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Inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in ready-to-bake cookie dough by gamma and electron beam irradiation

Seul-Gi Jeong^a, Dong-Hyun Kang^{a, b, *}

^a Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, <mark>Center for Food and Bioconvergence</mark>, Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, 08826, Republic of Korea ^b Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do, 25354, Republic of Korea

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ABSTRACT

This study was conducted to investigate the efficacy of gamma and electron beam irradiation to inactivate foodborne pathogens in ready-to-bake cookie dough and to determine the effect on quality by measuring color and texture changes. Cookie dough inoculated with *Escherichia coli* O157:H7, *Salmonella* Typhimurium, or *Listeria monocytogenes* was subjected to gamma and electron beam irradiation, with doses ranging from 0 to 3 kGy. As the radiation dose increased, the inactivation effect increased among all tested pathogens. After 3.0 kGy of gamma and electron beam irradiation, numbers of inoculated pathogens were reduced to below the detection limit (1 log CFU/g). The D₁₀-values of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in cookie dough treated with gamma rays were 0.53, 0.51, and 0.71 kGy, respectively, which were similar to those treated by electron beam with the same dose. Based on the D₁₀-value of pathogens in cookie dough, *L. monocytogenes* showed more resistance to both treatments than did *E. coli* O157:H7 and *S.* Typhimurium. Color values and textural characteristics of irradiated cookie dough were not significantly (P > 0.05) different from the control. These results suggest that irradiation can be applied to control pathogens in ready-to-bake cookie dough products without affecting quality.

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1. Introduction

Due to increased consumer demand for home-baked foods, ready-to-bake cookie dough has become very popular. Although it is not a ready-to-eat food, risky eating behaviors, such as consumption of unbaked cookie dough, are widespread among consumers. A survey taken of risky eating behaviors among college students showed that 53% consumed unbaked products that are intended to be cooked before consumption. Some respondents said they purchased cookie dough with the intention of only eating it unbaked and had no plans to actually bake cookies (Byrd-Bredbenner et al., 2008). This practice is a health risk, because ready-to-bake cookie dough can be a reservoir of pathogens due to its various ingredients such as flour, eggs, chocolate chips,

E-mail address: kang7820@snu.ac.kr (D.-H. Kang).

molasses, sugar, margarine, baking soda, and vanillin/vanilla extract. It can also readily acquire and harbor foodborne pathogens during processing, preservation, and transportation (Neil et al., 2012).

In July 2009, a large multistate outbreak of *Escherichia coli* O157:H7 infections associated with ready-to-bake commercial prepackaged cookie dough occurred in the United States. During this outbreak, a reported 76 persons in 31 states became ill (Centers for Disease Control and Prevention, 2009). This outbreak was the first time refrigerated and prepackaged cookie dough or a similar product has been implicated as a vehicle for *E. coli* O157:H7 infections. *Salmonella* can be found in wheat flour which is main ingredient of cookie dough, and flour and flour-based mixtures have been implicated in *Salmonella* outbreaks (Guodong et al., 2007; New Zealand Food and Safety Authority, 2008; Sperber, 2007). No outbreaks involving *Listeria monocytogenes* in flour-based foods have been reported. However, *L. monocytogenes* could be a potential risk in refrigerated products like cookie dough due to its ability to grow at refrigeration temperature (Liu et al., 2002).







^{*} Corresponding author. Department of Agricultural Biotechnology, Seoul National University, Seoul, 08826, Republic of Korea.

For this reason, several cookie dough manufacturers have been advised by the U.S. Food and Drug Administration (FDA) that the use of pasteurized flour for ready-to-bake cookie dough products should be implemented during manufacturing (Neil et al., 2012). However, insufficient heating may happen or post-processing contamination may occur before packaging. Furthermore, according to the consumption pattern of cookie dough, it is likely to be consumed without cooking or baking, though label statements may warn against consuming unbaked product. Therefore, an intervention step, which is a process designed to control postprocessing contaminating pathogens before or after the products are packaged, is necessary to ensure the final microbiological safety of the products.

