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Microbial Safety and Physicochemical Characteristics of Electron Beam Irradiated Whole Egg Powder

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Abstract The effect of electron beam (EB) irradiation on inactivation of foodborne microorganisms and the quality of whole egg powder (WEP) was investigated. WEP sample was irradiated (0, 1, 2, and 4 kGy) and stored for 2 months at 25°C. The D_{10} values for *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* Typhimurium were 0.26, 0.13, and 0.26 kGy, respectively. The L* value increased, while the a* and b* values decreased with increasing irradiation dose. Foaming ability of irradiated samples increased at 0 and 1 months, but not at 2 month. The peroxide value of sample increased both with irradiation and storage. After 1 and 2 months, samples irradiated at 2 and 4 kGy had lower sensory scores for color than those irradiated at 1 kGy. These results suggested that the use of low-dose EB irradiation (≤2 kGy) could improve microbial safety and foaming ability of the WEP with minimal quality changes.

Keywords: whole egg powder, electron beam irradiation, microorganism, quality

Introduction

The consumption of egg products has been increasing worldwide (1). In particular, the use of egg powder has consistently increased owing to its convenience in commercial food processing and home preparation and relatively cheaper price. Egg powder is generally known to possess a long shelf-life and be stored conveniently because of pasteurization and drying (2). However, some microorganisms can survive, increasing the risks associated with consumption evidenced by several food poisoning outbreaks related to egg powder (3). Furthermore, chemical modifications could occur during these processes, thereby deteriorating the quality and functional properties naturally found in eggs (2).

Electron beam irradiation (EB) is an excellent method of inactivating microorganisms and thus prolonging the shelf life of eggs and egg products (4). Serrano *et al.* (5) have reported that contamination of shell eggs and liquid eggs were effectively reduced using 1.5 kGy of irradiation. Thus, the US Food and Drug Administration has approved the irradiation of shell egg at doses less than 3 kGy (6). In Korea, only egg powder including egg yolk powder, egg white powder, and whole egg powder, has approved the irradiation at doses less than 5 kGy (7). Huang *et al.* (8) showed that EB at 2.5 kGy did not affect any significant physico-chemical and functional changes in the egg yolk. Similarly, an irradiation of 1.5 kGy did not affect the color and thermal characteristics of shell eggs and liquid eggs (5). However, limited information is available about the physico-chemical and functional properties of EB-irradiated whole egg powder (WEP) during storage.

Therefore, the objective of this study was to investigate the effect of EB on microbial safety and physicochemical properties of WEP during ambient storage (25°C) over a 2-month period.

Materials and Methods

Sample preparation Commercial WEP (Edentown F&B, Jinchon, Korea) was purchased from a local market in Daejeon, Korea. Samples (5 g each) were vacuum-packaged in polyethylene/nylon bags (2 mL O_2/m^2 per day at 0°C, Kuk Young Export Packaging Co., Daejeon, Korea).

Sterilization, test microorganisms, culture conditions, and inoculation For the inoculation test, samples were randomly selected and sterilized using EB irradiation (35 kGy at 2.5 MeV) with a linear electron beam RF accelerator (EB tech, Daejeon, Korea). Three pathogens, *Escherichia coli* (KCTC 1682), *Listeria monocytogenes*

(KCTC 3569), and *Salmonella* Typhimurium (KCTC 1925), were obtained from the Korean Collection for Type Cultures (KCTC, Daejeon, Korea) for this study. The pathogens were grown in tryptic soy broth (Difco Laboratories, Detroit, MI, USA), tryptic soy broth containing 0.6% yeast extract (Difco Laboratories), and nutrient broth (Difco Laboratories), respectively. *E. coli* tested in the present study is non-pathogenic but can be used as a surrogate for the pathogenic *E. coli* O157:H7 in an inactivation treatment (9). All microorganisms were incubated at 37°C for 48 h. The activated cell cultures were centrifuged (2,795x*g* for 10 min) in a refrigerated centrifuge (Vs-5500; Vision Scientific Co., Seoul, Korea), and the cultures were washed twice with sterile saline solution. The pellet was finally suspended in sterile saline solution to a cell density of approximately 10^9 colony-forming units (CFU)/mL. The test culture suspension (100 μL) was spread onto the sterile WEP samples. Each sample was then resealed and shaken for homogenization.

