Quality Prediction of Eggs Treated in Combination with Gamma Irradiation and Chitosan Coating Using Response Surface Methodology

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ABSTRACT

The aim of this work was to determine the method and predict the optimum conditions for egg quality stored for 7 days when combination treatments of irradiation and chitosan coating were applied using response surface methodology (RSM). A central composite design was chosen for the RSM in this study and the factors were irradiation dose (0~2 kGy) and concentration of chitosan coating material (0~2%). Performance of the irradiation and chitosan coating were evaluated by analyzing the egg quality and functional property factors. The predicted maximum level of Haugh units and foaming ability calculated by a developed model were 74.19 at 0 kGy of irradiation with coating by 0.96% chitosan solution and 50.83 mm at 2.0 kGy with 1.01%, respectively. The predicted minimum value of foam stability and 2-thiobarbituric acid reactive substances (TBARS) value were 2.97 mm at 0.39 kGy with 0.21% and 0.54 mg malonaldehyde/kg egg yolk at 0 kGy with 0.90% of chitosan solution, respectively. Results clearly showed that gamma irradiation negatively affected the Haugh unit and TBARS but positively affected the foaming capacity. The estimated value from the developed model by RSM was verified by no statistical difference with observed value. Therefore, RSM can be a good tool for optimization and prediction of egg quality when 2 or more treatments are combined. However, one should decide the target quality first to achieve a successful implementation of this technology.

(**Key words** : Egg, Irradiation, Chitosan, Response surface methodology)

INTRODUCTION

Many pathogenic bacteria have been isolated from the chicken eggs including *Escherichia coli* O157:H7, *Salmonella* spp, *Campylobacter jejuni,* and *Listeria monocytogenes.* Especially, *Salmonella* Enteritidis has been the leading cause of all egg-related food borne illness (Wong et al., 2003). Usually eggs are infected in the upper oviduct of infected laying hens. *Salmonella* exists on the surface of the shell that can penetrate into the interior of eggs and contaminate the internal content (Ma et al., 1996). To improve egg safety, heat pasteurization is the only commercial process used to eliminate pathogen from egg products, but this technique is ineffective against transovarian and horizontal contamination, since the bacteria located in the albumen or yolk are sheltered from the heat treatment by the egg shell (Wong et al., 2003). Moreover, egg white proteins such as ovotransferrin can be denatured by heat treatments at lower temperature (Ko et al., 2011).

To achieve hygienic quality of egg, which cannot use high heat pasteurization, natural antimicrobial agents can be used for the treatment on egg shell. For example, Liu et al. (2009a) reported that chitosan coating on egg shell controlled the growth of *Salmonella* by 2% of solution concentration. Another advantage of antimicrobial coating on egg shell was the inhibition of moisture loss from eggs during storage (Liu et al., 2009a).

On the other hand, irradiation can eliminate foodborne pathogens in both inside and surface of shell eggs. Previous study indicated that irradiation of 2.5 kGy was enough to eliminate *Salmonella* (10^5 cells/mL) inoculated on egg surface (Liu et al., 2009a). Furthermore, irradiation improves functional properties of egg. Song et al. (2009) and Liu et al. (2009b) reported that foaming ability and viscosity of egg can be improved by 2 kGy of irradiation. However, irradiation has also some disadvantages. Especially, irradiation may decrease the Haugh unit which determines egg freshness, and increase egg yolk lipid oxidation. To pinpoint

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the best recipe, these two factors, gamma irradiation and chitosan coating needed to be set in a proper combination and it should be done by proper scientific methodology.

Response surface methodology (RSM) can be used for optimization of irradiation dose and chitosan concentration for target dependant variables such as Haugh unit, foaming ability, viscosity, and pathogen reduction. RSM is a collection of mathematical and statistical techniques using designing experiment, building models, and analyzing the effect of the several independent factors (Wang et al., 2008). The advantages of RSM were reduction of experimental number and evaluation of multiple factors and their interaction. RSM has been used to evaluate the effects of different factors on quality attributes and to optimize different process conditions (Su et al., 2009). Hence, the application of RSM may enhance the recipe (optimum conditions) and/or the manufacturing procedure to promote standardization and shelf-life extension of special products (Farris and Piergovanni, 2009).