One suitable intervention treatments is ionizing irradiation, which includes gamma or electron beam irradiation. It is approved by the FDA for use on meat, poultry, spices, and fresh produce at doses up to 8 kGy (Code of Federal Regulations, 2013). Inactivation of microorganisms by ionizing irradiation is mainly caused by DNA damage resulting from indirect action by the radiolysis of water molecules present in the cell or direct action by the absorption of radiation energy (Clavero et al., 1994; Farkas, 2006; Ray and Bhunia, 2007). This has caused ionizing irradiation to be explored as a nonthermal method for inactivating pathogens or spoilage microorganisms on foods. The advantages of ionizing irradiation include that it does not leave a residue, does not lead to thermal damage of food product, and is not limited to surfaces but also affects the inside of foods (Ray and Bhunia, 2007). Although consumers may be concerned about the wholesomeness of irradiated food due to lack of public knowledge, the World Health Organization (WHO) informed that irradiation treatment at doses up to 10 kGy presents no toxicological hazard or nutritional problems (World Health Organization, 1981). For these reasons, gamma and electron beam irradiation have been used to improve the microbiological safety of a wide range of foods.

Gamma rays and electron beam irradiation are produced by radioisotopes such as Cobalt-60 and a machine source based on electricity, respectively. A major difference between both types of irradiation is penetration depth; gamma rays have much higher penetrating power than electron beams, which facilitates the use of gamma irradiation for processing of bulk items. Electron beam irradiation is applied to both sides in order to overcome the problem of its limited penetration depth, but the maximum penetration depth is only about 8 cm in water for the maximum permitted energy of 10 MeV (Miller, 2005). Despite this limitation, electron beam treatment is efficient which comes from the switch-off capability and high dose-rate.

To date, there have been no studies on the inactivation of foodborne pathogens in cookie dough by ionizing irradiation. Thus, the overall objective of this study was to evaluate the potential use of gamma or electron beam irradiation for reducing *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in ready-to-bake cookie dough by comparing the efficacy of both treatments. Also, the radiation resistance of each pathogen subjected to each treatment was investigated by calculating the D₁₀-value.

2. Materials and method

2.1. Bacterial strains

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889 and ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971 and ATCC 700408), and *L. monocytogenes* (ATCC 7644, ATCC 19114 and ATCC 19115) were used. Each strain was obtained from the Department of Food and Animal Biotechnology culture collection at Seoul National University (Seoul, South Korea) for this study. Stock

cultures were prepared by growing strains in 5 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD, USA) at 37 °C for 24 h (to stationary phase) then combining 0.7 ml of culture with 0.3 ml of 50% glycerol and storing at -80 °C. Working cultures were prepared by streaking onto tryptic soy agar (TSA; Difco), incubating at 37 °C for 24 h, and maintaining at 4 °C for not more than 1 week.

2.2. Inoculum preparation

Each strain of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was grown in 5 ml of TSB at 37 °C for 24 h and tested separately for gamma or electron beam irradiation. Also, a 3-pathogen mixed culture cocktail was used in subsequent experiments, because inoculation with several strains or pathogens would more closely approximate reality. The cultures were harvested by centrifugation at 4000 × g for 20 min at 4 °C, and washed three times with 0.2% peptone water (PW; Difco). The final pellets were resuspended in 0.2% PW, corresponding to approximately 10^7-10^8 CFU/ml.

2.3. Sample inoculation

Commercially prepackaged cookie dough was purchased at a local grocery store (Seoul, South Korea). The composition of the cookie dough is shown in Table 1. Twenty-five grams samples were placed in sterile stomacher bags (Labphas, Inc., Sainte-Juilie, Quebec, Canada). One ml of inoculum was added to the samples, gently mixed by hand for 1 min to allow even distribution of inoculum, spread into a thin layer approximately 10 mm in thickness, and then dried for 30 min inside a biosafety hood with the fan running until the a_w of the sample equaled that of a non-inoculated sample (ca. 0.86). The final cell concentration was 10^6-10^7 CFU/g. After drying, all sample bags were individually sealed, packed in a cooler with frozen gel packs, and stored at 4 °C during transportation to the irradiation processing facility (Korea Atomic Energy Research Institute, Jeongeup, South Korea).

2.4. Irradiation

Samples were irradiated at doses ranging from 0 to 3 kGy using two devices: a cobalt-60 gamma irradiator (point source AECL, IR-79; MDS Nordion International Co., Ltd., Ottawa, ON, Canada) with a source strength of 320 kCi and a dose rate of 20 kGy/h, and a single-sided linear electron beam accelerator (UELV-10-10S; NIIFFA, Moscow, Russia) with an acceleration voltage of 10 MeV. The absorbed doses were measured by alanine dosimeters (ES 200-2106, batch No. T020604; Bruker Biospin, Rheinstetten, Germany) and an e-scan alanine dosimeter reader (Bruker Biospin) (Table 2). For electron beam irradiation, the arithmetic average of the entrance and exit doses received by the sample was used to represent the actual measured dose. For simplicity, the authors refer only the target dose for both types of irradiation. Following

Table 1	
Formulation of cookie dough product used in this study.	

