

Salmonella Enteritidis가 접종된 비세척 계란의 품질 유지를 위한 적절 유통온도

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Appropriate Distribution Temperature for the Quality of Unwashed Eggs Inoculated with *Salmonella* Enteritidis onto Shells

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ABSTRACT - This study aimed to assess the effect of temperature alterations on the preservation of egg quality and determine suitable temperature management practices for unwashed eggs contaminated with *Salmonella* Enteritidis on their shells in an actual distribution environment. Unwashed eggs inoculated with *Salmonella* Enteritidis were stored for 7 d under six different conditions, constant temperature storage at 25°C and five different temperature-changing storage conditions. For the temperature-changing conditions, the eggs were initially stored at 25°C, and then the temperature was changed to either 10 or 35°C. The indicators of egg quality, air cell height, weight loss, and specific gravity were preserved in the initial measurements when the storage temperature was lowered from 25 to 10°C from day 3 to 4 after inoculation with *Salmonella* Enteritidis. In addition, the thick albumen ratio did not show significant alteration caused by the storage conditions when compared with that of fresh eggs. These findings indicate that lowering the storage temperature from 25 to 10°C is appropriate for the safety management of unwashed eggs during actual distribution.

Keywords: Storage temperature; Unwashed egg; *Salmonella* Enteritidis; Quality indicator

The infection of *Salmonella* spp. leading to salmonellosis manifests in various acute symptoms, including fever, abdominal pain, nausea, diarrhea, and vomiting¹. Over the past 5 years, *Salmonella* spp. emerged as the predominant foodborne pathogen, accounting for 26% of food poisoning cases in Korea². Moreover, according to the Centers for Disease Control and Prevention (CDC), non-typhoidal *Salmonella* was estimated to be ranked second in causing annual cases of foodborne illnesses (11%), first in terms of hospitalizations (35%), and first in causing deaths associated

with foodborne pathogens (28%) in the United States³.

Among the *Salmonella* serotypes, *S. enterica* serovar Enteritidis (SE) accounts for the majority causing salmonellosis and is a frequent contaminator of eggs and egg products^{4,5}. There are two possible routes involved in the contamination of SE in eggs⁶. Vertical transmission results from the colonization of ovaries by SE, leading to contamination of the contents and eggshell before oviposition⁷. Horizontal transmission is the other route that results from SE penetration through the eggshell following the bacterial attachment to the eggshell surface⁸. While the dominant route of SE contamination in eggs is still uncertain, a study conducted by Martelli and Davies⁹ demonstrated that the majority of SE contamination in eggs occurred on the eggshells rather than within the contents in 1989-2009. In the case of horizontal transmission, cross-contamination can also occur due to the detachment of pathogens from the eggshell during distribution or cooking^{10,11}. Thus, eliminating

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pathogens on eggshells could be an important consideration to prevent cross-contamination of foods.

Eggshell contamination can result in the invasion of bacteria through the pore, but the albumen possesses antimicrobial properties, which are difficult to survive pathogens within the egg^{12,13}. However, bacteria that survived within the albumen can metabolize the nitrogen and carbon sources in the egg contents by producing hydrolytic enzymes, leading to spoilage¹⁴. This spoilage is also related to the storage temperature, and results in rotten eggs, characterized by abnormal odor, color, and albumen quality¹⁴. Egg spoilage by SE is not certainly identified, but Huang et al.¹⁵ reported that SE can survive in egg white through gene regulation. This survival of SE within eggs can cause spoilage, and it is necessary to comprehensively assess the quality deterioration by storing SE-contaminated eggs onto shell.

Air cell height, weight loss, specific gravity, and thick albumen ratio are closely related to egg quality^{16,17}. In the egg industry of the United States and Korea, air cell height is widely used for the assessment of egg quality^{18,19}. Generally, egg quality deterioration is caused by the release of carbon dioxide and moisture, resulting in an increase in the albumen pH^{20,21}. The emission of moisture from the egg contents causes oxygen to enter, leading to increased weight loss and decreased specific gravity and albumen volume. The decrease in albumen volume results in an increase of air cell height²². Ovomucin is a glycoprotein that provides gel-like properties to albumen^{22,23}. During storage, albumen thinning occurs due to the weakened interaction between ovomucin and lysozyme under alkaline pH conditions^{21,24,25}. This thinning leads to a reduction in thick albumen ratio¹⁶. Storage temperature and period play major roles in egg quality deterioration^{26,27}.