Electron beam (EB) irradiation Each prepared sample was irradiated on both sides in a linear EB radio frequency accelerator (energy, 2.5 MeV; beam power, 40 kW, EB Tech.). The beam current was 0-4.5 mA. Irradiation was performed with a conveyor velocity of 10 m/min and a dose rate of 1.1-4.4 kGy/s. Because the incident EB had low penetration power, all the egg powder samples were prepared to a thickness of 0.7 cm to enhance the effectiveness of irradiation. To confirm the target dose, we attached alanine dosimeters to the top and bottom surfaces of the sample packs, and the results were read using a 104 Electron Paramagnetic Resonance unit (Bruker Instruments Inc., Billerica, MA, USA). The doses used in this study were 0, 0.5, 1, 2, 3, and 4 kGy. After irradiation, the samples were immediately stored under commercial storage conditions (25°C) until further analysis was performed.

Microbial analysis Samples (5 g each) were homogenized for 2 min in sterile Stomacher bags containing 45 mL of sterile saline solution using a Stomacher BagMixer® 400 (Interscience Co., Saint-Nom-La-Breteche, France). Then, the samples were serially diluted in sterile saline (0.85%) solution, and each diluent (0.1 mL) was spread. For inoculation tests, tryptic soy agar (Difco Laboratories) was used for *E. coli*, tryptic soy agar containing 0.6% yeast extract for *L. monocytogenes*, and nutrient agar (Difco Laboratories) for *S.* Typhimurium. Plates were incubated at 37°C for 48 h, and microbial counts were expressed as CFU/g. The radiation sensitivity of the microorganisms was calculated as D_{10}, a value that represents the dose required to inactivate 90% of the microbial population.

Storage stability EB irradiation Each prepared sample (non-sterilized) was irradiated using same conditions previously described above. The applied doses were 0, 1, 2, and 4 kGy. After irradiation the samples were immediately stored under commercial storage conditions at 25°C during 2 months.

Microbial analysis Samples (5 g) were homogenized with 45 mL of sterile saline solution using a Stomacher BagMixer® 400 (Interscience Co.). The homogenized samples were serially diluted in sterile saline and plated by spreading on each bacterial media in the same way as microbial analysis of inoculation test. Total plate count agar (Difco Laboratories) and eosin methylene blue agar (Difco Laboratories) were used for total aerobic bacteria counts and total coliform counts, respectively. Plates were then incubated at 37°C for 48 h. For enumerating mold count, samples were plated onto yeast/malt extract agar (containing 10% citric acid, pH 3.6, Difco Laboratories), and the plates were incubated for 5 days at 25°C.

Color measurement The color of the egg powder was evaluated using a color difference meter (Spectrophotometer CM-3500d; Konica Minolta Sensing, Inc., Osaka, Japan), and Hunter color L* (lightness), a* (redness), and b* (yellowness) values were determined. The instrument was calibrated with standard black and white plates before analysis. The color values were monitored using a computerized system using spectra magic software (Konica Minolta Sensing, Inc.), and the measurements were performed in triplicate.

Foaming ability The egg powder was added to distilled water in a 1:1 (w/v) ratio. A 30 mL solution was poured into a 100 mL cylinder and whipped for 30 s with a homogenizer (Model T25 basic; IKA Works, Kuala Lumpur, Malaysia) at 1,900 rpm. The foaming ability was defined as the foam volume (mL) measured at 1 min after the end of whipping.

Peroxide value (POV) For POV analysis, the lipid extraction of egg powder was modified using Folch's extraction method (10). The extracted lipid sample was treated with 35 mL of a solvent mixture (acetic acid:chloroform, 3:2) and shaken thoroughly, followed by the addition of 0.5 mL of a saturated potassium iodide solution. The resulting mixture was kept in the dark for 5 min and then 75 mL of distilled water were added, followed by vigorous mixing. A starch solution in phosphate buffer (2.5 mL, 1%, w/v) was employed as an indicator. The POV was determined by titrating the iodine liberated from potassium iodide against a standardized 0.005 N sodium thiosulfate solution. The POV was calculated using the following equation:

$$\text{POV (meq/kg)} = \frac{(V_1 - V_0) \times F \times 0.01}{S} \times 1000$$

V_1: titration volume (mL) of 0.005 N $Na_2S_2O_3$ for the samples
V_0: titration volume (mL) of 0.005 N $Na_2S_2O_3$ for the blank
F: factor of 0.005 N $Na_2S_2O_3$ solution
S: sample weight (g)

Sensory evaluation Sensory evaluation was performed by 10 panelists with previous experience in analyzing off-odor induced by

irradiation and quality of WEP. The samples were assessed for their color and odor. A seven-point hedonic scale, wherein seven represented strong liking and 1 represented strong disliking, was employed for evaluating all the sensory parameters. This method of sensory evaluation was conducted three times for the same storage month independently.