	Amount per serving ^a	% Daily value ^b
Calories (kcal)	355	
Total fat (g)	22	43
Total carbohydrate (g)	36	11
Protein (g)	3	5
Sodium (mg)	140	7
Cholesterol (mg)	11	4

^a Serving size is 60 g per person.

^b Percent daily values are based on a 2000 calorie diet.

Table 2
Actual measured doses for gamma and electron beam irradiation. ^a

Target dose (kGy)	Actual measured dose (kGy)			
	Gamma ray	Electron beam		
0.5	0.53 ± 0.00			
1	1.04 ± 0.01	1.04 ± 0.05		
1.5	1.52 ± 0.01			
2	2.06 ± 0.03	1.98 ± 0.02		
2.5	2.73 ± 0.02			
3	3.33 ± 0.02	3.10 ± 0.01		

^a Means \pm standard deviations from three replications.

irradiation treatment, the samples were repacked and shipped to Seoul National University (held at 4 °C for 6 h). Non-irradiated controls were also kept under the same storage and transport conditions as the irradiated samples. The total time from inoculation of the samples to commencement of microbial analyses was 24 h. Microbial survival over this time was verified, with no significant (P > 0.05) differences in pathogen populations in the control samples during this time interval.

2.5. Bacterial enumeration

At selected dose intervals, irradiated 25 g samples were diluted in 225 ml of 0.2% PW and homogenized with a stomacher (EASY MIX, AES Chemunex, Rennes, France) for 2 min. After homogenization, 1-ml aliquots of sample were 10-fold serially diluted in 9-ml of 0.2% PW, and 0.1 ml of appropriate diluent was spread-plated onto each selective medium. Sorbitol MacConkey agar (SMAC; Difco), xylose lysine desoxycholate agar (XLD; Difco), and Oxford agar base with Bacto Oxford antimicrobial supplement (OAB; Difco) were used as selective media for the enumeration of E. coli O157:H7, S. Typhimurium, and L. monocytogenes, respectively. When low populations of surviving cells were anticipated, 1-ml aliquots of the original homogenate were equally divided between four plates of each medium and spread-plated (detection limit, 1 log CFU/g). All plates were incubated at 37 °C for 24 h and typical colonies were counted. In order to confirm identify of the pathogens, random colonies selected from the enumeration plates were subjected to serological and biochemical tests. These tests consisted of the E. coli O157:H7 latex agglutination assay (Oxoid, Ogdensberg, NY), Salmonella latex agglutination assay (Oxoid), and API Listeria (bio-Mérieux, Hazelwood, MO) for E. coli O157:H7, S. Typhimurium, and L. monocytogenes, respectively.

2.6. D_{10} -value calculation

Viable-count reductions of each pathogen following three repeated irradiation treatment were plotted on a logarithmic scale against the measured dose. Regression analyses were conducted to determine regression coefficients and slopes. D_{10} -value, the dose required to decrease microbial population by 90% (1 log), was calculated as follows (equation (1)):

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

where *N* is the number of surviving cells in irradiated samples, N_0 is the initial cell population, *D* is the D₁₀-value, and *d* is the irradiation dose.

2.7. Color measurement

To measure the effect of gamma or electron beam irradiation on

the color of ready-to-bake cookie dough, the color changes of irradiated samples were measured using a Minolta colorimeter (CR400; Minolta Co., Osaka, Japan). CIELAB color space using illuminant C and 2° viewing angle were used to quantify color attributes and measurements were taken from irradiated and non-irradiated uninoculated cookie dough at random locations. L*, a*, and b* values indicate color lightness, redness, and yellowness of the sample, respectively.

2.8. Texture measurement

Changes in texture of irradiated cookie dough were evaluated with a Brookfield texture analyzer (CT3-10k; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with a cylinder set probe (TA10; Brookfield Engineering Laboratories, Inc.). Texture profile analysis (TPA) was conducted according to the method adapted from Zoulias et al. (2000). The dough disks ($40 \times 10 \text{ mm}$) were placed onto the press holder, and a cylinder was moved down at 2 mm/s. The analyzer was set at a distance of 2.4 mm, a trigger load of 5 g, and a load cell of 10000 g. The samples were applied two compressions in a reciprocating motion. Based on the force-time curve recorded by TexturePro CT software (version 1.2; Brookfield Engineering Laboratories, Inc.), hardness, adhesiveness, cohesiveness, and resilience of the cookie dough were quantified. Hardness, adhesiveness, cohesiveness, and resilience represent the force required to compress a sample on the first compression, the ease of removal of the sample from a surface after the first compression, the capacity to withstand a second deformation, and the recovery ability against the first compression, respectively.

