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Effect of Cold Chain on Chicken Egg Quality in a Simulated Post Washing Processing and Consumer Storage Model

ABSTRACT

This study assessed the effect of the cold chain on egg quality in a model simulation of post-washing processing and consumer storage. Post-washed eggs were assigned to 12 groups that simulated the conditions of temporary storage after washing (step 1; 7°C or 25°C for 1 day), transportation (step 2; 7°C or 30°C for 8 h), and selling or storage (step 3; 7°C, 25°C or 30°C for 4 weeks). The freshness and microbial characteristics of the eggs were analyzed for 4 weeks. High-temperature conditions in steps 1 or 2 resulted in reduced quality and more bacteria on eggshells, and this egg quality deterioration worsened after storage for over 2 weeks. In step 3, the quality of the eggs stored at 7°C was maintained during the entire storage, whereas the eggs stored at 25°C had lower quality and broken vitelline membranes in week 4, and the eggs stored at 30°C were spoiled. Eggs should be stored from post-washing until storage by consumers in a cold environment without interruption of temperature control to maintain quality and safety. Consumers must be aware that eggs should be stored at refrigerator temperature.

INTRODUCTION

Eggs provide plenty of nutrients and great culinary versatility at a low economic cost (Miranda *et al.*, 2015). Post-washing egg procedures commonly involve temporary storage, transportation, selling, and storage until consumption. According to regulation (EC) No. 853/2004, eggs “must be stored and transported at a temperature, preferably constant, that is best suited to assure optimal conservation of their hygiene properties.” Because of the risk of Salmonella infection, eggs should be refrigerated until consumption (Gross *et al.*, 2015). The Food Safety and Inspection Service (FSIS) of the US Department of Agriculture (USDA) requires packed eggs to be stored and transported under refrigeration at a temperature lower than 7.2°C. The US Food and Drug Administration’s Egg Safety Rule requires transported eggs to maintain at 7.2°C beginning 36 hours after laying (USDA-FSIS, 2011).

In the food industry, the cold chain refers to the entire agricultural production supply process after harvesting or slaughter, passing through primary production or manufacturing and ending with food that is placed in retail stores, domestic refrigerators, or catering containers by consumers (Kuo & Chen, 2010). The shelf life and safety of perishable foods, such as eggs, dairy, meat, poultry, and fish, are strongly related to environmental factors and temperature conditions. If the temperature of chilled foods exceeds a specific limit, microorganisms may grow and thus deteriorate them, spoiling and poisoning foods (Aung & Chang, 2014). Many researchers have investigated the effects of incorrect temperature in a cold chain on perishable foods, such as fresh pork and



poultry (Bruckner *et al.*, 2012), sliced ham (Derens-Bertheau *et al.*, 2015), and vacuum-packed pork ham slices (Geczi *et al.*, 2017).

Koppel *et al.* (2014, 2015, 2016) surveyed consumers' egg purchase, storage, and preparation behaviors in selected countries. Koppel *et al.* (2016) indicated that almost all US consumers purchased chilled eggs and stored them in a refrigerator. Most Argentinian and Colombian consumers (91% and 84%, respectively) bought eggs stored at room temperature, and more than half (76% and 54%, respectively) stored them in a refrigerator. In Russia and Estonia, consumers purchased both refrigerated and room-temperature eggs, whereas people in Italy (84%) and Spain (87%) typically purchased eggs stored at room temperature; almost all consumers in these countries kept eggs in a refrigerator (Koppel *et al.*, 2015). Consumers in South Korea generally buy refrigerated eggs, whereas consumers in India (100%) and Thailand (36%) buy eggs that are stored at room temperature. After purchasing, most consumers in South Korea (89%), Thailand (74%), and India (80%) stored eggs in a refrigerator (Koppel *et al.*, 2014). Considering the average annual temperature of these countries, keeping eggs at ambient temperature would strongly affect egg shelf life (Koppel *et al.*, 2016).

Simsiri *et al.* (2021) concluded that in simulated post-washing processing, maintaining washed eggs at 7°C throughout the cold chain contributed to superior quality during a 4-week storage. Any interruption of the low temperature in the cold chain resulted in varying degrees of quality deterioration. Observational consumer food-handling studies have shown that consumers often make food-handling mistakes at home, increasing the risk of foodborne illnesses (Anderson *et al.*, 2004). Therefore, the present study was a simulation spanning from post-washing

processing until products reach the consumers. We assessed the effect of the cold chain on washed egg quality using post post-washing processing and consumer storage simulations.

