pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 38, No. 5, pp. 332~337 (2023)
https://doi.org/10.13103/JFHS.2023.38.5.332

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Salmonella Enteritidis가 접종된 비세척 계란의 품질 유지를 위한 적절 유통온도

안지훈[1] · 이희석[1,2]*
[1]중앙대학교 식품안전규제과학과
[2]중앙대학교 식품과학생명공학과

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

Ji-Hoon An[1], Hee-Seok Lee[1,2]*
[1]*Department of Food Safety and Regulatory Science, Chung-Ang University, Anseong, Korea*
[2]*Department of Food Science and Biotechnology, Chung-Ang University, Anseong, Korea*

(Received July 14, 2023/Revised October 10, 2023/Accepted October 11, 2023)

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 vomiting[1]. Over the past 5 years, *Salmonella* spp. emerged as the predominant foodborne pathogen, accounting for 26% of food poisoning cases in Korea[2]. 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 States[3].

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 eggs[6]. Vertical transmission results from the colonization of ovaries by SE, leading to contamination of the contents and eggshell before oviposition[7]. Horizontal transmission is the other route that results from SE penetration through the eggshell following the bacterial attachment to the eggshell surface[8]. While the dominant route of SE contamination in eggs is still uncertain, a study conducted by Martelli and Davies[9] 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

*Correspondence to: Hee-Seok Lee, Department of Food Safety and Regulatory Science, Chung-Ang University, Anseong, 17546, Korea
Tel: +82-31-670-3258, Fax: +82-31-675-3108
E-mail: hslee0515@cau.ac.kr

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 spoilage[14]. This spoilage is also related to the storage temperature, and results in rotten eggs, characterized by abnormal odor, color, and albumen quality[14]. 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 height[22]. 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 ratio[16]. 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 eggs[28]. 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, respectively[29]. Egg distribution in the United States requires washing before distribution and storage at temperatures below 7°C within 36 h of laying[30]. 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°C[31].

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 Korea[35]. 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 Korea[19]. 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 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

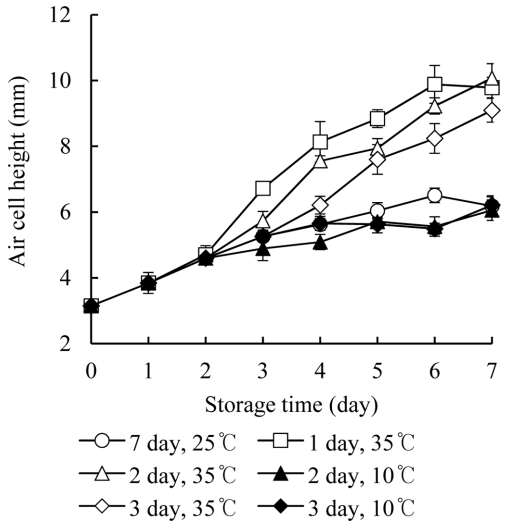

Fig. 1. Air cell height changes by the storage conditions. Data represent mean ± SEM.

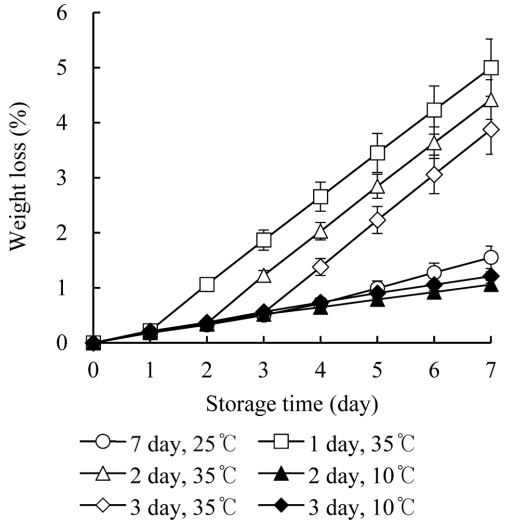

Fig. 2. Changes in weight loss of eggs by the storage conditions. Data represent mean ± SEM.