2.9. Statistical analysis

All experiments were replicated three times with duplicate samples. Data were analyzed by the analysis of variance procedure using Statistical Analysis System (SAS Institute, Cary, NC), and Duncan's multiple range tests was used to determine significant differences (P < 0.05) in mean values.

3. Results

3.1. Inactivation of pathogenic bacteria by gamma irradiation

The reductions (log CFU/g) of E. coli O157:H7, S. Typhimurium, and L. monocytogenes in ready-to-bake cookie dough treated with gamma irradiation are depicted in Fig. 1. The reduction of pathogens increased with increasing radiation dose from 0.5 to 3 kGy. The initial populations of E. coli O157:H7, S. Typhimurium, and L. monocytogenes in cookie dough were 7.51, 7.82, and 5.96 log CFU/ g, respectively. The levels of E. coli O157:H7 were reduced by 1.23, 2.76, 4.04, 4.49, and 5.15 log CFU/g at 0.5, 1, 1.5, 2, and 2.5 kGy, respectively. S. Typhimurium in cookie dough presented similar reduction patterns to E. coli O157:H7. The number of S. Typhimurium cells experienced reductions of 1.40, 2.51, 3.15, 4.41, and 5.34 log CFU/g at 0.5, 1, 1.5, 2, and 2.5 kGy, respectively. For L. monocytogenes, the reduction was 0.81 log CFU/g at 0.5 kGy and 3.49 log CFU/g at 2.5 kGy. After 3 kGy gamma irradiation exposure, the levels of surviving cells of the three pathogens were reduced to below the detection limit.

3.2. Inactivation of pathogenic bacteria by electron beam irradiation

Fig. 2 shows the inactivation effect of electron beam irradiation on *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in readyto-bake cookie dough. The overall reduction patterns of pathogens

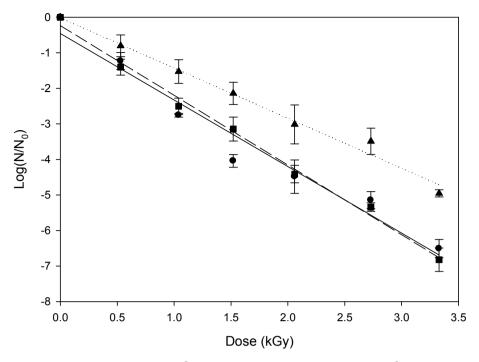


Fig. 1. Standardized reductions of *Escherichia coli* 0157:H7 (\bullet , solid line, R² = 0.98), *Salmonella* Typhimurium (\blacksquare , dashed line, R² = 0.99), and *Listeria monocytogenes* (\blacktriangle , dotted line, R² = 0.98) in cookie dough treated with gamma irradiation. Error bars indicate standard deviations calculated from triplicates.

irradiated by gamma rays were similar to those subjected to electron beam irradiation. As the radiation dose increased from 1 to 3 kGy, populations of these pathogens decreased more effectively with reductions at 1, 2, and 3 kGy of 2.98, 5.07, and 6.13 log CFU/g, respectively, for *E. coli* O157:H7; 3.07, 4.98, and 6.35 log CFU/g, respectively, for *S.* Typhimurium; and 2.14, 3.77, and 4.84 log CFU/g, respectively, for *L. monocytogenes*. The electron beam irradiation dose required to reduce the three pathogens to undetectable levels was the same as the gamma irradiation dose. At all tested radiation dosages, reduction of *L. monocytogenes* was significantly (P < 0.05) smaller than that of *E. coli* O157:H7 and *S.* Typhimurium.

3.3. Comparison of bactericidal effect between gamma and electron beam irradiation

Table 3 shows the decimal reduction dose (D₁₀-value) of

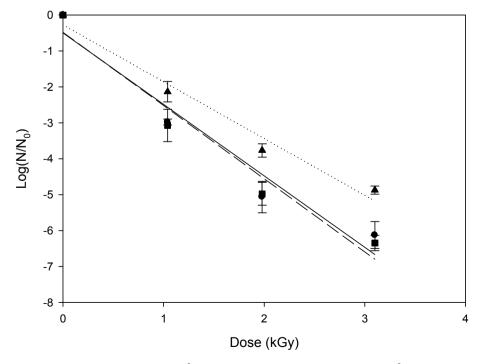


Fig. 2. Standardized reductions of *Escherichia coli* 0157:H7 (\bullet , solid line, R² = 0.96), *Salmonella* Typhimurium (\blacksquare , dashed line, R² = 0.97), and *Listeria monocytogenes* (\blacktriangle , dotted line, R² = 0.98) in cookie dough treated with electron beam irradiation. Error bars indicate standard deviations calculated from triplicates.

