

Effects of chitosan coating and storage with dry ice on the freshness and quality of eggs

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ABSTRACT To develop a method that can maintain egg freshness during practical storage conditions, eggs were coated with chitosan and stored with or without dry ice. The physicochemical and microbiological qualities of eggs were evaluated during 14 d of storage at 4 and 23°C without dry ice and at 23°C with dry ice. The combination of chitosan coating and dry ice significantly inhibited a Haugh unit decrease during storage at 23°C. No difference in functional properties, such as foaming ability, foam stability, and viscosity, among treatments was observed, but chitosan coating and

storage with dry ice decreased the rate of pH increase and moisture loss in albumen at d 7 and 14. The eggs treated with chitosan coating and storage with dry ice had a significantly lower number of *Salmonella* Typhimurium inoculated on the egg surface than did control eggs during storage at 23°C. Results revealed that the combination of chitosan coating and storage with dry ice limited the moisture loss, CO₂ emission, and pH increase, which helped maintaining the freshness of eggs. Microbial growth was also inhibited during storage at 23°C.

Key words: chitosan coating, dry ice, storage, quality, functional property

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INTRODUCTION

Various quality attributes of egg whites and yolks are lost as eggs age. A reduction in the rates of CO₂ emission and moisture loss from eggs can extend their shelf life. Curtis et al. (1995) found higher Haugh units for eggs cryogenically cooled with CO₂ gas, as compared with those traditionally cooled, after 30 d of storage. Cryogenic cooling of shell eggs using CO₂ resulted in lower *Salmonella enterica* serovar Enteritidis levels during storage, higher internal quality, and longer shelf life than those obtained by traditional cooling (Hughes et al., 1999). Jones et al. (2002) also reported no significant difference between CO₂ cryogenic cooling and traditional cooling in visible egg quality, such as cracks, stuck yolks, or color changes. The use of a CO₂ cryogenic freezer, however, may be limited because of the high cost of the design and limited availability in a

small transporter. A simpler and less expensive industrial method for transporting and preserving shell eggs is needed.

Film and coating can act as barriers against moisture, gas, and aroma transfer (Wan et al., 2005). Chitosan is a natural biopolymer derived by deacetylation of chitin, a major component of the shells of crustaceans such as crab, shrimp, and crawfish. It has been documented that crustacean shells possess a film-forming property that can be used in edible films or coatings (Butler et al., 1996; Nadarajah et al., 2006), and they also possess antibacterial properties (Wang, 1992). Recent studies (Lee et al., 1996; Bhale et al., 2003; No et al., 2005; Liu et al., 2009a) have revealed that chitosan coating is effective in preserving the internal quality of eggs without affecting consumer acceptance. Chitosan coating decreased weight loss and increased the shelf life of eggs by 2 to 3 wk when compared with noncoated eggs at 25°C (Bhale et al., 2003; No et al., 2005).

The aim of this study was to develop a method that could preserve the freshness of eggs by combining a chitosan coating and storage with dry ice. The physicochemical and functional properties and microbial qual-

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ity of eggs were evaluated during storage at 4 and 23°C without dry ice and at 23°C with dry ice.

MATERIALS AND METHODS

Sample Preparation

Clean, large (65 ± 2 g) 7-d-old eggs were purchased from a local market (Daejeon, Korea). Chitosan powder (molecular weight approximately 40,000) was obtained from Kumhohwaseong Co. Ltd. (Seoul, Korea). A chitosan solution (1%) was prepared in apple cider vinegar (approximately 6 to 7%), and pH was adjusted to 5.0 with 6 N NaOH. Eggs were dipped in the prepared chitosan solution and allowed to dry under a fan (Hanil Electronics, Seoul, Korea) for 1 h. Dry ice was obtained from a local market (Dajeon, Korea). The dry ice (0.7 kg) was placed in a Styrofoam box ($37 \times 32 \times 24$ cm), and a 4-cm-thick Styrofoam divider was placed between the dry ice and the eggs to inhibit shell damage caused by freezing. Boxes of egg cartons containing 15 eggs were then placed on top. Silica gel blue (10 g in a paper envelope, Samchun, Seoul, Korea) was attached to the lid of the Styrofoam boxes to adsorb moisture because a preliminary test showed that fungal growth was observed on the surface of chitosan-coated eggs during storage because of moisture condensation (data not shown). The boxes were closed and sealed with adhesive tape. The internal temperature of the box of cartons was 11°C at the beginning, was increased to 18°C after 4 h, and was then further increased to room temperature (23°C) after 18 h of storage. Chitosan-coated and noncoated eggs (270 eggs in total) were stored at 4 and 23°C without dry ice and at 23°C with dry ice for 14 d.

