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Effects of gamma irradiation for inactivating *Salmonella* Typhimurium in peanut butter product during storage

Ga-Hee Ban, Dong-Hyun Kang *

Department of Food and Animal Biotechnology, School of Agricultural Biotechnology, Center for Food and Bioconvergence, Seoul National University, Seoul 151-921, South Korea
Center for Food and Bioconvergence, Seoul National University, Seoul 151-921, South Korea

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ABSTRACT

Three types (A, B, and C) of peanut butter product with different water activities (0.18, 0.39, and 0.65 a_w) inoculated with a 3-strain mixture of *Salmonella* Typhimurium were subjected to gamma irradiation (^{60}Co) treatment, with doses ranging from 0 to 3 kGy. The inactivation of *S.* Typhimurium in the 3 types of treated peanut butter product over a 14 day storage period and the influence of storage temperature at 4 (refrigerated) and 25 °C (ambient), and peanut butter product formulation were investigated. Three types of peanut butter product inoculated with *S.* Typhimurium to a level of ca. 6.6 log CFU/g and subjected to gamma irradiation experienced significant ($p < 0.05$) reductions of 1.3 to 1.9, 2.6 to 2.8, and 3.5 to 4.0 log CFU/g at doses of 1, 2, and 3 kGy, respectively. The time required to reduce *S.* Typhimurium in peanut butter product to undetectable levels was 14, 5, and 5 days at 25 °C after exposure to 3 kGy for products A, B, and C, respectively, and 7 days at 25 °C following exposure to 2 kGy for product C. During storage at 4 and 25 °C, survival of *S.* Typhimurium was lowest in product C compared to products A and B. Water activity (a_w) of peanut butter product was likely the most critical factor affecting pathogen survival. When a_w is reduced, radiolysis of water is reduced, thereby decreasing antimicrobial action. Overall, death was more rapid at 25 °C versus 4 °C for all peanut butter products during 14 day storage. Following gamma irradiation, acid values of peanut butter product were not significantly different from the control, and general observations failed to detect changes in color and aroma, even though lightness observed using a colorimeter was slightly reduced on day 0. The use of gamma irradiation has potential in preventing spoilage of post-packaged food by destroying microorganisms and improving the safety and quality of foods without compromising sensory quality.

1. Introduction

Salmonella Typhimurium produces diarrhea, fever, and abdominal cramps 12 to 72 h after infection (Baird-Parker, 1990; Blaser and Newman, 1982). Salmonellosis incidents are known to be linked to consumption of not only animal products such as poultry, meat or eggs, and fresh produce, but also foods of low water activity (a_w) (Shachar and Yaron, 2006). Furthermore, low concentrations of *Salmonella* have been implicated in outbreaks caused by consumption of contaminated high fat and low a_w foods, such as peanut butter (Gill et al., 1983) or chocolate (Scheil et al., 1998). Recently, multistate outbreaks involving *S.* Typhimurium in peanut butter products have been reported in the United States from 2008 to 2009 (CDC, 2009). These salmonellosis outbreaks were accountable for illnesses in 714 people resulting in more than 150 hospitalizations and 9 recorded deaths (CDC, 2010).

Salmonella can be introduced into peanut butter processing plants through various vehicles of contamination, such as raw peanuts contaminated during growth, harvest, or storage, water, animals, humans, or other vectors (Sheth et al., 2011). Commonly, peanut butter experiences heat treatment between 70 and 75 °C for approximately 20 min before packaging. *Salmonella* spp. develop an increased heat tolerance within a high fat and low a_w environment (Shachar and Yaron, 2006). *Salmonella* can survive in nuts or low a_w foods for long periods, although optimal growth of *Salmonella* strains happens at a a_w of 0.99 (Shachar and Yaron, 2006). Researchers discovered that *Salmonella* survived for at least 32 weeks after inoculation onto pecan halves (Beuchat and Heaton, 1975) and more than 24 weeks in peanut butter (Burnett et al., 2000).

To inactivate *Salmonella* in peanut butter, several methodologies such as thermal inactivation (Ma et al., 2009), high pressure (Grasso et al., 2010), and electron beam (e-beam) (Hvizdzak et al., 2010) exposure have been evaluated. However, long holding times and/or increased temperatures would likely cause undesirable changes in flavor, texture, and overall quality (Shachar and Yaron, 2006). Many researchers have been searching for an alternative

* Corresponding author at: Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, South Korea. Tel.: +82 2 880 4927; fax: +82 2 883 4928.
E-mail address: kang7820@snu.ac.kr (D.-H. Kang).

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method because thermal inactivation decreases the sensory quality of peanut butter, but none have been fully developed yet.