Table 3

D₁₀-values of Escherichia coli O157:H7. Salmonella Typhimurium, and Listeria monocytogenes in cookie dough exposed to gamma and electron beam irradiation.²

Pathogen	D ₁₀ -value (kGy)			
	Gamma ray	Electron beam		
E. coli O157:H7 S. Typhimurium L. monocytogenes	0.53 ± 0.02 Ba 0.51 ± 0.01 Ba 0.71 ± 0.06 Aa	0.50 ± 0.04 Ba 0.49 ± 0.01 Ba 0.63 ± 0.02 Aa		

^a Means + standard deviations from three replications. Means with the same uppercase letter in the same column are not significantly different (P > 0.05). Means with the same lowercase letter in the same row are not significantly different (P > 0.05).

pathogens in cookie dough. Because R² values ranged from 0.96 to 0.99, indicating a very good linear regression for each pathogen, D₁₀-values for the entire data set were calculated. Statistically significant (P < 0.05) differences between the D₁₀-values in gammairradiated and electron beam-irradiated cookie dough were not observed. However, D₁₀-values varied depending on type of pathogen. L. monocytogenes was the pathogen most resistant to both types of radiation and had the highest D₁₀-values.

3.4. The effect of gamma and electron beam irradiation on product quality

The color and texture values of cookie dough samples after gamma and electron beam irradiation at different doses are summarized in Tables 4 and 5. Color parameters are one of the critical attributes for determining the commercial quality of foods. L^{*}, a^{*}, and b^{*} values of irradiated (3 kGy) cookie dough were not significantly (P > 0.05) different from those of non-irradiated samples. Since the TPA parameters of cookie dough, including hardness, adhesiveness, cohesiveness, and resilience, reflect the development of the gluten network resulting in the structure of baked products, retention of these characteristics during a given process is very important in the baking industry. There were no significant (P > 0.05) differences in textural properties between non-irradiated and irradiated cookie dough.

4. Discussion

Recently, the FDA has amended its regulation on food irradiation evolving from a formal request for approval of irradiated foods (Code of Federal Regulations, 2013). However, the petition for approval of irradiation of complex multi-component food products has been still pending since 1999 (National Food Processors Association Food Science, 1999). Also, the Centers for Disease Control did not estimate the effects of irradiation of complex foods such as cookie dough (Sommers and Boyd, 2006). This study confirmed that gamma and electron beam irradiation is effective at reducing populations of E. coli O157:H7, S. Typhimurium, and L. monocytogenes in cookie dough. We found that the higher the radiation dose, the greater the inactivation of these pathogens. When samples were subjected to 3 kGy of gamma ray or electron beam irradiation, more than 5 log CFU/g reductions were achieved.

Most comparative studies on the effect of gamma and electron beam irradiation have focused on the differences in the penetration depth between the two radiation sources. Unlike gamma rays, electrons have shorter penetration depth (Ray and Bhunia, 2007). Waje et al. (2009) reported that gamma irradiation was more effective than electron beam irradiation in reducing pathogens in broccoli and red radish sprouts and their seeds due to the shorter penetration depth of electrons resulting in insufficient treatment. Similar results were observed in salted, seasoned, and fermented oysters (Song et al., 2009). The lack of bactericidal effect of electron beams may be due to bacteria surviving in regions beyond the reach of electron beams. Thus, there is a need to assess and statistically compare the sterilization efficacy between gamma and electron beam irradiation with identical penetrating depth.

Assuming that the penetration depth (d_p) of electron beam irradiation is given by the equation $d_p = \frac{(0.524E - 0.1337)}{d}$, where *E* is the beam energy (MeV) and \hat{d} is the density (g/ml) (Leek and Hall, 1998), and without considering the absorption of the film from the

Table 4

Color values and textural properties of cookie dough following treatment with gamma irradiation.^a