The aim of this work was to determine and predict the optimum conditions for egg quality stored for 7 days when combination treatments of irradiation and chitosan coating were applied using response surface methodology.

MATERIALS AND METHODS

1. Experimental design and optimization

A central composite design (CCD) was chosen for the RSM in this study, which is well suited for fitting a quadratic surface and usually works well for the process optimization (Tiwari et al. 2008). The CCD is an effective experimental design that is ideal for sequential experimentation and allows a reasonable amount of information for testing lack of fit while not involving an unusually large number of design points (Tiwari et al. 2008). Therefore, Box-Benhnken CCD with two factors was applied. The bounds of the factors are irradiation dose (0~2 kGy) and concentration of chitosan coating material (0~2%) as shown in Table 1. Performance of the irradiation dose and chitosan coating

levels were evaluated by analyzing the response on egg quality and functional property.

2. Sample preparation

Chitosan powder (molecular weight approximately 40,000) was obtained from *Kumhaohwaseong* Co, Ltd (Seoul, Korea). Chitosan solution was prepared in apple vinegar (6~7%) at 0, 0.5, 1, 1.5, and 2% concentration. The chitosan solutions were adjusted to pH 5.0 with 6 N-NaOH. Eggs were dipped into the prepared chitosan solution and allowed to dry under a fan for 1 hr. The eggs wrapped in paper box, coated by chitosan solution or not, were irradiated in a cobalt-60 gamma irradiatorat the Advanced Radiation Technology Institute, Jeongeup, Korea. The applied doses in this study were 0, 0.5, 1.0, 1.5 and 2.0 kGy. The source strength was approximately 42 kCi with a dose rate of 20 kGy/h at $12 \pm 0.5 ℃$. 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. The dosimeters were calibrated against an international standard set by the International Atomic Energy Agency (Vienna, Austria).

3. Measurement of egg quality and functional properties

Egg yolk color and Haugh units were determined using QCM+System (Technical Services and Supplies, York, England) as described by Liu et al. (2009a). Emulsion capacity of egg yolk was determined according to Cho's method (1999). Yolk (0.6 g) was added into 10 mL of deionized distilled water (DDW) and 10 mL of corn oil. The mixture was homogenized at $19,000 \times g$ for 1 min (T25B, IKA, Staufen, Germany) and centrifuged at $6,400 \times g$ for 20 min at room temperature. Released liquid was subtracted from total weight and calculated as a percentage of initial weight.

Foaming capacity and foaming stability of egg white were measured using the modified method of Philips et al. (1990).

Table 1. Levels of irradiation dose and chitosan coating concentration for egg quality when stored for 7 days at 20℃ based on central composite design

Independent variable	Levels				
	−2	−1	0	1	2
Irradiation (kGy)	0	0.5	1	1.5	2
Chitosan coating concentration (%)	0	0.5	1	1.5	2

Egg white (25 mL) was mixed with 25 mL DDW in a 100 mL-mess cylinder and then homogenized at $19,000 \times g$ for 30 sec. The height of the foam produced was measured as foaming capacity. Foam stability was determined by measuring increased water surface after 30 min at room temperature.

The pH of egg white and yolk was determined using a pH meter (Model 750 P, iSTEC, Seoul, Korea) after diluting the samples with 9 volumes of DDW. Egg white or yolk (200 mL) was diluted with DDW (200 mL) and then viscosity was measured using a viscometer (Model VT-03F, Rion, Tokyo, Japan).