MATERIALS AND METHODS

Cold chain preparation and sampling

Six-hundred freshly laid chicken eggs obtained from a single flock were transported to a commercial washing plant (Taichung, Taiwan). After being washed, the eggs were delivered to our laboratory within 20 min and assigned to 12 groups (Table 1). The post-washing processing consisted of three processing steps to simulate the typical commercial egg handling in Taiwan. Briefly, after washing, eggs were stored at 25°C or 7°C for one day for step 1 (i.e., post-washing temporary storage). The eggs were then stored at 7°C or 30°C for 8 h for step 2 (i.e., transportation). Finally, the eggs were held at 7°C, 25°C, or 30°C for four weeks for step 3 (i.e., selling and storage). The eggs were held at 30°C in an incubator (Universal Oven, Model UN 110, Memmert, Schwabach, Germany), at 25°C in an air-conditioned room, or at 7°C in a refrigerator during processing, and were analyzed every two weeks.

Egg quality determination

The cumulative weight loss of the eggs was determined using the method reported by Liu *et al.* (2016). During storage, each egg was weighed using a scale. The cumulative weight loss (%) was calculated using the following formula: current weight – weight in week 0. The eggs were then manually shelled and placed on an egg quality measurement stand (NFN-381, FHK Fujihira Industry Co. Ltd., Tokyo, Japan). The heights of the thick albumen were determined

Table 1 – Description of post-washing handling of the eggs in the study.

Step 1	Step 2	Step 3	Code of treatment
Cold temperature (7 °C)	Cold temperature (7 °C)	Selling and storage (0-4 week)	
		Cold temperature (7 °C)	CCC
		Room temperature (25 °C)	CCR
	High temperature (30 °C)	High temperature (30 °C)	CCH
		Cold temperature (7 °C)	CHC
		Room temperature (25 °C)	CHR
Room temperature (25 °C)	Cold temperature (7 °C)	High temperature (30 °C)	CHH
		Cold temperature (7 °C)	RCC
		Room temperature (25 °C)	RCR
	High temperature (30 °C)	High temperature (30 °C)	RCH
		Cold temperature (7 °C)	RHC
		Room temperature (25 °C)	RHR
		High temperature (30 °C)	RHH



according to the method of Tan *et al.* (2022), using an egg quality gauge (FHK NFR3, Ozaki Manufacturing, Japan) to calculate the Haugh unit (HU) as follows: $HU = 100\log(h - 1.7w^{0.37} + 7.6)$, where *h* and *w* are the height of the albumen (mm) and weight of the egg (g), respectively. The yolk index (YI) was calculated using the method reported by Liu *et al.* (2016), measuring the width and height of the yolk using the same micrometer; the formula was $YI = (\text{yolk height})/(\text{yolk width})$. After pouring the albumen through a 2 mm mesh nylon sieve, the weights of the filtrate (thin albumen) and residue (thick albumen) were recorded and used to calculate the thick albumen ratio as follows: $\text{ratio} = (\text{weight of thick albumen})/(\text{weight of thin albumen})$ (Wan *et al.*, 2019). The albumen moisture content was measured using the method of the Association of Official Analytical Chemists (1990). The pH of the homogenized albumen and yolk (BagMixre, InterScience, Saint-Nom-la-Br eteche, France; for 30 s) was measured with a pH meter (PHM210 Standard, Radiometer, Villeurbanne Cedex, France).

Microbiological analyses

Changes in microorganisms on the eggshell surface and the egg content during storage were determined using the method reported by Cader *et al.* (2014). Each egg was aseptically placed in a sterile plastic bag containing 10 mL of 0.1% peptone solution. The bag was then softly hand-shaken for 1 min to release bacteria from the eggshell surface. After spraying the eggshell with 75% ethanol, the egg was manually cracked. The yolk and albumen were homogenized in a 1:10 dilution with 0.1% peptone water using a stomacher (BagMixre, InterScience, Saint-Nom-la-Br eteche, France) for 30 s. Serial dilutions were performed in 0.1% peptone water. Viable cells of the eggshell and egg content (log CFU/mL) were enumerated on plate count agar using the pour plate method, and incubated at 35°C for 48 h to determine the total plate count (TPC). The presence or absence of *Salmonella* spp. in each egg was determined using the 3M Petrifilm *Salmonella* Express system according to the method of Tan *et al.* (2022).