Egg Quality Measurements

Egg weight (g), shell color, albumen height (mm), yolk color, shell weight (g), eggshell thickness (mm), and Haugh units were determined using a QCM+ System (Technical Services and Supplies, York, UK; Liu et al., 2009b). Eggshell hardness was determined using an eggshell force gauge (Model-II, Robotmation Co. Ltd., Tokyo, Japan).

Functional Properties of Egg Yolk and White

Foaming capacity and foam stability of egg whites were measured using the modified method of Phillips et al. (1990). Egg whites (25 mL) were mixed with 25 mL of deionized distilled water (DDW) in a 100-mL mass cylinder and then homogenized using a polytron instrument (T25B, IKA, Staufen, Germany) at 19,000 rpm for 30 s. The height of the foam produced was measured as the foaming capacity. Foam stability was determined by measuring the increase in water surface after 30 min at room temperature. The pH of egg whites and yolks was determined using a pH meter (Model 750 P, iSTEC, Seoul, Korea) after diluting the samples with 9 vol of DDW. The egg whites or yolks (200 mL) were diluted with DDW (200 mL), and then viscosity was measured using a viscometer (Model VT-03F, Rion, Tokyo, Japan).

Salmonella Typhimurium Inoculation Test

The dried surfaces of chitosan-coated and noncoated eggs were washed with 70% ethanol and then placed under UV light for 30 min to eliminate the possible contamination of eggshells. *Salmonella enterica* serovar Typhimurium (KCTC 1925) was inoculated in 100 mL of tryptic soy broth medium (Difco Laboratories, Livonia, MD) and incubated at 37°C for 20 h with constant shaking at 190 rpm. Chitosan-coated and noncoated eggs were dipped into the *Salmonella* Typhimurium culture medium (10^{10} cells/mL, tryptic soy broth) for 10 min. They were transferred to a sterile rack for air drying at room temperature. This resulted in approximately 10^5 cfu/mL of bacterial density on the surface of each treated egg. The inoculated eggs were placed at room temperature and stored for 14 d. At 0, 7, and 14 d of storage, the eggs were broken and separated from the eggshells, and the eggshells were transferred to sterile Stomacher (Embo_Mixer, Seoul, Korea) bags containing 60 mL of DDW. The samples were placed at room temperature for another 10 min with regular stirring. The solution was then serially diluted using a glass tube and plated on TSA (Difco Laboratories). The total plate count was obtained after incubation at

Table 1. General quality of the shell eggs treated with a combination of chitosan coating and dry ice storage at d 0

Chitosan coating	Storage condition	Egg weight (g)	Eggshell hardness (kg)	Eggshell color	Albumen height (mm)	Haugh units	Egg yolk color	Eggshell thickness (mm)
Not coated	23°C	64.69	3.05	29.00	4.93	65.31	11.22	0.35
	4°C	65.10	2.67	29.78	6.51	78.10	11.22	0.39
	Dry ice + 23°C	63.35	2.94	28.67	5.56	69.24	11.56	0.36
Coated	23°C	66.04	3.08	31.22	6.08	73.69	10.89	0.35
	4°C	63.65	3.29	30.78	6.27	76.57	11.67	0.36
	Dry ice + 23°C	64.64	3.22	30.22	5.94	73.58	11.11	0.35
SEM ¹		1.329	0.315	2.117	0.501	4.117	0.281	0.015

¹n = 15.

Table 2. General quality of the shell eggs treated with a combination of chitosan coating and dry ice storage at d 7

Chitosan coating	Storage condition	Egg weight (g)	Eggshell hardness (kg)	Eggshell color	Albumen height (mm)	Haugh units	Egg yolk color	Eggshell thickness (mm)
Not coated	23°C	63.04	3.68	29.22	3.57 ^c	50.42 ^d	11.11 ^b	0.34
	4°C	64.83	3.67	27.78	6.18 ^a	75.61 ^{ab}	11.89 ^{ab}	0.33
	Dry ice + 23°C	61.94	3.61	30.22	5.02 ^b	65.84 ^{bc}	11.67 ^{ab}	0.34
Coated	23°C	63.56	3.18	30.89	4.39 ^{bc}	59.34 ^{cd}	11.22 ^a	0.34
	4°C	63.44	3.24	29.89	7.23 ^a	83.96 ^a	11.56 ^{ab}	0.35
	Dry ice + 23°C	63.59	3.22	29.22	4.84 ^b	65.02 ^{bc}	12.44 ^a	0.33
SEM ¹		1.173	0.293	1.873	0.388	3.988	0.407	0.012

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

¹ $n = 15$.