Dose (kGy)	Color ^b			Texture ^c			
	L*	a*	b*	Hardness (g)	Adhesiveness (g)	Cohesiveness	Resilience
0	78.46 ± 1.82 a	-2.84 ± 0.23 a	29.71 ± 0.52 a	396 ± 113 a	116 ± 27 a	0.50 ± 0.05 a	0.093 ± 0.006 a
0.5	78.23 ± 1.72 a	-2.71 ± 0.10 a	29.48 ± 0.41 a	$462 \pm 52 a$	109 ± 22 a	0.51 ± 0.04 a	0.087 ± 0.012 a
1	79.54 ± 0.45 a	-2.71 ± 0.08 a	30.07 ± 0.27 a	512 ± 24 a	127 ± 17 a	0.56 ± 0.05 a	0.080 ± 0.010 a
1.5	79.92 ± 0.63 a	-2.64 ± 0.24 a	29.02 ± 1.20 a	486 ± 99 a	102 ± 21 a	0.50 ± 0.02 a	0.087 ± 0.006 a
2	78.92 ± 0.50 a	-2.71 ± 0.08 a	29.73 ± 1.19 a	484 ± 24 a	105 ± 19 a	0.53 ± 0.03 a	0.090 ± 0.000 a
2.5	79.11 ± 1.02 a	-2.65 ± 0.07 a	29.01 ± 0.45 a	496 ± 30 a	140 ± 33 a	0.56 ± 0.04 a	0.083 ± 0.006 a
3	79.23 ± 1.67 a	-2.68 ± 0.18 a	29.12 ± 0.50 a	399 ± 51 a	98 ± 3 a	0.53 ± 0.06 a	0.093 ± 0.006 a

^a Means \pm standard deviations from three replications. Values followed by the same letters within the column are not significantly different (P > 0.05). ^b Color parameters are L^{*} (lightness), a^{*} (redness), b^{*} (yellowness).

^c Textural properties are represented by texture profile analysis (TPA) parameters; hardness, adhesiveness, cohesiveness, resilience.

Table 5

	following treatment with electron beam irradiation. ^a

Dose (kGy)	Color ^b			Texture ^c			
	L*	a [*]	b*	Hardness (g)	Adhesiveness (g)	Cohesiveness	Resilience
0	77.44 ± 0.31 a	-2.70 ± 0.10 a	29.16 ± 0.44 a	520 ± 98 a	75 ± 16 a	0.50 ± 0.04 a	0.087 ± 0.006 a
1	77.83 ± 1.14 a	-2.67 ± 0.13 a	29.23 ± 0.40 a	573 ± 40 a	66 ± 14 a	0.45 ± 0.04 a	0.093 ± 0.006 a
2	78.27 ± 0.42 a	-2.66 ± 0.22 a	29.46 ± 0.20 a	514 ± 17 a	91 ± 6 a	0.48 ± 0.02 a	0.085 ± 0.009 a
3	78.88 ± 1.18 a	-2.66 ± 0.05 a	29.26 ± 0.30 a	566 ± 15 a	77 ± 5 a	0.50 ± 0.01 a	$0.090 \pm 0.010 a$

^a Means \pm standard deviations from three replications. Values followed by the same letters within the column are not significantly different (P > 0.05).

^b Color parameters are L^{*} (lightness), a^{*} (redness), b^{*} (yellowness).

^c Textural properties are represented by texture profile analysis (TPA) parameters; hardness, adhesiveness, cohesiveness, resilience.

plastic bag, in the present study, the electron beam had a penetration depth of approximately 44 mm, which was enough to penetrate cookie dough which has a thickness of 10 mm. The D₁₀value of E. coli O157:H7, S. Typhimurium, and L. monocytogenes in cookie dough did not show any statistically significant (P > 0.05) differences. Our results agree with a previous study by Kim et al. (2010) which concluded that the effect of electron beam irradiation on inactivating Staphylococcus aureus inoculated into sliced pizza cheeses was similar to that of gamma irradiation at similar dose. On the other hand, Murano et al. (1999) showed significantly (P < 0.05) higher D₁₀-values for *E. coli* O157:H7 in ground beef patties exposed to gamma rays compared to electron beam treatment of identical penetrating depth. Therefore, it is a challenge to compare and explain the effect of the two irradiation sources on the survival of microorganisms. Several factors such as oxygen concentration, availability of water, and type of food substrates affect the dose-rate effects and the resultant gamma and electron beam irradiation (Murano et al., 1999).