Statistical analysis

The means of the data were compared using a one-way analysis of variance with a 5% level of significance. Means were separated by performing the Scheff e test. All statistical analyses were performed using Statistical Analysis System software.

RESULTS AND DISCUSSION

Changes in Haugh unit

We classified egg grades according to the parameters of the US Department of Agriculture (USDA, 2000). Eggs with HU above 72, 71–60, 59–31, and below 31 were classified into quality grades AA, A, B, and C, respectively. When considering the effect of storage temperature in steps 1, 2, and 3 (*i.e.*, post-washing storage, transportation, and selling and storage, respectively), the HUs of the eggs stored at 7°C were higher than those of the eggs held at 25°C or 30°C (Table 2). Throughout storage, we divided the decreasing patterns of the HUs into three groups according to the step 3 condition. In the first group, the HUs of the eggs stored at 7°C (CCC, CHC, RCC, and RHC) slowly decreased from week 0 to week 4; and only the CCC, CHC, and RCC eggs remained at grade A in week 4. RHC eggs were probably not grade A in week 4 because they were stored at a low temperature

Table 2 – Changes in Haugh unit and USDA egg quality standards of the eggs during storage.

Storage condition and treatments	Storage time (weeks)		
	0	2	4
CCC	89.66 ± 2.34 ^{Aa} AA	68.92 ± 5.06 ^{Ab} A	66.07 ± 7.81 ^{Ab} A
CCR	85.26 ± 0.58 ^{Ba} AA	34.16 ± 9.43 ^{Cb} B	ND
CCH	80.40 ± 1.38 ^{EDa} AA	22.97 ± 3.80 ^{DEb} C	S
CHC	83.77 ± 1.74 ^{CBa} AA	65.72 ± 4.53 ^{ABb} A	62.18 ± 6.35 ^{Ab} A
CHR	81.54 ± 0.65 ^{CDa} AA	33.40 ± 2.52 ^{Cb} B	ND
CHH	76.98 ± 0.88 ^{Fa} AA	21.15 ± 4.34 ^{Eb} C	S
RCC	81.20 ± 1.27 ^{CDa} AA	62.91 ± 2.09 ^{ABb} A	61.84 ± 1.12 ^{Ab} A
RCR	78.21 ± 0.88 ^{EFa} AA	32.22 ± 3.55 ^{Cb} B	ND
RCH	76.58 ± 0.31 ^F AA	ND	S
RHC	79.61 ± 1.86 ^{EDa} AA	60.27 ± 1.48 ^{Bb} A	59.20 ± 8.73 ^{Ab} B
RHR	76.44 ± 1.86 ^{Fa} AA	29.63 ± 3.25 ^{CDb} C	ND
RHH	75.90 ± 0.39 ^F AA	ND	S

Abbreviations are the same used in Table 1.

^{A-F} Means in the same column with different lowercase letters are significantly different ($p < 0.05$).

^{a-b} Means in the same row with different capital letters are significantly different ($p < 0.05$).

*USDA egg quality standards: grade AA, A, B, and C = HUs of >72, 71–60, 59–31 and <31, respectively.

**ND: Not determined due to weakened or broken vitelline membrane

***S: Spoiled egg



only in step 3, but not in steps 1 and 2. For the second group (CCR, CHR, RCR, and RHR), HUs decreased rapidly from week 0 to 2 and became undetectable in week 4. For the third group (CCH, CHH, RCH, and RHH), HUs decreased rapidly, and eggs became spoiled by week 4. Step 3 was designed to simulate the selling and storage of eggs and their handling by retailers and consumers. Consumers worldwide have various egg purchase, storage, and handling practices (Koppel *et al.*, 2014; Koppel *et al.*, 2015; Koppel *et al.*, 2016); they are often the last people to have contact with food before its consumption and are ultimately responsible for any mishandling practices at home (Koppel *et al.*, 2015).