4. 2-thiobarbituric acid reactive substances (TBARS) value

Egg yolk was mixed by hand for 30 sec and used for the TBARS value determination (Jung et al., 2010). Egg yolk (5 g) and butylated hydroxytoluene (50 L, 7.2%) was added into 15 mL of DDW and homogenized at high speed/short time. The homogenate (1 mL) was added into 2 mL of TBA solution (20 mM TBA in 15% trichloroacetic acid) and heated for 15 min at 90℃. The reaction mixture was centrifuged at $2400 \times g$ for 15 min at 4℃. and the absorbance of supernatant was measured with a spectrophotometer (Beckman, Fullerton, CA, USA) at 532 nm.

5. *Salmonella* Typhimurium inoculation test

Dried surface of eggs (coated by 0~2% of chitosan solution) were washed with 70% ethanol, and then placed under UV light for 15 min to eliminate possible contamination. The *S.* Typhimurium (KTCT 1925) were seeded in 100 mL of tryptic soy broth medium (Difco laboratories, Livonia, MD, USA) and incubated at 37℃ for 20 h with constant shaking at 190 $\times g$. The eggs were dipped into *S.* Typhimurium cultured medium (10^{10} cells/mL, tryptic soy broth) for 10 min. They were, then, transferred to a sterile rack for air drying at 23 ℃. This resulted in approximately 10^5 CFU/mL of bacterial density on the surface of each treated eggs. Inoculated eggs were then irradiated at 0~2 kGy by gamma rays. Then, the eggs were placed at room temperature (20℃) and stored for 7 days. At day 7, the eggs were broken, the egg shell was separated, and the egg shell was transferred to sterile stomacher bags containing 10 mL of DDW. The samples were placed at room temperature for another 10 min with regular stirring. Then, the solution was serially diluted with glass tube and seeded on tryptic soy agar (Difco laboratories, Livonia, MD, USA). The total plate count was obtained after incubation at 37℃ for 48 h.

6. Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) for different treatment combinations, and when significant differences were detected, the differences among mean values were identified by Tukey's multiple range test using SAS software with the confidence level at P<0.05. Mean values and standard errors of the means are reported.

The linear, quadratic and interaction coefficients obtained from experimental data (Tables 2 and 3) to the quadratic model for each response. The regression coefficients not significant at 95% level were removed and the model refitted to the data. Only the other ones inside this confidence interval were selected for developing the models. Once models were obtained, ANOVA was calculated to verify their capability to represent the data.

To verify the developed model, a theoretical predicted data was compared with experimental data. Experiments for verification were repeated three times and subjected to one-way ANOVA with the Tukey's multiple range test to detect the differences between predicted and observed values.

RESULTS AND DISCUSSION

The Haugh units of eggs weresignificantly decreased by irradiation ($P < 0.05$, Table 2). Haugh unit was scored 0 when irradiation dose was 1 kGy or above, regardless of chitosan coating concentration. The previous study demonstrates that irradiation of 0.5 kGy also decrease the Haugh unit significantly right after irradiation and during storage (Liu et al., 2009a). The yolk color of egg seems to be decreased with the increase in irradiation dose but there was no statistical difference (Table 2). The pH of egg white and yolk did not show any difference in different experimental conditions. There was no difference in emulsion stability by the combination of irradiation dose and chitosan coating concentration.

Foaming capacity was improved with the increase in irradiation dose in the egg white stored for 7 days at room temperature ($P < 0.05$, Table 2). In contrast, foam stability

Table 2. Quality factors of the shell egg treated by the combination of chitosan-coating and irradiation and stored for 7 days at 20℃

Exp. No	Irradiation dose (kGy)	Chitosan coating (%)	Haugh units	Yolk color	pH (white)	pH (yolk)	Emulsion stability (%)	Foaming ability (mm)	Foam stability (mm)
1	1.5	1.5	0^c	7.10	9.43	6.39	48.79	36.0^a	8.0^b
2	1.5	0.5	0^c	7.30	9.45	6.30	45.36	36.0^a	10.0^b
3	0.5	1.5	28.44^b	7.30	9.32	6.37	44.34	11.0^b	7.5^b
4	0.5	0.5	32.36^b	7.50	9.34	6.37	44.91	10.0^b	5.5^b
5	1.0	1.0	0^c	7.90	9.34	6.33	46.88	12.0^b	7.5^b
6	1.0	1.0	0^c	8.20	9.32	6.38	45.47	11.5^b	8.5^b
7	2.0	1.0	0^c	7.10	9.38	6.32	44.72	46.0^a	18.5^a
8	0.0	1.0	74.74^a	7.90	9.38	6.34	49.41	11.5^b	5.5^b
9	1.0	2.0	0^c	7.10	9.34	6.30	43.69	14.0^b	8.5^b
10	1.0	0.0	0^c	7.70	9.34	6.24	46.99	9.5^b	6.0^b
SEM			2.51	0.349	0.045	0.015	3.056	3.571	1.323