Among suitable alternative treatments, gamma irradiation is an established technology of well-documented safety and efficacy and an alternative to thermal processing for inactivation of microorganisms and insects. It is approved by the U. S. Food and Drug Administration (FDA) for use on meat, poultry, spices, fresh fruits, and vegetables (FDA, 2009). Its efficacy comes from the fact that its activity is not limited to surfaces but also the insides of foods, being able to penetrate the product and eliminate microorganisms (Prakash et al., 2008). The process involves exposing foods to a specific dose of ionizing irradiation from, for instance, Cobalt-60, a radioisotope of cobalt as a gamma ray source. Irradiation is known to operate through direct action by the absorption of radiation energy or indirect action by the radiolysis of water leading to the impairment of structural or metabolic functions, such as the fragmentation of DNA and the eventual death of microbial cells, hence improving the microbiological safety of foods by reducing the population of spoilage and pathogenic microorganisms (Clavero et al., 1994; Diehl, 1990; Moseley, 1989). For this reason, gamma irradiation may be effective in reducing *S.* Typhimurium in peanut butter. Moreover, like other bacteria, *Salmonella* spp. display increased heat resistance in low-a_w or high lipid foods (Mattick et al., 2000). However, no research studies to date have reported on the inactivation of *S.* Typhimurium in peanut butter, or understanding survival dynamics related to temperature and food ingredients during the storage period following gamma irradiation.

Therefore, the objective of this study was to evaluate and determine the effectiveness of gamma irradiation for reducing *S.* Typhimurium in peanut butter product and its survival characteristics in three kinds of peanut butter product at refrigerator (4 °C) and room (25 °C) temperature during 14 day storage.

2. Materials and methods

2.1. Bacterial strains and inoculum preparation

Three strains each of *S.* Typhimurium (ATCC 19585, ATCC 43971, DT 104) were obtained from the bacterial culture collection of Seoul National University (Seoul, Korea) for this study. Each strain of *S.* Typhimurium was grown in 15 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) at 37 °C for 24 h and harvested by centrifugation at 4000 ×g at 4 °C for 20 min, then washed three times with buffered peptone water (BPW; Difco). Cells in the final pellet were resuspended in sterile BPW, corresponding to approximately 10^7–10^8 CFU/ml and combined to construct a three-strain culture cocktail.

2.2. Sample preparation and inoculation

Three types (A, B, and C) of commercially processed peanut butter product were purchased from a retail supermarket. The composition of these peanut butter products is shown in Table 1. The a_w of each of the peanut butter products was measured using an Aqua Lab Model 3TE water activity meter (Decagon Devices Inc., Seoul, Korea). Twenty five gram samples of each peanut butter product were placed in sterile stomacher bags. Inocula (100 μl) were added to the samples, gently hand massaged for 1 min to ensure even distribution of the culture, and then spread into a thin layer to promote even gamma irradiation absorption. The peanut butter was then dried for 30 min inside a biosafety hood with the fan running until the a_w of the sample equaled that of non-inoculated sample. After drying, no significant differences in a_w of peanut butter were observed. The inoculated peanut butter product was labeled to receive one of four different gamma irradiation doses: 0 (control), 1, 2, and 3 kGy. The sample bags were individually sealed and placed into a larger SealPAK bag to reduce the possibility of leakage or contamination. The bags were then packed in plastic containers and stored at 25 °C until transportation to the irradiation facility. Samples were maintained at ambient temperatures for the length of the study, as per manufacturer's recommendations for optimum product quality.

Table 1
Formulations of three different peanut butter products used in this study.

Ingredient (per 32 g)	Product code		
	A	B	C
Calories (kcal)	190	190	130
Total fat (g)	16	16	5
Total carbohydrate (g)	7	7	18
Protein (g)	7	7	2
Sodium (mg)	150	120	6
Water activity (a_w)	0.18	0.39	0.65

2.3. Gamma irradiation

Samples were shipped to a gamma irradiation processing facility (Jung-eup, Korea). At the irradiation facility, samples were maintained at room temperature (25 °C) until treatment with gamma irradiation. The following target doses were applied: 0 (control), 1, 2, and 3 kGy, with actual doses being within ±5% of the target dose. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free-radical signal was measured using a Bruker EMS 104 EPR Analyzer. On conclusion of gamma irradiation treatment, the samples were repacked and shipped (held at 25 °C for 6 h). Once back at Seoul National University, samples were placed and stored in an incubator at 25 °C.

The total time from inoculation of the samples to commencement of microbial analyses was 18–20 h. The samples were prepared and inoculated for 2 h the day before. After 8 h, those were shipped and irradiated for 9 h the following day. Microbial survival over this time was monitored, with no significant ($p > 0.05$) reduction occurring in the control samples.

2.4. Bacterial enumeration

Three types of peanut butter samples were analyzed for survival of *S.* Typhimurium: not treated, immediately after irradiation (0 day), stored at 4 and 25 °C for 1, 3, 5, 7, and 14 days. Two hundred twenty five milliliters of buffered peptone water (Difco) was added to stomacher bags containing peanut butter samples and homogenized for 2 min with a stomacher (EASY MIX, AES Chemunex, Rennes, France). After homogenization, aliquots (1 ml) of stomached samples were tenfold serially diluted in 9 ml blanks of 0.1% peptone water, and 100 μl of appropriate dilutions spread-plated onto xylose lysine desoxycholate agar (XLD; Difco), a selective medium for enumeration of *Salmonella* spp. When low bacterial numbers were anticipated, 250 μl of undiluted stomacher bag contents were plated onto each of four petri dishes for a total of 1 ml. All plates were incubated at 37 °C for 24 h, and then colonies enumerated. Random presumptive *Salmonella* colonies from XLD were subjected to serological confirmation using the *Salmonella* Latex Agglutination Test (Oxoid, UK).