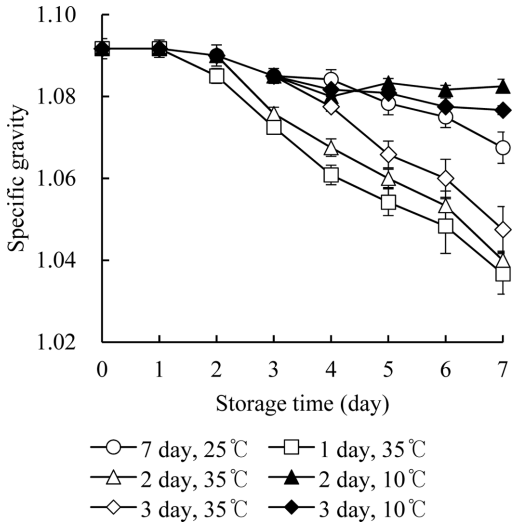

Fig. 3. Changes in specific gravity of eggs by the storage conditions. Data represent mean ± SEM.

35°C on day 2, 3, and 4, decreasing the specific gravity were significant on day 7, respectively. Aygun and Sert[39] 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

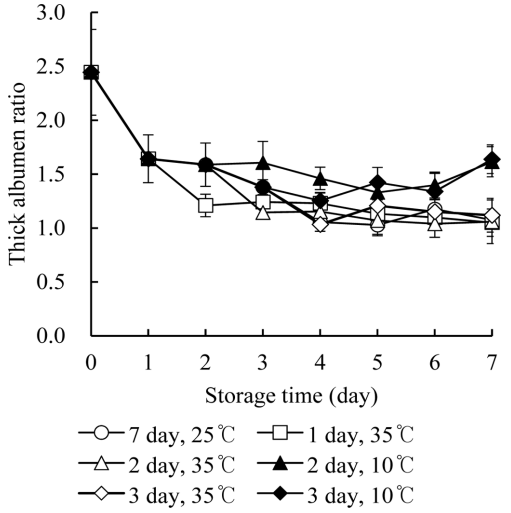

Fig. 4. Thick albumen ratio changes by the storage conditions. Data represent mean ± SEM.

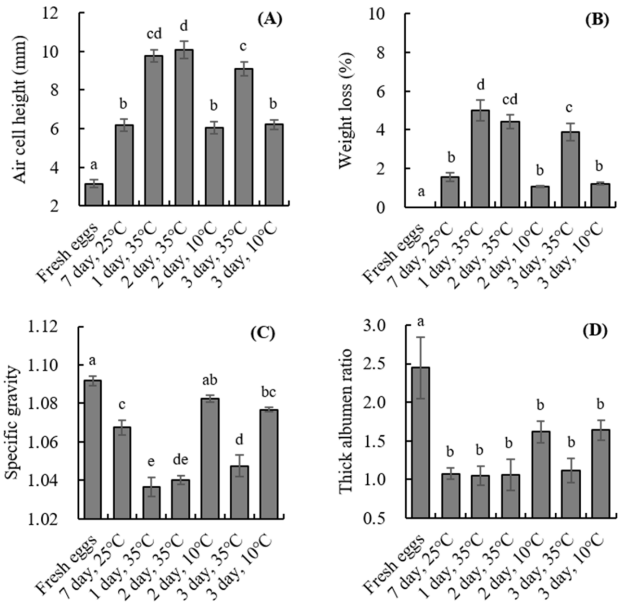

Fig. 5. Appropriate temperature to maintain the quality of unwashed eggs. (**A**) Air cell height, (**B**) Weight loss, (**C**) Specific gravity, (**D**) Thick albumen ratio. Data represent mean ± SEM. [a-e] Significant differences within the same quality indicator are indicated by different letters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

Acknowledgments

This work was supported by a grant (21153MFDS605) from the Ministry of Food and Drug Safety.

국문요약

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

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

Conflict of interests

The authors declare no potential conflict of interest.

ORCID

Ji-Hoon An https://orcid.org/0009-0001-5049-856X
Hee-Seok Lee https://orcid.org/0000-0001-5879-7997