Statistical analysis Statistical analysis was performed with one-way analysis of variance. When significant differences were detected, the differences among the mean values were identified with the Student-Newman-Keul multiple range test using SAS software (Version 9.2) at a confidence level of $p<0.05$. Mean values and standard errors of the mean are reported.

Results and Discussion

Inoculation test The microorganisms inoculated into the commercial WEP are shown in Table 1. All microorganisms were initially loaded at 10^9 CFU/g for inoculation. Irradiation of 1 kGy showed an approximate 4 decimal reduction of *E. coli*. No viable cells were observed in 2- and 4-kGy irradiated samples. Irradiation of 0.5 kGy caused an approximate 4 log reductions in the number of *L. monocytogenes*, and no viable cells were observed after irradiation at 1 kGy at detection limit less than 10^1 CFU/g. The original number of *S.* Typhimurium cells inoculated onto the sample was 7.82 log CFU/g, and irradiation at 1 kGy resulted in an approximate 2.5 decimal reduction.

Several studies on the inactivation of microorganisms in eggs and egg products by irradiation have been conducted. Narvaiz *et al.* (3) have shown that irradiation higher than 2 kGy effectively reduced *Salmonella* in egg powder. *L. monocytogenes, E. coli,* and *S.* Typhimurium were undetected when the inoculated shell eggs were treated with 2 kGy of electron beam dosage and storage for 7 days and *S.* Typhimurium is eliminated from shell eggs by 3 kGy of irradiation, whereas *E. coli* and *Staphylococcus sciuri* were eliminated at 5 kGy (11,12). Liu *et al.* (13) also reported that inoculated *S.* Typhimurium in liquid egg white and yolk was eliminated by 2 kGy of gamma irradiation.

Table 2 shows the calculated D_{10} values of EB on WEP. D_{10} values of bacteria in food are affected by a number of factors including water activity, food composition, irradiation or storage temperature, and presence of oxygen (14). Certain components in food system, such as proteins, interact with radiolytic free radicals which can reduce the effect of radiation damage and render the organisms more radiation-resistant (15).

Matic *et al.* (16) have reported that the D_{10} value of gamma-irradiated egg powder was 0.8 kGy for a mixture of the strains, *Salmonella* Lille, *S.* Enteritidis, and *S.* Typhimurium. Carbo Verde *et al.* (17) have shown that D_{10} values in whole eggs vary between 0.31-0.26 kGy and 0.20-0.18 kGy for *S.* Typhimurium and *S.* Enteritidis and between 0.20-0.18 kGy and 0.07-0.09 kGy for *Campylobacter coli* and *C. jejuni* for shell eggs and yolks and whites, respectively. Radiation D_{10} values in liquid whole eggs have been reported to be 0.18, 0.39, and 0.49 kGy for *Aeromonas hydrophila*, *S.* Enteritidis, and *L. monocytogenes*, respectively (18). The major extracellular environmental factors that influence the survival of irradiated organisms are temperature, pH, phase, gaseous environment, water activity, and chemical composition of foods (19). The present study indicated that the dried state of WEP enhanced the radiation sensitivity of the tested microorganisms.

Table 1. Effects of the electron beam irradiation on different microorganisms inoculated into whole egg powder

Irradiation dose (kGy)	Pathogens (log CFU/g)		
	Escherichia coli	*Listeria monocytogenes*	*Salmonella* Typhimurium
0	7.87[a]	7.89[a]	7.82[a]
0.5	5.11[b]	3.21[b]	6.16[b]
1	3.30[c]	ND[c2)]	5.32[c]
2	ND[d]	ND[c]	ND[d]
3	ND[d]	ND[c]	ND[d]
4	ND[d]	ND[c]	ND[d]
SEM[1)]	0.054	0.030	0.037

*Initial population: *Listeria monocytogenes*, 9.98 log CFU/mL; *Escherichia coli,* 9.99 log CFU/mL; *Salmonella* Typhimurium, 9.55 log CFU/mL
[1)]Standard errors of the mean (n=24)
[2)]Viable with no growth at a detection limit < 10^1 CFU/g
[a-d]Values with different letters within the same column differ significantly ($p<0.05$).

Table 2. D_{10} values of the different microorganisms inoculated into whole egg powder

Pathogens	D_{10} value (kGy)
Listeria monocytogenes	0.13[b]
Escherichia coli	0.26[a]
Salmonella Typhimurium	0.26[a]
SEM[1)]	0.018

[1)]Standard errors of the mean (n=12)
[a,b]Values with different letters within the same column differ significantly ($p<0.05$).