2.5. Acid value

Indicators of lipid oxidation in gamma irradiated peanut butter product were measured by acid value. Acid value (AV, mg KOH/g sample) titrations were determined according to the American Oil Chemists' Society Official Method Cd 3d-63 (AOAC, 1998). Acid value is the amount in milligrams of potassium hydroxide necessary to neutralize free fatty acids in 1 g of a sample. Acid value for the control and all treatments was measured after storage at 4 and 25 °C for 0, 7, and 14 days. Analyses were done in triplicate.

2.6. Color measurement

Color assessments were measured using a Minolta colorimeter (Model CR-400; Minolta Camera Co. Ltd., Osaka, Japan). Measurements were taken from irradiated and non-irradiated uninoculated samples measured at random locations on peanut butter product on days 0, 7, and 14, and averaged. L* (lightness; scale: 0–100), a* (intensity of redness; scale: −60 to +60), and b* (intensity of yellow color; scale: −60 to +60) values were measured in triplicate for each treatment and day.

2.7. Statistical analysis

All experiments were done in triplicate. The data were analyzed by ANOVA using Statistical Analysis System (SAS Institute, Cary, NC, USA) and the separation of means was tested by Duncan's multiple range test at a probability level of $p < 0.05$. All counts were normalized to 1 cm^2 areas and transformed into log_{10} values.

3. Results and discussion

3.1. Inactivation of S. Typhimurium in 3 types of peanut butter product

The effectiveness of gamma irradiation for reduction of *S.* Typhimurium in 3 types of peanut butter product at 4 or 25 °C for up to 14 day storage was tested, and results are shown in Table 2. The initial inoculum levels of *S.* Typhimurium in peanut butter products A, B, and C were 6.8, 6.4, and 6.8 log CFU/g, respectively. There were no significant ($p > 0.05$) reductions of *S.* Typhimurium populations during 14 day storage at 4 °C in peanut butter products A and B (untreated controls), while significant decreases of *S.* Typhimurium in all three peanut butter products occurred after 14 day storage at 25 °C. These results are similar to those of another study reporting the survival of *Salmonella* Tennessee in peanut butter over a 14 day storage period; after 14 day storage at 4 °C, there were no significant decreases in microbial populations (Park et al., 2008). In contrast, Burnett et al. (2000) reported no significant reduction of *Salmonella* in reduced-fat peanut spread products after 2 weeks of storage at 21 °C due to the presence of proteins, vitamins, and mineral supplements present in peanut butter spread. However, there were significant reductions in *Salmonella* cells after 6 week storage, and further reductions of up to 4.3 logs were observed after 24 week storage, possibly due to depletion of nutrients necessary for survival (Burnett et al., 2000). Although present in low numbers (32 CFU/g), *Salmonella* was detected in all samples and one of three samples stored at 5 and 21 °C, respectively, after 24 week storage (Burnett et al., 2000).

In this present study, results show that there were significant ($p < 0.05$) reductions in microbial populations after gamma irradiation doses of 1, 2, and 3 kGy in 3 types of peanut butter product on day 0. As treatment irradiation doses were increased, the rate of *S.* Typhimurium inactivation also increased, with reductions at 1, 2, and 3 kGy of 1.9, 2.8, and 3.9 log CFU/g, respectively, in product A; 1.3, 2.6, and 3.5 log CFU/g, respectively, in product B; and 1.8, 2.6, and 4.0 log CFU/g, respectively, in product C. The time required to reduce *S.* Typhimurium to undetectable levels (<1 log CFU/g) in peanut butter product was 14, 5, and 5 days at 3 kGy at 25 °C for products A, B, and C, respectively, and 7 days at 2 kGy at 25 °C for product C. Reductions of *S.* Typhimurium obtained from the different gamma irradiation dosages in this study were lower compared to e-beam treatment in another similar study. Hvizdzak et al. (2010) reported that *S.* Typhimurium was reduced by 4.9 log on XLD at the approximate e-beam dose of 3.0 kGy. However, the reduction rates of *S.* Typhimurium in gamma irradiated-peanut butter were similar to, or higher than e-beam treatment during 14 day storage.

In this current study, regardless of treatment dose over the storage period at 4 and 25 °C, survival of *S.* Typhimurium was the lowest in product C compared to products A and B. Peanut butter is a colloidal suspension of small but non-colloidal peanut particles in peanut oil (Citerne et al., 2001). When *Salmonella* is inoculated into peanut butter, the cells tend to aggregate or clump within or near the water phase (Burnett et al., 2000). Water activity (a_w) of peanut butter was likely the most critical factor affecting pathogen survival. Ionizing radiation is associated with the microbial inactivation of free radicals generated by water radiolysis (Stewart, 2001). Free radicals are transient in nature, and disappear by reacting with each other, microbial cells, and/or food ingredients (Simic, 1983). If free radicals are bound, their reaction with microbial cells decreases, and when a_w is reduced, radiolysis of water is reduced, hence decreasing the indirect mechanism to overall antimicrobial action (Black and Jaczynski, 2008). Black and Jaczynski (2008) reported that regardless of the species, a_w reduction increased most e-beam resistance of *Escherichia coli*, and even small reductions in a_w significantly increased D_{10}-values. These studies support our observations that product C, having markedly higher water activity than

Table 2
Survival of *Salmonella* Typhimurium in peanut butter products (A, B, and C) stored at 4 and 25 °C for up to 14 days.