Maintaining rigorous temperature control during transportation and storage is necessary to minimize the growth of pathogens on eggshell and preserve the quality of eggs²⁸. In Korea, both washed and unwashed eggs are permitted to be distributed, and the storage temperature should be at 0-10 and 1-35°C, respectively²⁹. Egg distribution in the United States requires washing before distribution and storage at temperatures below 7°C within 36 h of laying³⁰. In the European Union, Class A eggs should not be washed or cleaned, except in authorized Member States where washing is permitted. Moreover, Class A eggs should not be artificially maintained at temperatures lower than 5°C³¹.

A number of studies have assessed the relationship between temperature and the deterioration of egg quality^{16,27,32}. However, unwashed eggs can be exposed to various temperatures during distribution^{33,34}, and the assessment of the quality deterioration of eggs as affected by temperature

changes is insufficient.

Thus, this study aimed to evaluate the effect of temperature alterations on egg quality maintenance by assessing quality indicators of eggs inoculated with SE onto shell, including air cell height, weight loss, specific gravity, and thick albumen ratio. Moreover, we intended to determine suitable temperature management practices for unwashed eggs contaminated with SE on shell in an actual distribution environment.

Materials and Methods

Preparation of unwashed eggs and SE inoculation

The unwashed eggs were obtained from a poultry farm in April, 2023 (JH egg farm, Anseong, Korea). The protocol by Park et al.³⁵ with minor modifications was followed for SE inoculation. SE (ATCC 13076) was incubated in tryptic soy broth (Difco, Becton Dickinson, Sparks, MD, USA) at 37°C for 24 h. Subsequently, the culture was centrifuged at 5,000×g, 4°C for 5 min, following which the supernatant was removed. The remaining SE cells were washed and resuspended in 10 mL of phosphate buffer (LPS Solution, Daejeon, Korea). Twenty microliters of an SE suspension with a concentration of 8 log CFU/mL were inoculated on 10 random spots of the eggshell. Afterward, the SE-inoculated eggs were air-dried in a biosafety cabinet for 1 h and then stored under each condition.

Incubation conditions

To identify appropriate storage conditions during distribution of unwashed eggs, SE-inoculated eggs were stored for a total of 7 days under six different conditions. The initial storage temperature was set at room temperature (25°C) because unwashed eggs are generally distributed at ambient temperature (15-25°C) in Korea³⁵. These conditions consisted of one condition of constant temperature storage at 25°C and five conditions of temperature-changing storage. For the temperature-changing conditions, the eggs were initially stored at 25°C, and the temperature was changed to either 10°C or 35°C.

Quality assessment

The protocol of previous studies with minor modifications was followed for quality assessment^{16,27,32}. Weight loss (%) was determined by calculating the difference in weight compared to the initial whole weight (g), expressed as a percentage. Specific gravity was assessed by placing the eggs in saline solutions with varying specific gravities ranging from 1.02 to 1.10 in increments of 0.005. The height of the air cell (mm) was measured using a tripod micrometer (Digital Haugh Tester, Orka Food Technology, West

Bountiful, UT, USA). Subsequently, the albumen was poured onto a 2-mm mesh sieve to separate the thick albumen and thin albumen, and the volumes of thick and thin albumen (mL) were measured. The thick albumen ratio was calculated using Eq. (1).

$$\text{Thick albumen ratio} = \frac{\text{Volume of thick albumen}}{\text{Volume of thin albumen}} \quad (1)$$

Statistical analysis

The SPSS software package (SPSS 26.0, IBM Corp., New York, NY, USA) was used to analyze the collected data. One-way ANOVA was conducted to determine the effect of storage time and conditions on air cell height, weight loss, specific gravity, and thick albumen ratio. The mean values were compared using Duncan's multiple range test to determine significant differences ($P < 0.05$).

Results and Discussion

Effect of storage temperature on air cell height

The effect of storage temperature on air cell height of SE-inoculated unwashed eggs is shown in Fig. 1. Under constant temperature storage at 25°C, the air cell height significantly increased to 6.18 mm on day 7, corresponding to a grade B quality classification in Korea¹⁹). Similarly, when the storage temperature was lowered to 10°C on days 3 and 4, the air cell height increased to 6.06 mm and 6.21 mm on day 7 (grade B), respectively. However, when the storage temperature was changed to 35°C, the air cell height showed significant increases to 9.10-10.07 mm on day 7 (grade C). Kim et al.³⁶) reported that air cell height