A breakdown in temperature control at any stage of a cold chain affects the final product quality; therefore, the quality and safety of perishable food products must be continually monitored throughout the entire cold chain (Aung & Chang, 2014). Anderson *et al.* (2008) indicated that refrigerated transportation should be a critical component in assessing egg safety. Mercier *et al.* (2017) reported that each step of the

cold chain could remarkably affect final food quality. A temperature fluctuation occurring at any point in the cold chain can lead to food waste or raise safety issues. In the current study, we discovered that the CCC treatment was optimal for maintaining egg HU, probably because the eggs were stored at a low temperature in each step of the cold chain without any temperature fluctuation. By contrast, RHH eggs had the lowest HUs and reduced shelf life, probably because of the cumulative damage under high-temperature storage. Any incorrect temperature during the cold chain process led to reduced shelf life.

Changes in egg quality during storage

As seen with the HU trend, chilled eggs had higher quality as indicated by a higher albumen ratio, albumen moisture, YI and lower albumen pH, and yolk pH (Figures a-e). After two weeks, eggs stored at a high temperature in step 3 had undeterminable egg quality, probably due to broken vitelline membranes. Jones & Musgrove (2005) explained that vitelline membrane strength decreases during storage because

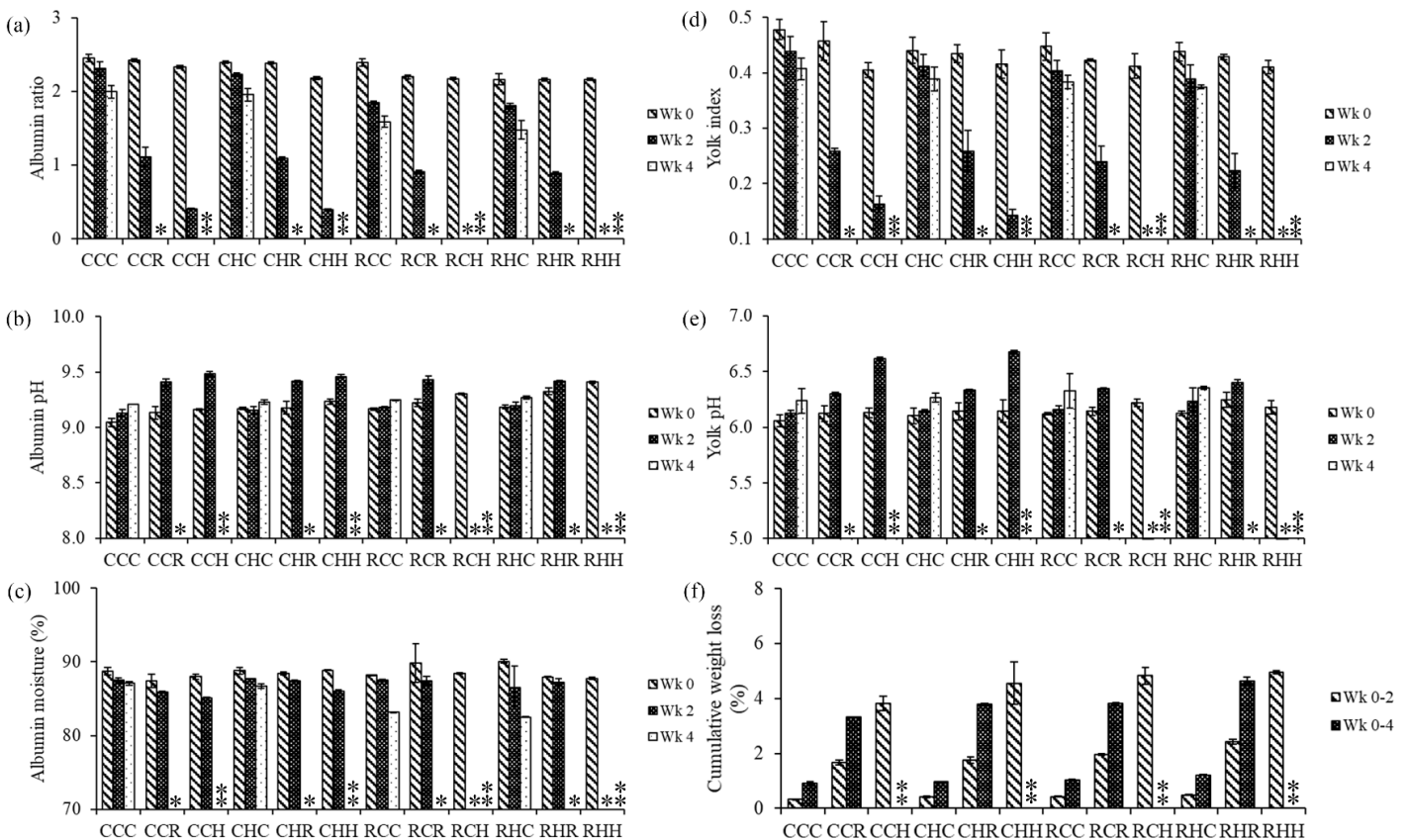


Figure – Changes in (a) albumen ratio, (b) albumen pH, (c) albumen moisture, (d) yolk index, (e) yolk pH, and (f) cumulative weight loss of eggs during storage.