Storage stability Microbial analysis The total aerobic bacterial population in the commercial WEP was 3.30 log CFU/g. No viable cells were detected for 2 months in samples irradiated at 1 kGy or higher with a detection limit of <10^1 CFU/g (Table 3). Molds and coliform bacteria were not detected from the initial stage or during 2 months at 25°C (data not shown). Kim *et al.* (4) have reported that the total aerobic bacterial count in shell eggs after EB (2 kGy) was not detected after 7 and 14 days of storage. Many studies have reported that the inactivation of microorganisms by EB is attributable to the inhibition of DNA repair mechanism owing to the increased energy demands of homeostasis on the cell (20,21).

Color Hunter color L* value of the WEP increased, while the a* and b* values decreased with increasing irradiation dose. These values

Table 3. Total aerobic bacterial count (log CFU/g) of whole egg powder after electron beam irradiation during storage at 25°C

Irradiation dose (kGy)	Storage periods (month)		
	0	1	2
0	3.30[a]	4.17[a]	4.33[a]
1	ND[b2)]	ND[b]	ND[b]
2	ND[b]	ND[b]	ND[b]
4	ND[b]	ND[b]	ND[b]
SEM[1)]	0.022	0.009	0.016

[1)]Standard errors of the mean (n=12)
[2)]Viable with no growth at a detection limit < 10^1 CFU/g
[a,b]Values with different letters within the same column differ significantly (p<0.05).

Table 4. Hunter color value of whole egg powder after electron beam irradiation during storage at 25°C

Hunter color	Irradiation dose (kGy)	Storage periods (month)		
		0	1	2
L*	0	82.53[b]	81.45[c]	81.40[c]
	1	83.85[a]	81.98[b]	81.61[bc]
	2	84.04[a]	82.18[ab]	81.92[ab]
	4	83.98[a]	82.61[a]	82.05[a]
	SEM[1)]	0.385	0.136	0.119
a*	0	8.16[a]	8.39[a]	8.36[a]
	1	7.51[b]	7.17[b]	7.08[b]
	2	6.58[c]	6.32[c]	6.31[c]
	4	6.19[d]	6.09[c]	5.95[d]
	SEM	0.080	0.080	0.040
b*	0	34.60[a]	35.21[a]	34.88[a]
	1	32.34[b]	31.99[b]	31.63[b]
	2	29.38[c]	29.05[c]	28.93[c]
	4	28.27[d]	28.10[d]	28.04[d]
	SEM	0.168	0.175	0.118

[1)]Standard errors of the mean (n=12)
[a-d]Means within the same column with different superscript differ significantly (p<0.05).

remained consistent over a 2-month period (Table 4). This radiation-induced discoloration of the egg yolk has been observed in previous studies (13,22,23). Dvořák *et al.* (24) reported that the levels of the parameters L*, a*, and b* showed a significantly decreased when the eggs were irradiated using Co-60 at doses of 1, 2.5 kGy, and 5 kGy. Du and Ahn (25) also showed that a* and b* values of egg yolk powder was continuously decreased during storage with proportional to irradiation dose by destruction of pigment such as carotenoids in egg yolk. The color change could have been caused by destruction of carotenoid in the egg yolk, the decay of which was proportional to the radiation dose (26). Egg yolk color is changed by yolk carotenoids which contain unsaturated double bonds and able to be oxidized as lipid oxidation does (25). However, Serrano *et al.* (5) indicated that 1.5 kGy of EB did not cause any adverse changes in the color of either the egg white or the yolk.

Foaming ability The foaming ability of egg white could be influenced by various factors including methods of beating, pretreatments, and addition of ingredient (27). Wong and Kitts (12) reported that electron beam doses of 0, 2, 3, and 4 kGy did not affect the foaming capacity of irradiated egg whites (p<0.05). In the present study, however, an increase in the foaming ability of irradiated samples was observed at months 0 and 1, but not 2 during storage (p<0.05) (Table 5). The previous study indicated that the foaming ability of egg whites was enhanced by gamma irradiation (28). This increase in the foaming ability after irradiation was due to conformational changes in the proteins of egg whites that increased the surface hydrophobicity and lowered the viscosity (23,29). Clark *et al.* (30) and Liu *et al.* (29) also reported improved functional properties in spray-dried egg whites irradiated at ≥2 kGy, because irradiation caused changes in the secondary structure (from an α-helix to a random coil) and disulfide bond, thereby enhancing some functional properties. Song *et al.* (28) reported that an angel cake prepared from irradiated liquid egg whites and egg white powder had greater volume and height, but lower hardness, which supported the increase in the foaming ability of final products that underwent irradiation. In contrast, Min *et al.* (31) reported that the oxidative changes of proteins by irradiation, especially globulins, ovomucin, and lysozyme, would result in deterioration of foam properties in egg white.