	Day	4 °C				25 °C			
		0 kGy	1 kGy	2 kGy	3 kGy	0 kGy	1 kGy	2 kGy	3 kGy
(A)	0	6.74 ± 0.05^{Aa}	4.89 ± 0.31^{Ab}	3.98 ± 0.14^{Ac}	2.86 ± 0.53^{Ad}	6.74 ± 0.05^{Aa}	4.89 ± 0.31^{Ab}	3.98 ± 0.14^{Ac}	2.86 ± 0.53^{Ad}
	1	6.28 ± 0.57^{Aa}	4.69 ± 0.18^{ABb}	3.86 ± 0.22^{ABc}	2.75 ± 0.40^{Ad}	5.83 ± 0.59^{Ba}	4.64 ± 0.19^{ABb}	3.84 ± 0.13^{Ac}	2.18 ± 0.26^{ABd}
	3	6.14 ± 0.64^{Aa}	4.64 ± 0.14^{ABb}	3.77 ± 0.26^{ABCc}	2.63 ± 0.35^{Ad}	5.38 ± 0.66^{BCa}	4.19 ± 0.64^{ABCb}	3.46 ± 0.56^{ABb}	2.01 ± 0.38^{ABc}
	5	5.93 ± 0.82^{Aa}	4.50 ± 0.15^{BCb}	3.69 ± 0.22^{ABCb}	2.50 ± 0.43^{Ac}	5.21 ± 0.60^{BCa}	3.83 ± 0.73^{BCDb}	3.39 ± 0.55^{ABb}	1.07 ± 0.92^{BCc}
	7	5.89 ± 0.82^{Aa}	4.44 ± 0.21^{BCb}	3.54 ± 0.14^{BCb}	2.40 ± 0.53^{Ac}	4.91 ± 0.22^{Ca}	3.55 ± 0.49^{CDb}	3.01 ± 0.31^{BCb}	1.07 ± 0.92^{BCc}
	14	5.80 ± 0.87^{Aa}	4.25 ± 0.16^{Cb}	3.41 ± 0.03^{Cb}	1.13 ± 0.98^{Bc}	4.78 ± 0.19^{Ca}	3.12 ± 0.46^{Db}	2.36 ± 0.56^{Cc}	$<1^{Cd}$
(B)	0	6.36 ± 0.05^{Aa}	5.06 ± 0.37^{Ab}	3.77 ± 0.12^{Ac}	2.91 ± 0.31^{Ad}	6.36 ± 0.05^{Aa}	5.06 ± 0.37^{Ab}	3.77 ± 0.12^{Ac}	2.91 ± 0.31^{Ad}
	1	6.15 ± 0.56^{Aa}	4.86 ± 0.44^{Ab}	3.71 ± 0.16^{ABc}	2.67 ± 0.49^{Ad}	5.84 ± 0.55^{ABa}	4.65 ± 0.21^{ABb}	3.41 ± 0.29^{ABc}	2.04 ± 0.38^{Bd}
	3	6.10 ± 0.50^{Aa}	4.75 ± 0.47^{Ab}	3.57 ± 0.16^{ABCc}	2.59 ± 0.43^{Ad}	5.59 ± 0.37^{ABCa}	4.41 ± 0.20^{Bb}	3.09 ± 0.39^{Bc}	1.13 ± 0.98^{Cd}
	5	5.91 ± 0.40^{Aa}	4.69 ± 0.43^{Ab}	3.50 ± 0.14^{BCc}	2.56 ± 0.38^{Ad}	5.30 ± 0.61^{BCa}	3.80 ± 0.43^{Cb}	3.00 ± 0.36^{Bc}	$<1^{Dd}$
	7	5.82 ± 0.42^{Aa}	4.65 ± 0.47^{Ab}	3.33 ± 0.12^{CDc}	2.53 ± 0.42^{Ad}	4.98 ± 0.35^{BCa}	3.61 ± 0.38^{Cb}	2.94 ± 0.35^{BCc}	$<1^{Dd}$
	14	5.73 ± 0.38^{Aa}	4.49 ± 0.58^{Ab}	3.18 ± 0.11^{Dc}	2.19 ± 0.11^{Ad}	4.74 ± 0.23^{Ca}	3.35 ± 0.05^{Cb}	2.42 ± 0.10^{Cc}	$<1^{Dd}$
(C)	0	6.78 ± 0.16^{Aa}	5.00 ± 0.44^{Ab}	4.23 ± 0.23^{Ac}	2.81 ± 0.19^{Ad}	6.78 ± 0.16^{Aa}	5.00 ± 0.44^{Ab}	4.23 ± 0.23^{Ac}	2.81 ± 0.19^{Ad}
	1	6.70 ± 0.22^{ABa}	4.98 ± 0.45^{Ab}	4.19 ± 0.13^{Ac}	2.72 ± 0.22^{Ad}	5.97 ± 0.56^{Ba}	4.73 ± 0.37^{Ab}	3.61 ± 0.16^{ABb}	1.13 ± 0.98^{Bc}
	3	6.58 ± 0.17^{ABa}	4.90 ± 0.34^{Ab}	4.16 ± 0.14^{Ac}	2.28 ± 0.20^{Ad}	5.44 ± 0.63^{Ba}	3.81 ± 0.25^{Bb}	3.01 ± 0.61^{BCb}	1.07 ± 0.92^{Bc}
	5	6.50 ± 0.19^{ABCa}	4.86 ± 0.34^{Ab}	4.03 ± 0.19^{ABc}	2.24 ± 0.19^{Ad}	4.38 ± 0.38^{Ca}	3.31 ± 0.36^{Bb}	2.40 ± 0.53^{Cc}	$<1^{Cd}$
	7	6.41 ± 0.22^{BCa}	4.83 ± 0.34^{Ab}	3.90 ± 0.38^{ABc}	1.99 ± 0.36^{Ad}	3.74 ± 0.40^{CDa}	2.41 ± 0.39^{Cb}	$<1^{Dc}$	$<1^{Cc}$
	14	6.20 ± 0.07^{Ca}	4.79 ± 0.30^{Ab}	3.58 ± 0.50^{Bc}	1.13 ± 0.98^{Bd}	3.27 ± 0.31^{Da}	1.80 ± 0.17^{Cb}	$<1^{Dc}$	$<1^{Cc}$