In the present study, the order of radiation sensitivity of tested pathogens was E. coli O157:H7 = S. Typhimurium > L. monocytogenes. This order was not influenced by the irradiation source used to expose cookie dough. This result is in agreement with earlier research which suggested that L. monocytogenes has a larger D₁₀-value than S. Typhimurium in legume seeds, sprouts, and Roma tomatoes (Mahmoud, 2010; Saroj et al., 2006). Gramnegative bacteria, such as E. coli O157:H7 and S. Typhimurium, are more sensitive to ionizing irradiation than Gram-positive bacteria, such as L. monocytogenes. The radiation resistance of Grampositive bacteria is caused by effective metabolic systems which repair cellular damage such as single- and double-strand breaks of DNA and base damage (Ray and Bhunia, 2007). However, Sommers and Boyd (2006) concluded that Salmonella spp. is the pathogen most resistant to irradiation among other common pathogens, including E. coli O157:H7 and L. monocytogenes, suspended in ready-to-eat food products. The radiation sensitivity of a given pathogen can be influenced by the food substrates even when tested under identical treatment conditions. It is well established that Salmonella suspended in buffer, broth, or deboned chicken (Thayer et al., 1990) and L. monocytogenes inoculated onto three types of sausages (Sommers et al., 2003) have different radiation sensitivities. The mechanism responsible for the effect of food substrate on radiation sensitivity is not identified obviously, but it is probably involved in the substrate's ability to protect microorganisms from the oxygen and hydroxyl radicals formed during the irradiation process (Farkas, 2007).

In addition, it is essential and advantageous to investigate the quality changes occurring during ionizing irradiation for commercial practical application of this intervention. In the current study, after the maximum treatment of 3 kGy, color values (L^{*}, a^{*}, and b^{*}) and texture values of samples were not significantly (P > 0.05) different from those of the control. These results suggest that gamma and electron beam irradiation can be applied to control foodborne pathogens in ready-to-bake cookie dough without affecting color and texture changes. Other researchers also reported that ionizing irradiation resulted in food products of superior quality. Park et al. (2010) found that gamma ray or electron beam-irradiated beef sausage patties maintained color, hardness, and sensory properties. Fernandes et al. (2012) reported that the ionizing irradiation method produced no loss in physico-chemical and nutritional properties in mushrooms.

Industrial scale of food irradiation for reducing foodborne pathogens in cookie dough should be based on commercial validation. Pathogen inactivation during irradiation is dependent on sample geometry, loading configuration, density, and certain other factors (Codex Alimentarius Commission, 2011). Further studies on optimization of the process parameters are needed to guarantee product safety and simultaneously ensure regulatory compliance. Irradiation at appropriate doses could be a very promising intervention strategy against the microbiological contamination of prepackaged cookie dough.