Table 5. Foaming ability (mL) of whole egg powder after electron beam irradiation during storage at 25°C

Irradiation dose (kGy)	Storage periods (month)		
	0	1	2
0	17.5[c]	18.3[b]	19.0[a]
1	19.0[b]	20.0[a]	19.7[a]
2	19.8[b]	19.7[a]	19.8[a]
4	21.5[a]	19.5[a]	20.5[a]
SEM[1)]	0.36	0.27	0.51

[1)]Standard errors of the mean (n=12)
[a-c]Means within the same column with different superscript differ significantly (p<0.05).

Table 6. Peroxide value (meq/kg) of whole egg powder after electron beam irradiation during storage at 25°C

Irradiation dose (kGy)	Storage periods (month)		
	0	1	2
0	0.13[a]	0.16[b]	0.14[b]
1	0.16[a]	0.21[b]	0.17[b]
2	0.20[a]	0.25[ab]	0.22[ab]
4	0.30[a]	0.36[a]	0.33[a]
SEM[1)]	0.075	0.040	0.043

[1)]Standard errors of the mean (n=12)
[a,b]Means within the same column with different superscript differ significantly (p<0.05).

Peroxide value (POV) Irradiation induces lipid oxidation by hydroxyl radicals generated by ionizing radiation in foods (32), and this adversely affects color, flavor, and texture of foods (33). Therefore, lipid oxidation is main a main factors in the quality irradiated egg

Table 7. Sensory attributes of whole egg powder after electron beam irradiation during storage at 25°C

Irradiation dose (kGy)	Color	Odor
	0 month	
0	4.9[a]	5.2[a]
1	4.9[a]	4.1[b]
2	4.8[a]	3.5[b]
4	4.0[a]	3.8[b]
SEM[1)]	0.62	0.54
	1 month	
0	5.4[a]	5.5[a]
1	5.7[a]	4.9[a]
2	4.3[b]	3.8[a]
4	4.0[b]	4.2[a]
SEM	0.43	0.51
	2 month	
0	5.6[a]	5.3[a]
1	5.6[a]	4.4[ab]
2	4.0[b]	4.5[ab]
4	4.0[b]	4.2[b]
SEM	0.49	0.47

[1)]Standard errors of the mean (n=12)
[a,b]Means within the same column with different superscript differ significantly (p<0.05).

products. An increase in the EB dose increased the POV (Table 6). This result was in agreement with earlier studies that reported the significance of irradiation in increasing lipid oxidation through the production of hydroxyl radicals from water radiolysis (29). Du and Ahn (25) also reported that the lipids in egg yolk powder can be oxidized during storage, and the changes can be accelerated by irradiation.

The POV of WEP increased during the first month of storage, followed by a decrease in the second month. The primary products of lipid oxidation were hydroperoxides, which are generally considered to be intermediate products in the oxidation process, which later degrade to secondary byproducts (34). Therefore, it is possible that the POV of the WEP decreased due to the longer storage period.

Sensory evaluation The sensory score for color was lower in the samples irradiated at 2 and 4 kGy than those irradiated at 0 and 1 kGy and stored for 1 and 2 months. In the beginning (0 month), the odor of the sample irradiated at ≥1 kGy was lower than that irradiated at 0 kGy. However, the odor was not different at 1 month, while only the sample irradiated at 4 kGy had a lower score than the other samples at 2 month (Table 7).

The destruction of carotenoids was closely correlated with irradiation dose, which implied that the dose-dependent production of free radicals contributed to the phenomenon (26). Other volatile compounds can be formed from egg proteins and lipids by irradiation via the radiolysis of amino acid side chains, followed by secondary reactions among the products of primary degradation (31). Ahn *et al.* (35) indicated that volatile sulfur compounds could have relevance to off-odor in irradiated animal origin foods.

Therefore, an appropriate processing technique such as packaging method, addition of antioxidants to prevent lipid oxidation, and certain flavors to mask or inhibit sensorial changes will be needed to efficiently apply irradiation for the production of WEP.

The present study indicated that low-dose electron beam irradiation (≤2 kGy) reduced or eliminated the risk of pathogens and enhanced the foaming ability of WEP. This low-dose electron beam irradiation resulted in minimum losses to physicochemical quality of whole egg powder during storage at ambient temperature.

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Disclosure The authors declare no conflict of interest.

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