37°C for 48 h, and the colony-forming units per gram were counted at 30 to 300 cfu/plate.

2-TBA Reactive Substances Value

Egg yolks were mixed by hand with a glass stirring rod for 30 s and used for TBA reactive substance (TBARS) analysis (Jo et al., 2002). Egg yolk (5 g) and 7.2% butylated hydroxytoluene (50 μ L) were added to 15 mL of DDW and subjected to high speed, short time homogenization. The homogenate (1 mL) was transferred to a test tube, and 2 mL of TBA-trichloroacetic acid solution was added (20 mM 2-TBA in 15% trichloroacetic acid) and heated for 15 min at 90°C. The sample was cooled in cold water for 10 min and centrifuged at $3,000 \times g$ for 15 min. The absorbance of the supernatant was then measured with a spectrophotometer (Beckman, Fullerton, CA) at 532 nm, and the lipid oxidation was reported as milligrams of malondialdehyde per kilogram of sample.

Statistical Analysis

Three independent trials were conducted. Statistical analysis was performed by one-way ANOVA. When significant differences were detected, the differences among mean values were identified by Duncan's multiple range test using SAS software (SAS Institute Inc., Cary, NC), with the confidence level at $P < 0.05$. Mean values and SEM are reported.

RESULTS AND DISCUSSION

General Egg Quality

Egg weight and eggshell hardness, color, and thickness were not affected by the chitosan coating and dry ice treatment until d 7 (Tables 1 and 2). However, Haugh units and albumen heights of eggs coated with chitosan and stored with dry ice at 23°C were significantly higher than those of the control group (Table 2). The addition of dry ice in the box and the chitosan coating on the egg surface significantly inhibited a decrease in Haugh units and improved the egg storage stability at 23°C. Egg yolk color was also significantly higher in the combined treatment with dry ice and chitosan coating than in the control group. At d 14, the eggs with chitosan coating and stored with dry ice showed significantly higher Haugh units compared with control eggs (Table 3). The combination of chitosan coating and storage with dry ice at 23°C achieved results similar to storing eggs at 4°C, based on Haugh units. Therefore, this method can be used as a new, economical way of storing eggs at higher than refrigeration temperatures. Jones et al. (2002) reported that cooling eggs with gaseous CO₂ improved Haugh units because the CO₂ gas entered the eggs during the cooling process, remained in the albumen, and slowed chemical degradation. Rocculi et al. (2009) also reported that the Haugh units of CO₂-packed eggs were higher than those of control eggs and those of eggs packed in air and in N₂. Haugh units were reduced because of a decrease in thick albumen height. Multiple hypotheses for this phenomenon have been

Table 3. General quality of the shell eggs treated with a combination of chitosan coating and dry ice storage at d 14

Chitosan coating	Storage condition	Egg weight (g)	Eggshell hardness (kg)	Eggshell color	Albumen height (mm)	Haugh units	Egg yolk color	Eggshell thickness (mm)
Not coated	23°C	59.58 ^b	3.62 ^a	32.67	2.53 ^d	37.00 ^d	11.33 ^b	0.31
	4°C	63.44 ^{ab}	3.07 ^{ab}	32.56	6.12 ^a	75.93 ^a	12.67 ^b	0.31
	Dry ice + 23°C	61.11 ^{ab}	3.55 ^a	31.67	3.69 ^c	53.11 ^{bc}	12.11 ^{ab}	0.31
Coated	23°C	62.59 ^{ab}	3.13 ^{ab}	32.67	3.02 ^{cd}	42.08 ^{cd}	11.33 ^a	0.31
	4°C	64.50 ^a	2.78 ^b	29.67	5.82 ^{ab}	73.16 ^a	11.89 ^{ab}	0.31
	Dry ice + 23°C	61.28 ^{ab}	3.16 ^{ab}	32.67	4.77 ^b	65.49 ^{ab}	12.78 ^a	0.31
SEM ¹		1.376	0.256	1.891	0.361	4.367	0.356	0.001

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

¹ $n = 18$.