References

1. World Health Organization (WHO), (2023, June 30). *Salmonella* (non-typhoidal). Retrieved from https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal)
2. Ministry of Food and Drug Safety (MFDS), (2023, June 30). Food poisoning statistics. Retrieved from https://www.foodsafetykorea.go.kr/portal/healthyfoodlife/foodPoisoningStat.do?menu_no=4425& menu_grp=MENU_NEW02
3. Centers for Disease Control and Prevention (CDC), (2023, May 17). Burden of foodborne illness: findings. Retrieved from https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html
4. European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC), The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA J.*, **16**, e05500 (2018).
5. CCenters for Disease Control and Prevention (CDC), (2023, June 17). Pathogen surveillance. Retrieved from https://wwwn.cdc.gov/foodnetfast/
6. Howard, Z.R., O'Bryan, C.A., Crandall, P.G., Ricke, S.C., Salmonella Enteritidis in shell eggs: current issues and prospects for control. *Food Res. Int.*, **45**, 755-764 (2012).
7. Liu, B., Zhang, X., Ding, X., Bin, P., Zhu, G., The vertical transmission of *Salmonella* Enteritidis in a one-health context. *One Health*, **16**, 100469 (2022).
8. Zhang, W., Zheng, J.X., Xu, G.Y., Toward better control of *Salmonella* contamination by taking advantage of the egg's self-defense system: a review. *J. Food Sci.*, **76**, R76-R81 (2011).
9. Martelli, F., Davies, R.H., *Salmonella* serovars isolated from table eggs: an overview. *Food Res. Int.*, **45**, 745-754 (2012).
10. Chousalkar, K.K., Khan, S., McWhorter, A.R., Microbial quality, safety and storage of eggs. *Curr. Opin. Food Sci.*, **38**, 91-95 (2021).
11. Kingsbury, J.M., Thom, K., Soboleva, T., Effect of storage temperature on the survival of New Zealand egg-associated *Salmonella* isolates in and on eggs. *J. Food Prot.*, **82**, 2161-2168 (2019).
12. Baron, F., Nau, F., Guérin-Dubiard, C., Bonnassie, S., Gautier, M., Andrews, S.C., Jan, S., Egg white versus *Salmonella* Enteritidis! A harsh medium meets a resilient pathogen. *Food Microbiol.*, **53**, 82-93 (2016).
13. Messens, W., Grijspeerdt, K., Herman, L., Eggshell penetration of hen's eggs by *Salmonella enterica* serovar Enteritidis upon various storage conditions. *Br. Poult. Sci.*, **47**, 554-560 (2006).
14. European Food Safety Authority (EFSA), Scientific opinion on the public health risks of table eggs due to deterioration and development of pathogens. *EFSA J.*, **12**, 3782 (2014).
15. Huang, X., Hu, M., Zhou, X., Liu, Y., Shi, C., Shi, X., Role of yoaE gene regulated by CpxR in the survival of *Salmonella enterica* serovar Enteritidis in antibacterial egg white. *mSphere*, **5**, e00638-19 (2020).
16. Liu, Y.C., Chen, T.H., Wu, Y.C., Lee, Y.C., Tan, F.J., Effects of egg washing and storage temperature on the quality of eggshell cuticle and eggs. *Food Chem.*, **211**, 687-693 (2016).
17. Lee, H.O., Kim, J.Y., Kim, B.S., Choosing quality indicators for quality prediction of egg in circulation. *Korean J. Food Preserv.*, **21**, 609-617 (2014).
18. United States Department of Agriculture (USDA), (2023, June 19). United States standards, grades, and weight classes for shell eggs AMS 56. Retrieved from https://www.ams.usda.gov/sites/default/files/media/Shell_Egg_Standard%5B1%5D.pdf
19. Ministry of Agriculture, Food and Rural Affairs (MAFRA), (2023, June 19). Detailed criteria for grading of livestock products. Retrieved from https://www.law.go.kr/%ED%96%89%EC%A0%95%EA%B7%9C%EC%B9%99/%EC%B6%95%EC%82%B0%EB%AC%BC%20%EB%93%B1%EA%B8%89%ED%8C%90%EC%A0%95%20%EC%84%B8%EB%B6%80%EA%B8%B0%EC%A4%80
20. Huopalahti, R., Anton, M., López-Fandiño, R., Schade, R., Bioactive egg compounds, Vol. 5, Springer, Berlin, Heidelberg, Germany, pp. 61-118 (2007).
21. Liu, M., Yu, W., Ren, F., Wu, J., Formation and characterization of peptides in egg white during storage at ambient temperature. *Food Chem.*, **263**, 135-141 (2018).
22. Kumari, A., Tripathi, U.K., Maurya, V., Kumar, M., Internal quality changes in eggs during storage. *Int. J. Sci. Environ. Technol.*, **9**, 615-624 (2020).
23. Omana, D.A., Wang, J., Wu, J., Ovomucin – a glycoprotein with promising potential. *Trends Food Sci. Tech.*, **21**, 455-463 (2010).
24. Shan, Y., Tang, D., Wang, R., Tu, A., Yi, Y., Wang, X., Liu, B., Zhou, Y., Huang, Q., Lü, X., Rheological and structural