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References

- Byrd-Bredbenner, C., Abbot, J.M., Wheatley, V., Schaffner, D., Bruhn, C., Blalock, L., 2008. Risky eating behaviors of young adults—implications for food safety education. J. Am. Dietetic Assoc. 108, 549–552.
- Centers for Disease Control and Prevention, 2009. Multistate Outbreak of E. coli 0157:H7 Infections Linked to Eating Raw Refrigerated, Prepackaged Cookie Dough. Available at: http://www.cdc.gov/ecoli/2009/0630.html.
- Clavero, M.R., Monk, J.D., Beuchat, L.R., Doyle, M.P., Brackett, R.E., 1994. Inactivation of *Escherichia coli* 0157: H7, Salmonellae, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. Appl. Environ. Microbiol. 60, 2069–2075.
- Code of Federal Regulations, 2013. 21 CFR part 179 irradiation in the production, processing and handling of food. U.S. Fed. Regist. 3, 455–457.
- Codex Alimentarius Commission, 2011. Code of Practice for Radiation Processing of Food (CAC/RCP 19-1979).
- Farkas, J., 2006. Irradiation for better foods. Trends Food Sci. Technol. 17, 148–152. Farkas, J., 2007. Physical methods of food preservation. In: Doyle, M.P., Beuchat, L.R.,
- Montville, TJ, (Eds.), Food Microbiology Fundamentals and Frontiers. American Society for Microbiology, Washington, D.C, pp. 497–519.
- Fernandes, Å., Antonio, A.L., Oliveira, M., Martins, A., Ferreira, I.C., 2012. Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushrooms: a review. Food Chem. 135, 641–650.
- Guodong, Z., Li, M., Patel, N., Swaminathan, B., Wedel, S., Doyle, M.P., 2007. Isolation of *Salmonella* Typhimurium from outbreak-associated cake mix. J. Food Prot. 70, 997–1001.
- Kim, H.J., Ham, J.S., Lee, J.W., Kim, K., Ha, S.D., Jo, C., 2010. Effects of gamma and electron beam irradiation on the survival of pathogens inoculated into sliced and pizza cheeses. Radiat. Phys. Chem. 79, 731–734.
- Leek, P., Hall, D., 1998. Portable electron beam systems. In: Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, vol. 82, pp. 1–4.
- Liu, S., Graham, J.E., Bigelow, L., Morse, P.D., Wilkinson, B.J., 2002. Identification of *Listeria monocytogenes* genes expressed in response to growth at low temperature. Appl. Environ. Microbiol. 68, 1697–1705.
- Mahmoud, B.S., 2010. The effects of X-ray radiation on *Escherichia coli* 0157: H7, *Listeria monocytogenes, Salmonella enterica* and *Shigella flexneri* inoculated on whole Roma tomatoes. Food Microbiol. 27, 1057–1063.
- Miller, R.B., 2005. Food irradiation using electron beams. In: Miller, R.B. (Ed.), Electronic Irradiation of Foods. Springer, New York, pp. 43–74.
- Murano, P.S., Brennan, R.E., Murano, E.A., 1999. Influence of various commercial packaging conditions on survival of *Escherichia coli* 0157: H7 to irradiation by electron beam versus gamma rays. J. Food Prot. 62, 10–15.
- National Food Processors Association Food Science, 1999. Food irradiation coalition petition to FDA to allow use of irradiation on variety of ready-to-eat foods. Food Irradiat. Coalit.
- Neil, K.P., Biggerstaff, G., MacDonald, J.K., Trees, E., Medus, C., Musser, K.A., Stroika, S.G., Zink, D., Sotir, M.J., 2012. A novel vehicle for transmission of *Escherichia coli* O157:H7 to humans: multistate outbreak of *E. coli* O157:H7 infections associated with consumption of ready-to-bake commercial prepackaged cookie dough–United States, 2009. Clin. Infect. Dis. 54, 511–518.
- New Zealand Food and Safety Authority, 2008. Flour Batch Believed Linked to Salmonella Outbreak. Available at: http://foodsafety.govt.nz/elibrary/industry/ Flour_Batch-Investigations_Into.htm.
- Park, J.G., Yoon, Y., Park, J.N., Han, I.J., Song, B.S., Kim, J.H., Kim, W.G., Hwang, H.J., Han, S.B., Lee, J.W., 2010. Effects of gamma irradiation and electron beam irradiation on quality, sensory, and bacterial populations in beef sausage patties. Meat Sci. 85, 368–372.
- Ray, B., Bhunia, A., 2007. Control by irradiation. In: Ray, B. (Ed.), Fundamental Food Microbiology. CRC press, Florida, pp. 411–416.
- Saroj, S.D., Shashidhar, R., Pandey, M., Dhokane, V., Hajare, S., Sharma, A., Bandekar, J.R., 2006. Effectiveness of radiation processing in elimination of *Salmonella* Typhimurium and *Listeria monocytogenes* from sprouts. J. Food Protection® 69, 1858–1864.
- Sommers, C., Fan, X., Niemira, B.A., Sokorai, K., 2003. Radiation (gamma) resistance and postirradiation growth of *Listeria monocytogenes* suspended in beef bologna containing sodium diacetate and potassium lactate. J. Food Protection®

66, 2051–2056.

- Sommers, C.H., Boyd, G., 2006. Variations in the radiation sensitivity of foodborne pathogens associated with complex ready-to-eat food products. Radiat. Phys. Chem. 75, 773–778.
- Song, H.P., Kim, B., Jung, S., Choe, J.H., Yun, H., Kim, Y.J., Jo, C., 2009. Effect of gamma and electron beam irradiation on the survival of pathogens inoculated into salted, seasoned, and fermented oyster. LWT-Food Sci. Technol. 42, 1320–1324.
- Sperber, W.H., 2007. Role of microbiological guidelines in the production and commercial use of milled cereal grains: a practical approach for the 21st Century. J. Food Prot. 70, 1041–1053.
- Thayer, D.W., Boyd, G., Muller, W.S., Lipson, C.A., Hayne, W.C., Baer, S.H., 1990. Radiation resistance of Salmonella. J. Industrial Microbiol. 5, 383–390.
- Waje, C.K., Jun, S.Y., Lee, Y.K., Kim, B.N., Han, D.H., Jo, C., Kwon, J.H., 2009. Microbial quality assessment and pathogen inactivation by electron beam and gamma irradiation of commercial seed sprouts. Food control. 20, 200–204.
- World Health Organization, 1981. Wholesomeness of irradiated food. WHO Tech. Rep. Ser. 659, 34.
- Zoulias, E.I., Piknis, S., Oreopoulou, V., 2000. Effect of sugar replacement by polyols and acesulfame-K on properties of low-fat cookies. J. Sci. Food Agric. 80, 2049–2056.