Table 4. Functional properties and moisture content of the shell eggs treated with a combination of chitosan coating and dry ice storage at d 0

Chitosan coating	Storage condition	Foaming capacity (cm)	Foaming stability (cm)	pH		Viscosity (mPa/s)		Moisture	
				Egg white	Egg yolk	Egg white	Egg yolk	Egg white	Egg yolk
Not coated	23°C	17.0	6.0	9.16	6.18	17.0	150.0	87.66	53.00
	4°C	16.5	5.5	9.16	6.16	17.5	157.5	88.22	53.55
	Dry ice + 23°C	17.0	6.5	9.16	6.16	22.0	175.0	88.18	53.90
Coated	23°C	17.0	6.5	9.16	6.15	16.5	165.0	88.26	53.11
	4°C	16.0	7.0	9.18	6.15	16.5	170.0	87.76	52.90
	Dry ice + 23°C	16.5	6.0	9.18	6.16	18.5	165.0	87.82	53.20
SEM ¹		2.92	2.50	0.033	0.031	3.11	24.54	0.574	0.244

¹n = 18.

proposed, including the breakdown of the ovomucin-lysozyme complex, the decreasing carbohydrate content of ovomucin during storage, and increasing pH (Burlley and Vadehra, 1989; Chen et al., 2005a). Kim et al. (2007) and Bhale et al. (2003) suggested that coating eggs with 2% chitosan increased the shelf life of eggs by approximately 3 wk (based on Haugh units) at 25°C. In addition, eggs with the chitosan coating and dry ice treatment showed less weight loss and less yolk color change after 14 d of storage. Li et al. (1985) showed that nonpacked eggs stored for 28 d at 25°C lost 10% of their weight, and Wong et al. (1996) reported that the weight losses of uncoated and mineral oil-coated eggs were 11 and 9.2%, respectively, after 28 d of storage at 4°C. Differences in weight loss between studies may be due to storage conditions, temperature, egg size, and shell porosity (Bhale et al., 2003; Caner, 2005).

Functional Properties of Eggs

The chitosan coating on the surface of eggs and storage with dry ice in a box did not show any effect on the foaming ability, foam stability, and viscosity of eggs during storage (Tables 4, 5, and 6). However, both the chitosan coating and dry ice treatments decreased the rate of pH increase and the moisture loss at d 7 and 14. The pH and moisture content of eggs treated with

the combination of chitosan coating and dry ice were significantly lower than those of the control group. The loss of moisture and CO₂ from inside the egg was directly related to the weight loss and pH increase, and it induced albumen migration, which lowered the albumen height and Haugh units. Rocculi et al. (2009) reported that the greatest weight loss in eggs in the control treatment was mainly due to water lost from the albumen. Lee (1999) indicated that CO₂ preservation and several coating methods may have limited the respiration from the inside to the outside of eggs and decreased the moisture and CO₂ loss during storage, which retarded the loss of weight and Haugh units. The author also indicated that the combination of CO₂ and coating was a good method of storing eggs.

The deterioration of quality, especially CO₂ emission and pH increase, occurs rapidly at the initial stage of storage. At this stage, the most important factor in minimizing the deterioration of quality is temperature. Addition of dry ice decreased the temperature in the box at the initial stage, thereby slowing the quality changes significantly.

Microbial Safety of the Eggshell Surface

The eggs with chitosan coating, those stored with dry ice, or both had significantly lower numbers of in-

Table 5. Functional properties and moisture content of the shell eggs treated with a combination of chitosan coating and dry ice storage at d 7

Chitosan coating	Storage condition	Foaming capacity (cm)	Foaming stability (cm)	pH		Viscosity (mPa/s)		Moisture	
				Egg white	Egg yolk	Egg white	Egg yolk	Egg white	Egg yolk
Not coated	23°C	16.5	6.0	10.66 ^a	6.43 ^a	19.0	142.5	86.79 ^b	53.00 ^b
	4°C	15.5	5.5	10.11 ^c	6.04 ^d	17.5	159.5	87.94 ^a	54.40 ^a
	Dry ice + 23°C	17.5	6.5	10.34 ^b	6.31 ^b	20.5	159.5	87.79 ^a	55.46 ^a
Coated	23°C	17.0	6.5	10.29 ^b	6.16 ^c	17.5	165.0	87.93 ^a	54.91 ^a
	4°C	16.5	7.0	10.06 ^c	6.07 ^{cd}	17.5	172.5	87.58 ^a	55.08 ^a
	Dry ice + 23°C	16.5	6.0	10.06 ^c	6.32 ^b	18.0	162.5	88.01 ^a	56.37 ^a
SEM ¹		2.38	2.50	0.031	0.025	3.65	20.04	0.154	0.191

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

¹n = 18.