Values are the mean, ±S.D. Means with the same uppercase letter in a data series relating to the same product and storage temperature within a column are not significantly different ($p < 0.05$); Means with the same lowercase letter in a data series relating to the same product and storage temperature within a row are not significantly different ($p < 0.05$).

either A or B, experienced the greatest microbial reduction during the course of our study. Thus, water activity is a major factor affecting the survival of *Salmonella* in peanut butter product during storage.

He et al. (2011) reported that lower carbohydrate content and higher storage temperatures were more effective at facilitating microbial inactivation in peanut butter with an a_w of 0.4. Furthermore, these authors observed that the presence of 0.25% salt in peanut butter led to greater log reduction compared to peanut butter without salt. And, peanut butter with high carbohydrate or low fat content protects *Salmonella enterica* from heat stress compared to the high fat and low carbohydrate formulation of conventional peanut butter (He et al., 2011). In this present study, *S.* Typhimurium in product C experienced higher microbial inactivation compared to products A and B with relatively low carbohydrate, high fat, and high sodium levels. Hence, the major control factor in product C may be high a_w in spite of high carbohydrates, low fat, and low sodium content. In addition, products A and B contain relatively more protein, which is thought to interact with radiolytic free radicals, thereby reducing the net effect of radiation damage and making the organisms somewhat more radiation-resistant (Clavero et al., 1994).

Overall, death was more rapid in peanut butter products stored at 25 °C compared with 4 °C in the three peanut butter products that were either treated or not treated with gamma irradiation during 14 day storage; with reductions in products A, B, and C of 0.6–1.7, 0.6–0.7, and 0.2–1.7 log CFU/g, respectively, at 4 °C; and 1.6–2.0, 1.4–1.8, and 1.8–3.5 log CFU/g, respectively, at 25 °C. Burnett et al. (2000) observed that an initial *Salmonella* population of 5.7 log CFU/g in peanut butter was decreased by 2.9 to 4.3 and 4.1 to 4.5 log CFU/g during storage for 24 weeks at 4 and 21 °C, respectively. Likewise, Park et al. (2008) reported that reductions of *S.* Tennessee increased more rapidly in peanut butter stored at 21 °C, compared with 4 °C.

3.2. Acid value analyses

Changes of oxidative rancidity of gamma irradiated peanut butter products over 14 day storage are shown in Table 3. Acid values of all products underwent significant ($p < 0.05$) increases in lipid oxidation at different doses on 0 day ($p < 0.05$). However, after 14 day storage, there were no differences between the control (0 kGy) and 3 kGy treatment as a function of time in all products. These results show that gamma irradiation up to 3 kGy had no direct effect on acid value, similar to the results of high fat content and low a_w peanut butter treated with e-beam radiation (El-Rawas et al., 2012). These results are in accord with Byun et al. (1995) who observed no significant differences of acid values, total lipid content, fatty acid composition, peroxide values (PV), and trans fatty acid content in soybean oil at different gamma irradiation doses (0–10 kGy). Additionally, El-Rawas et al. (2012) reported no significant ($p > 0.05$) changes of PV, TBARS, and fatty acid composition analyses in peanut butter subjected to e-beam treatment at doses up to 3.2 kGy. In contrast, Mexis and Kontominas (2009) found that PV increased significantly ($p < 0.05$), affecting the sensory property of hazelnuts.

Oxidative degradation of lipids can result in aroma and flavor deterioration of foods leading to reduced consumer preference. Sensory evaluation of pine nuts (Gölge and Ova, 2008), almonds (Mexis et al., 2009), and hazelnuts (Mexis and Kontominas, 2009) showed significant ($p < 0.05$) dose-dependent decreases in scores for aroma and taste. However, based on sensory analysis it is clear that doses higher than 3 kGy resulted in products of unacceptable organoleptic properties (Mexis et al., 2009). Likewise, general observations did not reveal noticeable differences in aroma though sensory analysis was not conducted in this present study.

