Effects of Electron Beam Irradiation on Pathogen Inactivation, Quality, and Functional Properties of Shell Egg during Ambient Storage

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Abstract

This study investigated the effects of electron beam irradiation on pathogens, quality, and functional properties of shell eggs during storage. A 1st grade 1-d-old egg was subjected to electron beam irradiation at 0, 1, 2, and 3 kGy, after which the number of total aerobic bacteria, reduction of inoculated Escherichia coli and Salmonella Typhimurium, egg quality, and functional properties were measured. Electron beam irradiation at 2 kGy reduced the number of E. coli and S. Typhimurium cells to a level below the detection limit (<102 CFU/g) after 7 and 14 d of storage. Egg freshness as measured by albumen height and the number of Haugh units was significantly reduced by 1-kGy irradiation. The viscosity of irradiated egg white was also significantly decreased by increased irradiation, whereas its foaming ability was increased. Electron beam irradiation also increased lipid oxidation in egg yolks. These results suggest that electron beam irradiation reduces the freshness of shell eggs while increasing the oxidation of egg yolk and improving important functional properties such as foaming capacity. Electron beam irradiation can also be applied to the egg breaking process since the irradiation reduces the viscosity of egg white, which can allow egg whites and yolks to be separated with greater efficiency.

Key words: egg, electron beam, pathogen, quality, functional property

Introduction

Eggs are consumed on a daily basis throughout the world (Cook and Briggs, 1986) and are a highly nutritious as well as low-cost source of protein (Theron et al., 2003). However, eggs are highly perishable, undergoing rapid degradation during the time period from collection to consumption. Bacterial contamination of egg has been shown to be mainly environmental, as aerosolized fecal particles can settle on eggs along with associated microorganisms (Theron et al., 2003). More importantly, bacteria on the surface of the egg can pass through pores in the shell and contaminate the interior, despite physical barriers that prevent microbial growth such as albumen in the egg white (Frazier and Westhoff, 1988).

Reports have found that undercooked and raw shell eggs are the major cause of Salmonella Enteritidis (SE) infection in humans (CDC, 2003). In addition, the frequency of salmonellosis caused by SE has steadily increased over the United States from 1985 to 1999, with 80% of all cases associated with eggs or egg-containing products (Patrick et al., 2004). To destroy bacteria on the egg surface at food processing level, many methods such as washing with antimicrobial solutions, heat, and UV irradiation have been applied. However, none of these techniques are effective against internal Salmonella contamination since the bacteria are sheltered from by the egg shell (Sommers and Fan, 2006).

Irradiation is a nonthermal method that can eliminate food-borne pathogens such as Salmonella, Escherichia coli, and Listeria from inside shell eggs. As a cold pasteurization technique, electron beam irradiation is more attractive for the elimination of pathogens in heat-sensitive products like fresh shell eggs (Tellez et al., 1995). Narvaiz et al. (1992) reported that irradiation above 2 kGy controls Salmonella and other pathogens in egg yolk, whereas Serrano et al. (1997) suggested that SE in...
shell eggs and liquid whole eggs could be effectively reduced (approximately 4 logs) by 1.5 kGy of irradiation. The US Food and Drug Administration approved the irradiation of shell egg at doses up to 3 kGy (USDA FSIS, 2000).

Among the sources of radiation used on food, the major limitation of electron beam irradiation is its limited penetration up to a maximum of about 8 cm at 10 MeV (Miller, 2005). Despite this, electron beam irradiation can be used on products such as grain or low-density foods such as ground spices, as well as for the removal of surface contamination on prepared meals. One consequence of the so-called “switch-off capability” of electron beam irradiation is that the technology can be integrated with food processing operations. Mitchell (1994) stated that although both radioisotopic (gamma ray) and machine sources (electron beam and X-ray) have the same level of impact on food, consumers would react more favorably to machine sources due to the association of isotopes with the nuclear industry. However, there is limited information available on the effect electron beam irradiation has on the safety, quality, and functional properties of shell egg.