Table 6. Functional properties and moisture content of the shell eggs treated with a combination of chitosan coating and dry ice storage at d 14

Chitosan coating	Storage condition	Foaming capacity (cm)	Foam stability (cm)	pH		Viscosity (mPa/s)		Moisture	
				Egg white	Egg yolk	Egg white	Egg yolk	Egg white	Egg yolk
Not coated	23°C	18.5	7.0	10.60 ^a	6.89 ^a	17.5	143.5	86.81 ^d	53.53 ^b
	4°C	16.5	6.5	10.04 ^d	6.16 ^d	17.0	158.5	87.83 ^{ab}	55.47 ^a
	Dry ice + 23°C	19.0	6.5	10.33 ^b	6.37 ^b	19.5	157.0	87.71 ^{ab}	54.99 ^a
Coated	23°C	18.0	7.0	10.15 ^c	6.24 ^c	18.0	162.5	87.25 ^{cd}	55.89 ^a
	4°C	17.0	6.5	10.03 ^d	6.19 ^{cd}	18.0	172.5	87.40 ^{bc}	55.85 ^a
	Dry ice + 23°C	17.5	7.0	10.02 ^d	6.36 ^b	18.5	157.5	87.85 ^a	55.16 ^a
SEM ¹		2.57	2.14	0.026	0.017	3.20	18.24	0.112	0.353

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

¹_n = 18.

oculated *Salmonella* Typhimurium recovered from the egg surface than did control eggs at d 14 (Table 7). Dry ice treatments also significantly decreased the bacterial numbers when compared with the control at d 7. In addition, the chitosan coating treatments increased bacterial death at all storage temperature conditions at d 7. Kim et al. (2007) suggested that the effect of chitosan as an antibacterial agent against *Salmonella* Enteritidis and as a coating material in preserving the internal quality of eggs can vary depending on the molecular weights and types of chitosan. Chen et al. (2005b) reported that the antimicrobial activity of chitosan coating was due to a change in cell permeability through the interactions between polycationic chitosan and the electronegative charge on the cell surfaces. Carbon dioxide preservation also limited bacterial growth (Lee, 1999). Garcia-Gonzalez et al. (2007) summarized the hypothetical inactivation mechanism of CO₂ as follows: 1) solubilization of pressurized CO₂ in the external liquid phase, 2) cell membrane modification, 3) decrease in intercellular pH, 4) inactivation of key enzymes or inhibition of cellular metabolism caused by a decrease in pH, 5) a direct effect of molecular CO₂ and HCO₃⁻ on metabolism, 6) disordering of the intracellular electrolyte balance, and 7) removal of vital constituents from cells and cell membranes. In the present study, the

sublimation of dry ice into CO₂ might have reduced the bacterial growth.

TBARS Values

Chitosan coating and storage with dry ice had no effect on the TBARS value of egg yolks (Table 8). Very few references were found in TBARS values of eggs after coating with chitosan and CO₂ treatment. Liu et al. (2009a) reported that TBARS values of egg yolks showed no difference because of chitosan coating, but irradiation of eggs increased the TBARS value, and a slight inhibition of TBARS was found with the chitosan coating.

In conclusion, the results revealed that the combination of chitosan coating and storage with dry ice limited the moisture loss, CO₂ emission, and temperature effect, thereby maintaining egg freshness and inhibiting microbial growth during storage, even when the eggs were stored at a high temperature (23°C). The most desirable way to maintain the freshness of eggs is by cold storage. However, if this is economically unfeasible, the combination of chitosan coating and storage with dry ice or storage with dry ice alone can help maintain egg freshness. These methods are expected to be very effective, especially in hot summer months.

Table 7. Effect of chitosan coating and dry ice on the number of *Salmonella* Typhimurium (log cfu/g) inoculated on eggshells during storage for 14 d

Chitosan coating	Storage condition	Storage (d)		
		0	7	14
Not coated	23°C	5.31	4.66 ^a	3.14 ^a
	4°C	5.40	3.90 ^{ab}	2.74 ^{bc}
	Dry ice + 23°C	5.45	3.30 ^{bc}	2.85 ^{ab}
Coated	23°C	5.35	2.48 ^c	2.01 ^d
	4°C	5.45	2.60 ^c	2.82 ^{abc}
	Dry ice + 23°C	5.28	2.57 ^c	2.47 ^c
SEM ¹		0.098	0.262	0.087

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

¹_n = 18.

Table 8. 2-Thiobarbituric acid reactive substance values (mg of malondialdehyde/kg) of the yolks of eggs treated with chitosan coating and dry ice during storage for 14 d

Chitosan coating	Storage condition	Storage (d)		
		0	7	14
Not coated	23°C	0.569	0.569	0.823
	4°C	0.536	0.55	0.825
	Dry ice + 23°C	0.527	0.611	0.809
Coated	23°C	0.569	0.58	0.748
	4°C	0.591	0.583	0.806
	Dry ice + 23°C	0.578	0.58	0.773
SEM ¹		0.079	0.031	0.036

¹n = 18.

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