$^{a-b}$ Means with different superscripts within a column are significantly different at $P \leq 0.05$.

[1] Standard errors of the mean (n = 30).

Table 3. Effect of chitosan coating and irradiation on the number of *Salmonella* Typhimurium (Log CFU/g) inoculated on egg shell and 2-thiobarbituric acid reactive substance (TBARS) value (mg malondialdehyde/ kg egg yolk) stored for 7 days at 20℃

Exp. No	Irradiation (kGy)	Chitosan solution concentration (%)	Number of *S.* Typhimurium	TBARS
1	1.5	1.5	−^{b}	1.38^{bc}
2	1.5	0.5	−^{b}	1.30^c
3	0.5	1.5	−^{b}	0.91^d
4	0.5	0.5	3.51[1]	0.73^f
5	1.0	1.0	−^{b}	1.26^c
6	1.0	1.0	−^{b}	1.30^c
7	2.0	1.0	−^{b}	1.67^a
8	0.0	1.0	−^{b}	0.59^f
9	1.0	2.0	−^{b}	1.37^{bc}
10	1.0	0.0	−^{b}	1.46^b
SEM			0.003	0.037

$^{a-f}$ Different superscripts within the same column differ significantly ($P < 0.05$).

[1] Viable cell was not detected at a detection limit $<10^2$.

was lower when 2 kGy irradiation and 1% of chitosan coating were treated ($P < 0.05$). From the results of different combination treatments, it can be proposed that irradiation affect the Haugh unit, foaming capacity, and foaming stability while chitosan coating did not affect the general quality factors of egg. Previous studies reported that irradiation decreased albumen height, Haugh units, yolk color, and foaming stability, but increased foaming capacity (Liu et al. 2009a; Liu et al., 2009b Min et al., 2005). Liu et al. (2009c) proposed that protein degradation caused by

irradiation may be a major reason of the foaming capacity improvement.

The counts of the inoculated *S.* Typhimurium on egg shell was significantly decreased by the combination treatment of irradiation and chitosan coating ($P < 0.05$, Table 3). Only in experiment 4, 0.5 kGy of irradiation and 0.5% of chitosan coating concentration showed viable cells (3.51 Log CFU/g) and all other experiment combinations showed no detected viable cells at detection limit less than 10^2 CFU/g. These results indicated that chitosan coating was effective in reducing and/or eliminating *S.* Typhimurium on egg shell. Previously, Liu et al. (2009a) reported that *S.* Typhimurium was survived at day 0 even if the egg was coated by chitosan solution (2%), but eliminated after 3 days of storage. The present study was carried out at 7 days after coating, the microorganism may be inactivated even though irradiation

was not applied (0 kGy, experiment no. 8 in Table 3).

TBARS values which assess lipid oxidation level, were higher when irradiation dose increased but there were inconsistent results in concentration of chitosan coating concentration ($P < 0.05$, Table 3). Badr (2006) indicated that contents of free fatty acids and peroxide values were increased by irradiation, while chitosan did not affect TBARS value of egg yolk.