Therefore, the objective of this study was to determine the safety level, quality, and functional properties of shell egg after electron beam irradiation and subsequent storage at ambient temperature.

Materials and Methods

Sample preparation and electron beam irradiation
Clean, medium-sized (65±2 g) 1-day-old eggs (the 1st grade) were purchased from Namsan poultry farm (Gongju, Korea). The eggs were irradiated in a paper box using a linear electron beam RF accelerator (Energy 2.5 MeV, beam power 40 kW, EB Tech, Daejeon, Korea) at 10±0.5°C. Irradiation was performed in the presence of air and at a conveyor velocity of 10 m/min. To confirm the target dose, alanine dosimeters attached to the top and bottom surfaces of the sample pack were read using a 104 Electron Paramagnetic Resonance unit (EMS-104, Bruker Instruments Inc., Bullerica, MA) after treatment.

Microbial analysis
The prepared sample (10 g) was homogenized using a stomacher (bag mixer® 400, Interscience Co, France) for 2 min in a sterile stomacher bag containing 90 mL of sterile saline solution. Media for the enumeration of total aerobic bacteria, yeast and mold were performed on total plate count agar (Difco Laboratories, Detroit, MI, USA), after which the plates were incubated at 37°C for 48 h. The colony forming units (CFU) per gram were counted at a dilution of 30 to 300 CFU per plate.

*Escherichia coli* (KCTC 1682) and *Salmonella Typhimurium* (KCTC 1925) were seeded in 100 mL of tryptic soy broth medium and incubated at 37°C for 20 h with constant shaking at 190 rpm. Bacteria were then transferred to a sterile rack for air-drying at room temperature, followed by electron beam irradiation for 1, 2, and 3 kGy under the same conditions explained in Section 2.1. The inoculated eggs were kept at room temperature, and the total plate count was measured at days 0, 3, and 7 following serial dilution and incubation at 37°C for 48 h on tryptic soy agar.

Egg quality and functional property
Yolk color, albumen height (mm), and Haugh units were measured using the QCM+ System (Technical Services and Supplies, York, England). Emulsion capacity of egg yolks was determined according to Jo *et al.* (2002). The foaming capacity and foaming stability of egg white were measured using the modified method of Phillips *et al.* (1990). Egg white (25 mL) was mixed with 25 mL of deionized distilled water in a 100 mL graduated cylinder, followed by homogenization at 24,200 g for 30 s using a homogenizer (T25B, IKA, Staufen, Germany). The foam height was measured as foaming capacity. Foam stability was determined by measuring the water content in the graduated cylinder after 30 min of foaming at room temperature. A higher value represents lower foam stability.

The pH values of egg white and yolk were determined using a pH meter (Model 750 P, iSTEC, Seoul, Korea) after diluting the samples with 9 volumes of DDW. Viscosity was measured using a viscometer (Model VT-03F, Rion, Tokyo, Japan).

Lipid oxidation of egg yolk
Egg yolk samples were mixed by hand for 30 s, and the 2-thiobarbituric acid reactive substance (TBARS) method was used to measure lipid oxidation (Jo *et al.*, 2002). Samples (5 g) in 15 mL of deionized distilled water (DDW) were homogenized using 50 µL of BHA (7.2%) for 15 s. Two milliliters of the homogenate was then transferred to a disposable test tube, after which 4 mL of 2-thiobarbituric acid (TBA)/trichloroacetic acid (TCA) (20 mM TBA in 15% TCA) solution was added. The mixture was blended and incubated in a boiling water bath for 15 min. The sample was then cooled in cold water for 10
min and then centrifuged for 15 min at 2,500 g and 4°C. Absorbance was measured at 532 nm, and lipid oxidation was reported as mg malondialdehyde/kg sample.

**Statistical analysis**

Statistical analysis was performed by one-way analysis of variance (ANOVA). Significant differences among mean values were identified by Duncan’s multiple range test using SAS software with a confidence level of \( p \leq 0.05 \). Mean values and standard errors of the mean are reported.