properties of ovomucin from chicken eggs with different interior quality. *Food Hydrocoll.*, **100**, 105393 (2020).
25. Wang, J., Omana, D.A., Wu J., Effect of shell eggs storage on ovomucin extraction. *Food Bioprocess Technol.*, **5**, 2280-2284 (2012).
26. Lee, M.H., Cho, E.J., Choi, E.S., Sohn, S.H., The effect of storage period and temperature on egg quality in commercial eggs. *Korean J. Poult. Sci.*, **43**, 31-38 (2016).
27. Kim, B.K., Park, C.E., Kim, B.S., Kim, Y.S., Quality factors affecting egg freshness and palatability during storage. *Korean J. Food Sci. Technol.*, **46**, 295-302 (2014).
28. Chousalkar, K., Gast, R., Martelli, F., Pande, V., Review of egg-related salmonellosis and reduction strategies in United States, Australia, United Kingdom and New Zealand. *Crit. Rev. Microbiol.*, **44**, 290-303 (2018).
29. Ministry of Food and Drug Safety (MFDS), (2023, June 27). Standards and specifications for foods. Retrieved from https://www.law.go.kr/%ED%96%89%EC%A0%95%EA%B7%9C%EC%B9%99/%EC%8B%9D%ED%92%88%EC%9D%98%EA%B8%B0%EC%A4%80%EB%B0%8F%EA%B7%9C%EA%B2%A9
30. Code of Federal Regulations (CFR), (2023, June 19). 21 CFR Part 118 Production, storage, and transportation of shell eggs. Retrieved from https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-118
31. European Commission, (2023, June 19). Commission Regulation (EC) No 589/2008 of 23 June 2008 laying down detailed rules for implementing council regulation (EC) No 1234/2007 as regards marketing standards for eggs. Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02008R0589-20171125&qid=1686796247412
32. Samli, H.E., Agma, A., Senkoylu, N., Effects of storage time and temperature on egg quality in old laying hens. *J. Appl. Poult. Res.*, **14**, 548-553 (2005).
33. Lee, S.M., Hong, C.H., Changes of egg quality according to eggshell treatment and storage condition. *Korean J. Vet. Serv.*, **28**, 225-234 (2005).
34. Lee, S.M., Yoo, H.S., Hong, C.H., Analysis of biological hazards and control points in layer houses. *Korean J. Vet. Res.*, **44**, 593-605 (2004).
35. Park, S., Choi, S., Kim, H., Kim, Y., Kim, B., Beuchat, L.R., Ryu, J., Fate of mesophilic aerobic bacteria and *Salmonella enterica* on the surface of eggs as affected by chicken feces, storage temperature, and relative humidity. *Food Microbiol.*, **48**, 200-205 (2015).
36. Kim, J.G., Park, J.Y., Kim, J.S., Effects of storage time and temperature on the hygienic quality of shell eggs. *J. Environ. Health Sci.*, **41**, 438-448 (2015).
37. Jin, Y., Lee, K., Lee, W., Han, Y., Effects of storage temperature and time on the quality of eggs from laying hens at peak production. *Asian-Australas. J. Anim. Sci.*, **24**, 279-284 (2011).
38. Jones, D.R., Ward, G.E., Regmi, P., Karcher, D.M., Impact of egg handling and conditions during extended storage on egg quality. *Poult. Sci.*, **97**, 716-723 (2018).
39. Aygun, A., Sert, D., Effects of vacuum packing on eggshell microbial activity and egg quality in table eggs under different storage temperatures. *J. Sci. Food Agric.*, **93**, 1626-1632 (2013).
40. Chen, G.Z., Chumngoen, W., Kaewkot, C., Sun, Y.M., Tan, F.J., Combination of sensory evaluation with conventional physiochemical analyses to evaluate quality changes during long-term storage and estimate the shelf life of chicken eggs. *Br. Poult. Sci.*, **64**, 594-604 (2023).