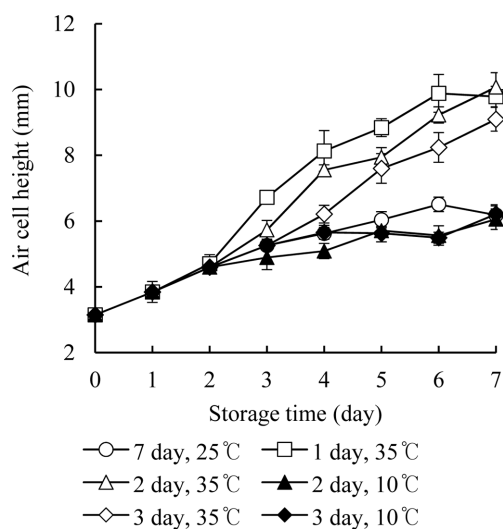


Fig. 1. Air cell height changes by the storage conditions. Data represent mean \pm SEM.

increased significantly from 1.4-1.8 mm to 4.3 mm on day 7 at room temperature (13.0-19.7°C), whereas an increase was not significant at refrigerated temperatures (4-5°C). In addition, Samli et al.³²) reported that air cell height increased significantly by 1.06, 2.51, and 4.64 mm at 5, 21, and 29°C on day 10, respectively.

Effect of storage temperature on weight loss

The effect of storage temperature on changes in weight loss is provided in Fig. 2. Without temperature change at 25°C, weight loss increased significantly by 1.55% on day 7. Similarly, when the storage temperature was lowered to 10°C on day 3 and 4, weight loss increased by 1.06 and 1.21%, respectively. However, with a change in storage temperature to 35°C, the weight loss showed a significant increase of 3.88-5.00%. In a study by Jin et al.³⁷) reported that increasing the weight were significant by 0.55, 1.18, and 3.67% at 5°C, 21°C, and 29°C on day 10, respectively. Moreover, Jones et al.³⁸) reported a significant difference in weight loss between the storage temperatures of 4°C and 22°C, with increases of 0.58% and 4.67%, respectively, during 4 weeks of storage.

Effect of storage temperature on specific gravity

The effect of storage temperature on specific gravity is presented in Fig. 3. The initial specific gravity was 1.092, and it decreased to 1.068 on day 7 under constant temperature storage. When the storage temperature was lowered to 10°C on day 3 and 4, specific gravity decreased to 1.083 and 1.077 on day 7, respectively. After lowering the storage temperature, there was no significant decline. However, when the storage temperature was changed to

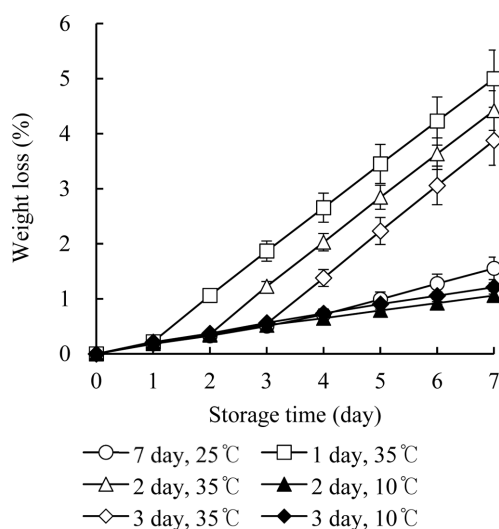


Fig. 2. Changes in weight loss of eggs by the storage conditions. Data represent mean \pm SEM.

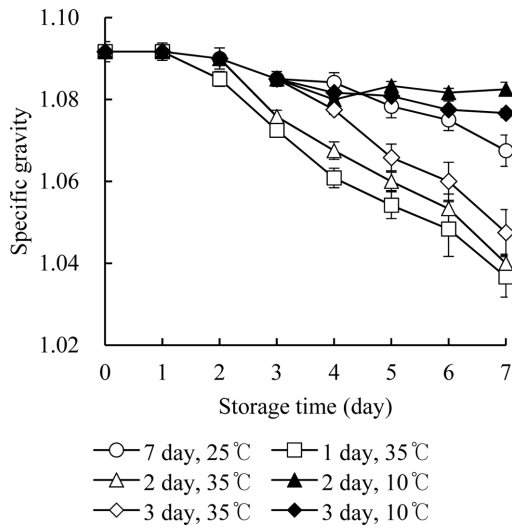


Fig. 3. Changes in specific gravity of eggs by the storage conditions. Data represent mean ± SEM.

35°C on day 2, 3, and 4, decreasing the specific gravity were significant on day 7, respectively. Aygun and Sert³⁹⁾ reported that specific gravity was significantly higher at 5°C than at 22°C during 6 weeks of storage, ranging between 1.045-1.074 and 1.001-1.037, respectively. Also, Samli et al.³²⁾ reported that specific gravity declined significantly from 1.086 to 1.080, 1.074, and 1.063 at 5, 21, and 29°C on day 10, respectively.