3.3. Color

Color values for gamma irradiated and non-irradiated peanut butter products are shown in Table 4. Peanut butter products darken as a function of dose with significant ($p < 0.05$) decreases in L*-values (lightness) being observed at doses greater than or equal to 2 kGy on day 0 in products A and C, and 3 kGy on day 0 in product B. Likewise, Mexis et al. (2009) observed that the effect of irradiation dose was statistically significant ($p < 0.05$) for L*-value of almonds at doses above 3 kGy. El-Rawas et al. (2012) reported significant ($p < 0.05$) reductions in L*-values of peanut butter when receiving electron beam treatment at doses >3.2 kGy on day 8, at doses >7 kGy at days 4, 6, and 14 and at a dose of 27.7 kGy for day 2. In the present study, significant ($p < 0.05$) differences in L*-values of products A and B were observed after 14 days compared to that of 0 day. Darkening may be explained by the breakdown of glycosidic and peptidic linkages into carbonyl and amino compounds which could then react in the Maillard reaction (non-enzymatic browning) and form colored compounds (Sirisoontaralak and Noomhorm, 2006). Generally, Maillard reactions require a heat source for carbonyl and amino compounds to react (El-Rawas et al., 2012). However, the browning of sugars and amino acids was reported during gamma irradiation at doses up to 30 kGy (Oh et al., 2006).

Value-a* (redness) showed no significant differences ($p > 0.05$) at all doses on day 0. However, dose-dependent significant ($p < 0.05$) differences in a*-values were observed in product A at 25 °C on day 7 and significant decreases were observed at 4 and 25 °C on day 14 compared to day 0. Differences in value-b* (yellowness) were insignificant though slight reduction occurred in all products at different doses over the storage period. Bhattacharjee et al. (2003) reported that yellowing of cashew nuts increased as a function of irradiation dose. In contrast, El-Rawas et al. (2012) found a reduction in b*-values ($p < 0.05$) at doses above 7 kGy, while remaining unaffected after irradiation of peanut butter at doses up to 3.2 kGy similar to the findings of this present study.

Table 3
Acid value of peanut butter products after exposure to a range of gamma irradiation doses (0–3 kGy) analyzed by dose and by day.

°C	kGy	(Product A)			(Product B)			(Product C)		
		0 day (control)	7 days	14 days	0 day	7 days	14 days	0 day	7 days	14 days
4	0	0.32 ± 0.03^{Abc}	0.31 ± 0.05^{Aa}	0.33 ± 0.03^{Aa}	0.36 ± 0.07^{Aa}	0.33 ± 0.05^{Aa}	0.36 ± 0.04^{Aa}	0.37 ± 0.02^{Aa}	0.41 ± 0.01^{Aa}	0.37 ± 0.12^{Aa}
	1	0.27 ± 0.06^{Bc}	0.31 ± 0.02^{ABa}	0.36 ± 0.02^{Aa}	0.32 ± 0.11^{Aa}	0.35 ± 0.06^{Aa}	0.48 ± 0.18^{Aa}	0.36 ± 0.13^{Aa}	0.37 ± 0.07^{Aa}	0.37 ± 0.03^{Aa}
	2	0.40 ± 0.06^{Aab}	0.32 ± 0.03^{Aa}	0.39 ± 0.08^{Aa}	0.46 ± 0.14^{Aa}	0.40 ± 0.04^{Aa}	0.38 ± 0.07^{Aa}	0.38 ± 0.07^{Aa}	0.42 ± 0.04^{Aa}	0.40 ± 0.05^{Aa}
	3	0.43 ± 0.04^{Aa}	0.38 ± 0.05^{Aa}	0.40 ± 0.05^{Aa}	0.44 ± 0.16^{Aa}	0.42 ± 0.04^{Aa}	0.43 ± 0.07^{Aa}	0.43 ± 0.13^{Aa}	0.45 ± 0.09^{Aa}	0.43 ± 0.02^{Aa}
25	0	0.32 ± 0.03^{Abc}	0.28 ± 0.04^{Ac}	0.34 ± 0.05^{Aa}	0.36 ± 0.07^{Aa}	0.32 ± 0.04^{Aa}	0.37 ± 0.16^{Aa}	0.37 ± 0.02^{Aa}	0.41 ± 0.01^{Aa}	0.36 ± 0.17^{Aa}
	1	0.27 ± 0.06^{Ac}	0.32 ± 0.04^{Abc}	0.34 ± 0.02^{Aa}	0.32 ± 0.11^{Aa}	0.38 ± 0.06^{Aa}	0.44 ± 0.14^{Aa}	0.36 ± 0.13^{Aa}	0.40 ± 0.10^{Aa}	0.44 ± 0.07^{Aa}
	2	0.40 ± 0.06^{Aab}	0.36 ± 0.00^{Aab}	0.34 ± 0.02^{Aa}	0.46 ± 0.14^{Aa}	0.39 ± 0.05^{Aa}	0.46 ± 0.18^{Aa}	0.38 ± 0.07^{Aa}	0.38 ± 0.05^{Aa}	0.40 ± 0.04^{Aa}
	3	0.43 ± 0.04^{Aa}	0.41 ± 0.04^{Aa}	0.41 ± 0.05^{Aa}	0.44 ± 0.16^{Aa}	0.40 ± 0.05^{Aa}	0.40 ± 0.08^{Aa}	0.43 ± 0.13^{Aa}	0.46 ± 0.05^{Aa}	0.42 ± 0.04^{Aa}

Values are the mean, ±S.D. Means with the same uppercase letter in a data series relating to the same product and storage temperature within a column are not significantly different ($p < 0.05$); Means with the same lowercase letter in a data series relating to the same product and storage temperature within a row are not significantly different ($p < 0.05$).