**Results and Discussion**

**Microbial analysis**

The total aerobic bacterial population of shell egg was 2.24 Log CFU/g (data not shown). No viable cells were observed immediately following electron beam irradiation or during storage. Kim *et al.* (2008) also reported that *S.* Typhimurium was eliminated from shell egg by gamma irradiation at 3 kGy, whereas *E.* coli and *S.* sciuri were eliminated by gamma irradiation at 5 kGy.

The original number of *E.* coli and *S.* Typhimurium cells inoculated on shell egg was 4.74 Log CFU/g, and irradiation at 3 kGy reduced this value by about 3 log cycles at day 0 (Table 1). Viable cell counts in egg at day 0 were lower compared to days 3 and 7. Shell egg acts as the first barrier against microbial attack due to its harsh conditions such as limited water and nutrient content (Liu *et al.*, 2009). A previous study indicated that inoculated pathogens such as *S.* Typhimurium are eliminated by 2 kGy of gamma irradiation (Liu *et al.*, 2009). Compared to the present result, gamma irradiation seems more effective than electron beam irradiation in eliminating microorganisms in shell egg. Generally, a somewhat weaker effect was observed in food irradiated with electron beams compared to gamma ray (Song *et al.*, 2009a) due to the varying depths of penetration between the two radiation sources. Electron beams unlike gamma ray have limited penetration (Miller, 2005) and higher dosages (Ito *et al.*, 1994), which may affect microbial inactivation on the size of packaging (Song *et al.*, 2009a). Another concern is that the energy level of the electron beam irradiator used in this study (2.5 MeV) could only provide limited penetration. Although shell eggs were placed evenly to improve the dose distribution, only one side was irradiated.

**Egg quality**

Table 2 shows the general quality of shell egg treated with electron beam irradiation. Irradiation did not have any effect on yolk color. In contrast, Huang *et al.* (1997) reported that egg yolk irradiated at 10 MeV had a significantly higher yolk color than non-irradiated samples. The color change could have been caused by the destruction of carotenoids, which decompose in proportion to the amount of radiation (Katusin-Razem *et al.*, 1992). However, this bleaching effect is insignificant compared to natural variations in pigment concentration that occur

<table>
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<th>Pathogens</th>
<th>Irradiation dose (kGy)</th>
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<th>7</th>
<th>14</th>
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<td></td>
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<td>4.05&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
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</table>

<sup>a</sup>Means with different superscripts within a column are significant at \( p \leq 0.05 \).

<sup>b</sup>Viable cells were not detected at a detection limit at 10<sup>5</sup> CFU/g.

<sup>c</sup>Standard errors of the mean (\( n = 12 \)).
among different samples (Brooks et al., 1959).

Albumen height and Haugh units were both reduced upon electron beam irradiation, thus indicating a loss of freshness. Ma (1996) reported that irradiation results in a decrease in the number of Haugh units at 1-3 kGy. The main explanation for this phenomenon is that the irradiation treatment ruptured the albuminous sac, resulting in the loss of thick albumen most likely due to the irradiation-induced scission of O-glycosides from ovomucin protein (Ma, 1996). This is one of the major changes in quality for irradiated shell egg (Liu et al., 2009).

**Functional properties**

The emulsion capacity of shell eggs did not change upon electron beam irradiation (Table 3). Huang et al. (1997) reported that egg yolk electron beam irradiated at 10 MeV had significantly higher emulsion capacity than non-irradiated samples, and the differences were significant within the first 7 d of frozen storage. Previous studies demonstrated that differences in emulsion capacity were observed for eggs treated with gamma irradiation (Liu et al., 2009; Ma et al., 1990). The major differences in the results must be due to the penetration power and dose rate.