From the data obtained from the experimental design only 4 variables including Haugh unit, foaming capacity, foam stability and TBARS values were significant at 95% level when eggs were stored for 7 days after the treatment of irradiation and chitosan coating. The second order polynomial equations were developed (Table 4) and the predicted maximum or minimum levels at different irradiation doses and chitosan concentrations were calculated (Table 5). Maximum

Table 4. The second order polynomials for egg quality by different irradiation dose and chitosan coating

Response	Second order polynomial equations	R^2	P>F
Haugh units	$Y = 76.990000\text{-}110.359524X_1\text{-}1.226190X_2+35.696429X_1^2+3.920000X_1X_2\text{-}1.673571X_2^2$	0.980	0.0018
Foaming capacity	$Y = 6.083333\text{-}8.857143X_1+6.809524X_2+14.928571X_1^2\text{-}1.000000X_1X_2\text{-}2.071429X_2^2$	0.903	0.0375
Foam stability	$Y = 1.250000+0.559524X_1+5.726190X_2+4.303571X_1^2\text{-}4.000000X_1X_2\text{-}0.446429X_2^2$	0.885	0.0513
TBARS value	$Y = 0.618333+0.821190X_1\text{-}0.268810X_2\text{-}0.093929X_1^2\text{-}0.100000X_1X_2+0.191071X_2^2$	0.896	0.0427

Y, dependent variable; X_1, irradiation dose; X_2, concentration of chitosan solution.

Table 5. Predicted level of treatment condition for the maximum or minimum responses of egg quality at different irradiation dose and chitosan concentration by the ridge analysis

Response variable	Irradiation dose (kGy)	Concentration of chitosan solution (%)	Estimated response	Morphology
Haugh units	0.0009	0.9584	74.1853 (Max)	Saddle
Foaming capacity (mm)	1.9999	1.0124	50.8255 (Max)	Saddle
Foam stability (mm)	0.3890	0.2083	2.9684 (Min)	Saddle
TBARS value (mg malonaldehyde/kg)	0.0051	0.8989	0.5348 (Min)	Saddle

Table 6. Regression analysis for regression model of egg quality at different irradiation dose and chitosan concentration

	Haugh units	Foaming capacity (mm)	Foam stability (mm)	TBARS value (mg malonaldehyde/kg)
Irradiation (kGy)	58.92***	11.38**	9.31**	10.53**
Chitosan (%)	0.09	0.10	0.57	0.50

** Significant at 5% level; *** Significant at 1% level

Table 7. Predicted values for response variables at a given condition[1] within the range of optimum condition for egg quality at 7 days at 20℃

Response variable	Predicted value	Observed value
Haugh units	0	0
Foaming capacity	21.10 ± 1.98	20.33 ± 1.53
Foam stability	8.61 ± 0.33	8.67 ± 1.53
TBARS value	1.30 ± 0.07	1.31 ± 0.05

[1] Given conditions of independent variables: irradiation dose (1.5 kGy), chitosan concentration (1.3%)
[2] Values are Mean ± S.D.(n=3)

Haugh unit was estimated as 74.2 by 0.0009 kGy of irradiation dose and 0.96% of chitosan solution. In practice, it is understood that no irradiation application will be best for Haugh unit. In contrast, foaming capacity can be maximized by 2.0 kGy irradiation with 1.01% of chitosan solution. As similar, the minimum response was estimated for foam stability and TBARS value.

From regression model it was clearly shown that Haugh units, foaming ability, foaming stability, and TBARS values were significantly affected by irradiation only (Table 6). From Table 3, only microbial inactivation was affected by concentration of chitosan solution.

To verify the developed model, a theoretical predicted data were compared with experimental ones (Table 7). Experiments based on the optimal conditions (irradiation dose 1.5 kGy and chitosan solution 1.3%) were carried out and the differences between predicted and observed values were detected. The obtained observed data were not significantly different ($P < 0.05$) from the estimated ones in all response variables. Therefore, the developed model can be used for the prediction of egg quality when combination of irradiation dose and chitosan coating is used.

In conclusion, RSM was a useful tool to pinpoint the best combination of irradiation dose and chitosan concentration on different egg quality parameters. However, it should be decided which quality factor (Haugh unit, foaming capacity, *Salmonella* inactivation and so on) is the most important for a user prior to the use of the model.

ACKNOWLEDGEMENT

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