Table 4
Color analysis* of gamma irradiated peanut butter products (A, B, and C) where L* is lightness, a* is redness, and b* is yellowness.

°C	kGy	L*			a*			b*		
		0 day	7	14	0	7	14	0	7	14
(A)										
4	0 (cont)	56.12 ± 0.33^{Aa}	55.24 ± 0.12^{ABa}	54.85 ± 0.71^{Ba}	8.68 ± 0.20^{Aa}	8.44 ± 0.07^{ABa}	8.34 ± 0.07^{Ba}	33.62 ± 0.48^{Aa}	32.11 ± 0.73^{Ba}	32.34 ± 0.44^{Ba}
	1	55.30 ± 0.17^{Aab}	54.05 ± 0.60^{Bb}	53.86 ± 0.25^{Bb}	8.33 ± 0.28^{Aa}	8.28 ± 0.06^{Aa}	8.50 ± 0.05^{Aa}	32.91 ± 0.71^{Aa}	32.17 ± 0.28^{Aa}	32.63 ± 0.13^{Aa}
	2	54.39 ± 0.87^{Abc}	53.49 ± 0.03^{Ab}	53.74 ± 0.06^{Ab}	8.57 ± 0.07^{Aa}	8.36 ± 0.05^{Aa}	8.21 ± 0.40^{Aa}	32.40 ± 0.87^{Aa}	31.99 ± 0.25^{Aa}	32.62 ± 0.80^{Aa}
	3	54.12 ± 0.50^{Ac}	53.58 ± 0.14^{Ab}	53.89 ± 0.25^{Ab}	8.39 ± 0.16^{Aa}	8.21 ± 0.21^{Aa}	8.19 ± 0.31^{Aa}	32.84 ± 0.09^{Aa}	32.36 ± 0.18^{Aa}	32.46 ± 0.67^{Aa}
25	0	56.12 ± 0.33^{Aa}	55.62 ± 0.05^{Ba}	55.37 ± 0.14^{Ba}	8.68 ± 0.20^{ABa}	8.84 ± 0.13^{Aa}	8.42 ± 0.14^{Ba}	33.62 ± 0.48^{Aa}	32.51 ± 0.83^{Aa}	33.25 ± 0.50^{Aa}
	1	55.30 ± 0.17^{Aab}	54.72 ± 0.65^{Ab}	55.00 ± 0.32^{Aab}	8.33 ± 0.28^{Aa}	8.20 ± 0.32^{Ab}	8.02 ± 0.10^{Ab}	32.91 ± 0.71^{Aa}	32.11 ± 0.20^{Aa}	32.72 ± 0.43^{Aa}
	2	54.39 ± 0.87^{Abc}	53.73 ± 0.50^{Ac}	54.84 ± 0.17^{Ab}	8.57 ± 0.07^{Aa}	8.46 ± 0.08^{Ab}	8.26 ± 0.09^{Bab}	32.40 ± 0.87^{Aa}	32.42 ± 0.17^{Aa}	32.71 ± 0.29^{Aa}
	3	54.12 ± 0.50^{ABc}	54.75 ± 0.03^{Ab}	53.76 ± 0.31^{Bc}	8.39 ± 0.16^{Aa}	8.34 ± 0.17^{Ab}	8.20 ± 0.17^{Aab}	32.84 ± 0.09^{Aa}	32.52 ± 0.25^{Aa}	32.78 ± 0.60^{Aa}
(B)										
4	0 (cont)	55.98 ± 0.58^{Aa}	55.15 ± 0.32^{Ba}	54.72 ± 0.11^{Ba}	8.54 ± 0.08^{Aa}	8.52 ± 0.17^{Aa}	8.43 ± 0.32^{Aa}	33.61 ± 0.32^{Aa}	32.87 ± 0.36^{Aa}	32.63 ± 1.13^{Aa}
	1	55.88 ± 0.55^{Aa}	54.58 ± 0.05^{Bab}	54.59 ± 0.18^{Ba}	8.37 ± 0.18^{ABa}	8.43 ± 0.12^{Aa}	8.15 ± 0.05^{Ba}	33.01 ± 0.45^{Aa}	32.68 ± 0.66^{Aa}	32.50 ± 0.29^{Aa}
	2	55.00 ± 0.63^{Aa}	54.61 ± 0.12^{Aab}	54.62 ± 0.37^{Aa}	8.43 ± 0.07^{Aa}	8.34 ± 0.09^{Aa}	8.19 ± 0.16^{Aa}	33.43 ± 0.13^{Aa}	32.44 ± 0.51^{Ba}	32.27 ± 0.67^{Ba}
	3	55.03 ± 0.04^{Ab}	54.44 ± 0.49^{Bb}	54.52 ± 0.49^{Ba}	8.37 ± 0.02^{Aa}	8.23 ± 0.20^{ABa}	8.11 ± 0.03^{Ba}	33.18 ± 0.35^{Aa}	32.24 ± 0.44^{Ba}	32.43 ± 0.37^{ABa}
25	0	55.98 ± 0.58^{Aa}	56.00 ± 0.55^{Aa}	55.12 ± 0.41^{Aa}	8.54 ± 0.08^{Aa}	8.30 ± 0.42^{Aa}	8.49 ± 0.14^{Aa}	33.61 ± 0.32^{Aa}	32.90 ± 0.49^{Aa}	32.66 ± 0.57^{Aa}
	1	55.88 ± 0.55^{Aa}	55.68 ± 0.36^{Aa}	55.38 ± 0.56^{Aa}	8.37 ± 0.18^{Aa}	8.45 ± 0.08^{Aa}	8.03 ± 0.09^{Ba}	33.01 ± 0.45^{Aa}	32.66 ± 0.91^{Aa}	32.16 ± 0.53^{Aa}
