.8 ± 0.09Ad	3.8 ± 0.49Bc

[a] Means of three replications ± standard deviation. Values followed by the same uppercase letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).

Table 3
D-values of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in black pepper and red pepper exposed to gamma irradiation.

Pathogen	D-value (kGy)[a]					
	Black pepper			Red pepper		
	Whole	707 μm to 1.19 mm	297 μm to 420 μm	Over 1.19 mm	707 μm to 1.19 mm	297 μm to 420 μm
Escherichia coli O157:H7	0.40 ± 0.03Aa	0.49 ± 0.05Ba	0.46 ± 0.05ABa	0.71 ± 0.12Aa	0.91 ± 0.06Ba	1.21 ± 0.05Ca
Salmonella Typhimurium	0.60 ± 0.05Ab	0.75 ± 0.08Bb	0.76 ± 0.03Bb	1.17 ± 0.10Ab	1.15 ± 0.13Ab	1.44 ± 0.27Aa

[a] Means of three replications ± standard deviation. Values followed by the same uppercase letters within a row and by the same lowercase letters within a column are not significantly different ($P > 0.05$).

pathogens in black pepper and red pepper. In the present study, the greater the irradiation dose, the greater the reduction of both *E. coli* O157:H7 and *S.* Typhimurium. When samples were treated with 5 kGy of gamma irradiation, 3.8 to >5.2 log CFU/g reductions were achieved.

Black pepper and red pepper are dried seasonings of low water activity. Pathogens in foods which have low water activity are extremely difficult to kill by conventional heat treatment. Thus several studies have been performed which applied irradiation to inactivate pathogens in low water activity foods. Saroj et al. (2007) reported that 2 kGy gamma irradiation treatment of mung beans (*Phaseolus aureus*) and chickpeas (*Cicer arietinum*) resulted in 4.6 and 4.8 log reductions, respectively, of *Salmonella*. Mukisa et al. (2012) reported that 10 + 25 kGy (35 kGy) of gamma irradiation treatment effectively reduced the total aerobic count and aerobic spore count in sorghum flour to under the detection limit (<1 log CFU/g). Prakash et al. (2010) reported that D-values of *S.* Enteritidis PT30, *S.* Anatum, *S.* Hartford and a cocktail of *S.* Anatum, *S.* Infintis, *S.* Newport and *S.* Stanley on almonds were 1.25, 1.23, 1.06 and 1.16 kGy, respectively, following gamma irradiation. Not only gamma irradiation but also X-rays effectively reduced foodborne pathogens in low water activity foods. Jeong et al. (2012) reported that D-values of *S.* Enteritidis PT30 and *S.* Tennessee were 0.226 and 0.256 kGy, respectively, in 0.2 A_w almonds and 0.474 and 0.554 kGy, respectively, in 0.2 A_w walnuts. Thus, irradiation is an effective pasteurization method for reducing populations of foodborne pathogens and microorganisms in low water activity foods.

The efficacy of the treatment varies with pathogen types. Monk et al. (1995) reported that *Salmonella* may be the most resistant pathogen to irradiation among gram-negative bacteria. Any irradiation sufficient to kill *Salmonella* would inactivate other gram-negative bacteria. Our study showed that *S.* Typhimurium has a larger D-value than *E. coli* O157:H7. In the case of 297 μm to 420 μm size red pepper, there was no significant difference. This was due to a large standard deviation in the D-value of *S.* Typhimurium.

During gamma irradiation treatment, injury of microorganisms can occur (Wu, 2008). Also foodborne pathogen such as *E. coli* O157:H7 and *S.* Typhimurium can be sublethally injured (Lucht et al., 1998). Injured cells cannot form colonies on selective agar because of selective agents such as antibiotics, dyes, or bile salts which interfere with growth of stressed or injured microorganisms (Prentice and Clegg, 1974). For these reasons, the inactivation effect of gamma irradiation on *E. coli* O157:H7 and *S.* Typhimurium could be overestimated. But some research studies show that during storage following gamma irradiation treatment, pathogens or microorganisms were reduced consistently (Song et al., 2006, 2007). This is due to the post-irradiation effect. Injured cells gradually died because they could not adapt to the surrounding environment (Byun et al., 2001).

Kim et al. (2005) reported that irradiation decreased the Hunter's a^*, and b^* values of dried red pepper. However, in the present study, there were no significant changes in L^*, a^*, and b^* values after irradiation of black pepper and red pepper except for the 297 μm to 420 μm size red pepper. The a^* value of 5 kGy treated 297 μm to 420 μm size red pepper was significantly different from those of untreated samples but it could not be detected visually.

In conclusion, we can recommend gamma irradiation as a pasteurization intervention for black pepper and red pepper. The D values of *E. coli* O157:H7 and *S.* Typhimurium in whole black pepper were smaller than those in ground black pepper. Also, the D value of *E. coli* O157:H7 in over 1.19 mm red pepper was smaller than those of smaller particle sizes. So, applying gamma irradiation before pepper is ground or fully crushed is recommended. Further investigation is needed to determine particle size dependency and the optimum size for better efficacy for reducing pathogens in black pepper and red pepper. Finally, the induction and subsequent resuscitation of injured cells in black pepper and red pepper upon gamma irradiation need to be studied.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (2012R1A1A2003838). This research was also supported by the Mid-career Research Program through a NRF grant funded by the Ministry of Education, Science, and Technology, Korea (2010-0026030).

Table 4
Hunter's color L (lightness), a (redness), and b (yellowness) values[a] of irradiated black pepper and red pepper.

Hunter's parameter[b]	Irradiation dose (kGy)	Black pepper			Irradiation dose (kGy)	Red pepper		
		Whole	707 μm to 1.19 mm	297 μm to 420 μm		Over 1.19 mm	707 μm to 1.19 mm	297 μm to 420 μm
L	0	22.26 ± 0.40a	32.06 ± 0.70a	41.97 ± 1.10a	0	26.51 ± 0.87a	27.33 ± 0.32a	33.76 ± 0.40a
	5	23.62 ± 1.24a	32.76 ± 1.40a	39.71 ± 0.96a	5	24.71 ± 1.15a	27.09 ± 0.94a	34.15 ± 0.23a
a	0	0.88 ± 0.33a	1.01 ± 0.10a	0.46 ± 0.05a	0	13.77 ± 0.76a	16.71 ± 0.31a	23.01 ± 0.40a
	5	0.50 ± 0.09a	1.01 ± 0.19a	0.55 ± 0.09a	5	12.54 ± 0.53a	16.33 ± 0.63a	22.14 ± 0.12b
b	0	2.64 ± 0.54a	5.46 ± 0.38a	9.73 ± 0.49a	0	8.14 ± 0.32a	9.79 ± 0.15a	15.50 ± 0.13a
	5	2.05 ± 0.72a	5.67 ± 0.85a	9.19 ± 0.80a	5	7.56 ± 0.38a	9.58 ± 0.51a	15.76 ± 0.16a

[a] Mean of three replications ± standard deviation. Values followed by the same letters within the column per parameter are not significantly different ($P > 0.05$).
[b] Color parameters are L^* (lightness), a^* (redness), and b^* (yellowness).

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