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

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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

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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.