	2	55.00 ± 0.63^{Aa}	55.32 ± 0.37^{Aa}	54.98 ± 1.22^{Aa}	8.43 ± 0.07^{Aa}	8.42 ± 0.09^{Aa}	8.17 ± 0.58^{Aa}	33.43 ± 0.13^{Aa}	32.66 ± 0.55^{Ba}	32.33 ± 0.32^{Ba}
	3	55.03 ± 0.04^{Ab}	54.37 ± 0.49^{Bb}	54.77 ± 0.86^{Ba}	8.37 ± 0.02^{Aa}	8.40 ± 0.11^{Aa}	8.16 ± 0.03^{Ba}	33.18 ± 0.35^{Aa}	32.50 ± 0.27^{Ba}	32.44 ± 0.39^{Ba}
(C)\										
4	0 (cont)	33.12 ± 0.29^{Aa}	32.38 ± 0.73^{Aa}	32.58 ± 1.01^{Aa}	10.57 ± 0.42^{Aa}	10.43 ± 0.41^{Aa}	10.22 ± 0.39^{Aa}	22.29 ± 0.33^{Aa}	21.20 ± 0.25^{Bb}	21.46 ± 0.27^{Ba}
	1	32.11 ± 0.68^{Aab}	31.87 ± 0.41^{Aa}	32.83 ± 1.19^{Aa}	10.52 ± 0.29^{Aa}	10.24 ± 0.64^{Aa}	10.20 ± 0.45^{Aa}	21.36 ± 0.84^{Aa}	21.48 ± 0.22^{Ab}	21.73 ± 0.26^{Aa}
	2	31.84 ± 0.98^{Ab}	32.37 ± 0.52^{Aa}	32.36 ± 1.35^{Aa}	10.60 ± 0.35^{Aa}	10.17 ± 0.33^{Aa}	10.08 ± 0.15^{Aa}	21.82 ± 0.82^{Aa}	21.80 ± 0.46^{Aab}	21.24 ± 0.60^{Aa}
	3	31.31 ± 0.32^{Bb}	32.49 ± 0.59^{Aa}	32.25 ± 0.61^{ABa}	10.22 ± 0.91^{Aa}	10.31 ± 0.34^{Aa}	10.35 ± 0.27^{Aa}	21.21 ± 0.58^{Aa}	22.24 ± 0.44^{Aab}	21.20 ± 0.49^{Aa}
25	0	33.12 ± 0.29^{Aa}	33.31 ± 0.74^{Aa}	32.10 ± 0.77^{Aa}	10.57 ± 0.42^{Aa}	10.33 ± 0.56^{Aab}	10.08 ± 0.58^{Aa}	22.29 ± 0.33^{Aa}	21.48 ± 0.31^{Ba}	21.34 ± 0.25^{Ba}
	1	32.11 ± 0.68^{Aab}	31.97 ± 0.33^{Ab}	31.57 ± 0.69^{Aa}	10.52 ± 0.29^{Aa}	10.87 ± 0.18^{Aa}	9.86 ± 0.34^{Aa}	21.36 ± 0.84^{Aa}	21.76 ± 0.19^{Aa}	21.02 ± 0.38^{Ba}
	2	31.84 ± 0.98^{Ab}	31.78 ± 0.68^{Ab}	31.41 ± 0.92^{Aa}	10.60 ± 0.35^{Aa}	10.06 ± 0.49^{Aa}	9.95 ± 0.36^{Aa}	21.82 ± 0.82^{Aa}	21.29 ± 0.70^{Aa}	21.35 ± 0.84^{Aa}
	3	31.31 ± 0.32^{Ab}	31.77 ± 0.54^{Ab}	31.83 ± 0.89^{Aa}	10.22 ± 0.91^{Aa}	10.33 ± 0.11^{Aab}	9.81 ± 0.49^{Aa}	21.21 ± 0.58^{Aa}	21.23 ± 0.35^{Aa}	21.28 ± 0.53^{Aa}

Values are the mean, ±S.D. Means with the same uppercase letter in a data series relating to the same product and storage temperature within a column are not significantly different ($p < 0.05$); Means with the same lowercase letter in a data series relating to the same product and storage temperature within a row are not significantly different ($p < 0.05$).

Color changes in gamma irradiated peanut butter products were not consistent and not always significant using spectrophotometric measurements. General observations by the experimental team were not able to detect color changes. Several sensory evaluations involving foods such as irradiated hazelnuts (Mexis and Kontominas, 2009), and almonds (Mexis et al., 2009) reported no significant ($p > 0.05$) differences in acceptance based on color in spite of significant ($p < 0.05$) color changes.

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