The viscosity of irradiated egg white decreased significantly with increased irradiation dose (Table 3). Chain scission reduces the number of peptide linkages in irradiated eggs, thereby decreasing the viscosity. This result is in accordance with previous studies using gamma ray (Ma et al., 1990; Pinto et al., 2004). The irradiation of proteins is known to cause denaturation as well as the formation of protein radicals due to interactions with water, resulting in reactions with constituent amino acid subunits (Stewart, 2001). Yang and Balwin (1995) suggested that changes in the viscosity of egg white were associated with the unfolding and aggregation of egg white proteins. They also showed that the viscosity of egg white is positively related to foaming ability, as most protein systems with high viscosity exhibit surface tension dependence (Phillips et al., 1994). Song et al. (2009b) found that irradiated liquid egg white with reduced viscosity may also have decreased surface tension, which creates a large surface area that is essential for foaming.

Protein foams are characterized by two factors, namely foaming ability and foam stability (Song et al., 2009b). Foaming ability was increased significantly while foam stability was decreased upon irradiation during storage at room temperature (Table 3). Ma et al. (1990) reported that foaming ability is improved by irradiation due to conformational changes of proteins in the egg white that increase surface hydrophobicity and lower viscosity. Clark et al. (1992) also reported improved functional properties for spray-dried egg white irradiated at 2 kGy or above. This is because irradiation causes small changes in secondary structures (i.e., from α-helix to random structure), thus enhancing some functional properties. Further, heat-treatment at high temperature (above 55°C) seems to enhance these protein-protein interactions at the interface.

### Table 3. Functional properties of shell egg treated with electron beam irradiation during ambient storage

<table>
<thead>
<tr>
<th>Storage period (d)</th>
<th>Irradiation dose (kGy)</th>
<th>Emulsion capacity (%)</th>
<th>Viscosity (white, mPa·s)</th>
<th>Foaming ability (mm)</th>
<th>Foam stability (mm)</th>
<th>pH (white)</th>
<th>pH (yolk)</th>
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*Mean values with different superscripts within a column are significant at p≤0.05.

aStandard errors of the mean (n=12).
Effects of Electron Beam Irradiation on Egg

possibly due to interactions between heat-exposed hydrophobic groups or sulfhydryl groups (Van der Planckt et al., 2006). Foam stability is important for the shell life and product appearance of food foams and must be maintained during exposure to a variety of processes such as heating, mixing, and cutting (Foegeding et al., 2006). Song et al. (2009b) obtained a similar result using gamma irradiated eggs, reporting that irradiation of egg white offers advantages for increasing foaming ability, improving the quality of final bakery products, and reducing the microbial load in egg white.

The pH values of egg yolk and egg white did not change upon electron beam irradiation (Table 3). In contrast, the pH levels of gamma irradiated egg white and egg yolk were increased by increased irradiation (Liu et al., 2009). Further, Huang et al. (1997) reported that the pH of electron beam irradiated (10 MeV) egg yolk was significantly higher than that of the non-processed sample.

2-Thiobarbituric acid reactive substance (TBARS) values

One of the concerns regarding irradiated foods rich in fat content is the acceleration of lipid oxidation. The TBARS values of egg yolk were increased by irradiation and also during storage (Table 4). Fresh shell egg lipids are not easily oxidized (Pike and Peng, 1985) since the structure of phospholipids in the yolk aids in the prevention of oxidation (Burley and Vadehra, 1989). However, Liu et al. (2009) reported that gamma irradiation increases the oxidation of polyunsaturated fatty acids in shell egg while chitosan coating may retard the development of lipid oxidation.

In conclusion, electron beam irradiation can, similar to the effects of gamma ray, reduce the freshness of shell egg while increasing the oxidation of egg white. However, this method may improve important functional properties such as foaming capacity. It also can be applied to the egg breaking process because the reduced viscosity of egg white can increase the separation efficiency of egg white and yolk.

Acknowledgement

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References


Table 4. 2-Thiobarbituric acid reactive substances values (mg malondialdehyde/kg) of egg yolk treated with electron beam irradiation during ambient storage

<table>
<thead>
<tr>
<th>Irradiation dose (kGy)</th>
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<td>0.03</td>
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</table>

¹SEM: Standard errors of the mean (n=12).
²Means with different superscripts within a column are significant at p≤0.05.

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References