Effect of storage temperature on thick albumen ratio

The effect of storage temperature on thick albumen ratio is shown in Fig. 4. The initial thick albumen ratio was 2.44, and it decreased to 1.64 on day 1, significantly. Regardless of the storage conditions, a significant decrease was not

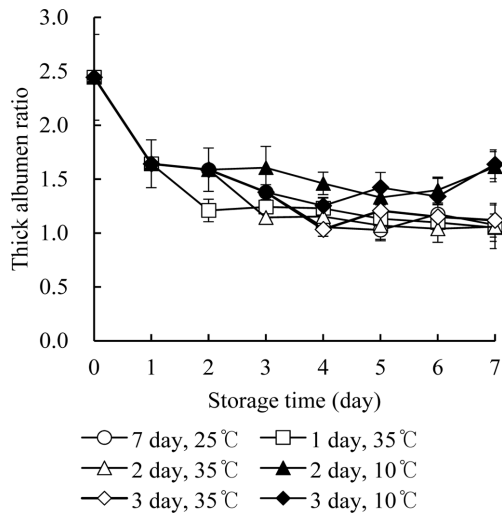


Fig. 4. Thick albumen ratio changes by the storage conditions. Data represent mean ± SEM.

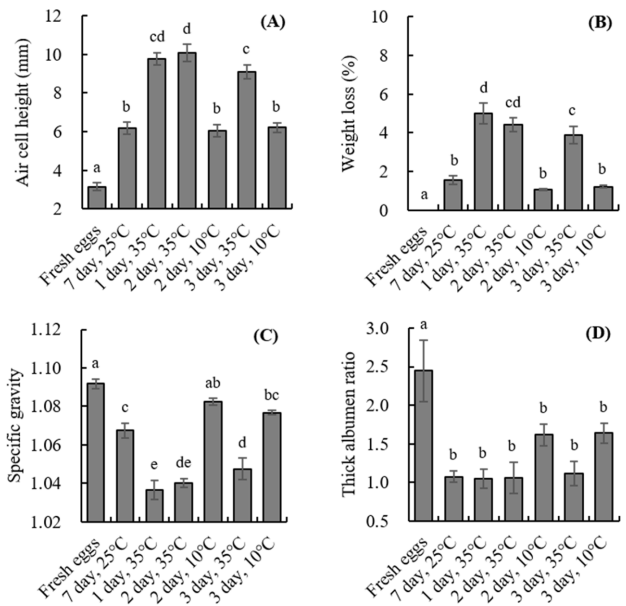


Fig. 5. Appropriate temperature to maintain the quality of unwashed eggs. (A) Air cell height, (B) Weight loss, (C) Specific gravity, (D) Thick albumen ratio. Data represent mean ± SEM. ^{a-e} Significant differences within the same quality indicator are indicated by different letters ($P < 0.05$).

observed from day 1 to day 7. When the storage temperature was lowered to 10°C on day 3 and 4, the thick albumen ratio was significantly higher than that of other conditions at 1.62 and 1.64 on day 7, respectively. Chen et al.⁴⁰⁾ the thick albumen ratio at 25°C decreased more at 25°C than 7°C over a storage period of 4 weeks, but a significant difference had been shown since week 2. Liu et al.¹⁶⁾ also reported a negative correlation between storage temperature and thick albumen ratio. Additionally, the thick albumen ratio showed high correlations with Haugh unit, albumen pH, and air cell height ($P < 0.01$).

In conclusion, when the storage temperature was lowered from 25°C to 10°C on day 3 and 4, the initial quality of the unwashed egg was preserved significantly higher compared to the other tested storage conditions (Fig. 5).

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국문요약

본 연구는 계란의 품질 유지를 위한 온도변화의 영향을 평가하고 실제 유통 환경에서 난각에 *Salmonella Enteritidis*가 오염된 비세척란의 적절한 온도 관리 방법을 결정하고자 하였다. *Salmonella Enteritidis*가 접종된 비세척란은 총

7일간 25°C 항온보관 및 5가지의 다른 온도변화조건에서 보관하였다. 온도변화조건은 계란을 초기 25°C에서 보관 중 온도를 10°C 또는 35°C로 변화하였다. 보관 중 기실의 높이, 중량감소율, 비중 및 농후난백 비율을 1일 간격으로 평가하였다. 기실의 높이, 중량감소율, 비중은 25°C 보관 3일 및 4일차에 10°C로 온도를 낮추었을 때 초기값이 유의적으로 보존되었다. 농후난백 비율은 초기 값과 비교하였을 때 보관 조건에 따른 유의한 차이를 나타내지 않았다. 이러한 결과는 25°C 보관 3일 및 4일차에 10°C로 낮추는 것이 실제 유통 시 비세척란의 안전관리에 적합함을 시사하였다.

Conflict of interests

The authors declare no potential conflict of interest.

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