Abbreviations are the same used in Table 1.

* not determined due to weakened or broken vitelline membrane

* not determined due to egg being spoiled



the yolk absorbs water molecules. Liu *et al.* (2016) reported that the HUs of washed eggs stored at 7°C slowly decreased after storage, whereas HUs rapidly decreased when eggs were held for only two weeks at 25 °C. Moreover, the HUs of eggs held at 30°C fell within a few days of the beginning of storage (Park *et al.*, 2003). The decline in the thick albumen during storage was probably due to the breakdown of carbonic acid in egg albumen, which produced carbon dioxide and water, leading to the albumen losing its gelatinous structure and becoming watery. This eventually reduced the thickness of the albumen and the HU of the eggs (Ragni *et al.*, 2007). During storage, the release of carbon dioxide from the eggs through eggshell pores increases albumen and yolk pH (Liu *et al.*, 2016). Changes in the functional properties of egg albumen and yolk during storage can be happen due to environmental conditions such as storage temperature, humidity, and duration (Qiu *et al.*, 2012).

The cumulative weight loss in all groups increased as the storage time increased in the current study (Figure-f). High temperature in either step 1 or 2 resulted in a more significant cumulative weight loss. Especially in step 3, the cumulative weight loss of the eggs stored at 7°C was markedly lower. The higher weight loss of the **CCH**, **CHH**, **RCH**, and **RHH** eggs could indicate quality deterioration (Park *et al.*, 2003; Aygun & Sert, 2013). The increase in weight loss during storage was mainly due to moisture evaporation through eggshell pores (Liu *et al.*, 2016; Jones *et al.*, 2018). When using scanning electron microscopy and cuticle staining of eggshells, Liu *et al.* (2016) discovered that the cuticle deterioration of eggs stored at 30°C and 25°C was more considerable than that of eggs stored at 7°C.

Changes in total eggshell plate count

In week 0, when considering the effect of step 1, significantly lower eggshell TPCs were found for the eggs stored at 7°C compared to the eggs stored at 25°C for the same duration (Table 3). When considering the effect of step 2, in week 0, we observed a more negligible difference in the microbial count between the 7°C and 25°C eggs, probably because of the short 8-h transportation duration. When considering the effect of step 3, the TPCs of eggs stored at 7°C were lower than those stored at 25°C and 30°C. Zhang *et al.* (2019) reported that an efficient cold chain could prevent or reduce microbial growth in foods and enhance food freshness and safety. We categorized microbial change patterns throughout storage into

Table 3 – Changes in total eggshell plate count (log CFU/mL) during storage for 4 weeks.

Storage condition and treatments	Storage time (Week)		
	0	2	4
CCC	1.63 ± 0.13 ^{EFa}	1.48 ± 0.00 ^{Ea}	1.10 ± 0.17 ^{Db}
CCR	1.48 ± 0.00 ^{Fb}	2.34 ± 0.11 ^{Ba}	2.36 ± 0.10 ^{Ca}
CCH	1.73 ± 0.05 ^{Ea}	1.63 ± 0.06 ^{DEa}	S
CHC	1.50 ± 0.17 ^{Fa}	1.20 ± 0.17 ^{Fb}	1.00 ± 0.00 ^{Db}
CHR	1.56 ± 0.07 ^{EFc}	2.31 ± 0.02 ^{Bb}	2.74 ± 0.04 ^{Ba}
CHH	1.73 ± 0.05 ^{Ea}	1.20 ± 0.17 ^{Fb}	S
RCC	2.21 ± 0.18 ^{Da}	1.97 ± 0.06 ^{Cb}	1.00 ± 0.00 ^{Dc}
RCR	2.61 ± 0.02 ^{Cc}	2.69 ± 0.01 ^{Ab}	2.86 ± 0.01 ^{ABa}
RCH	2.85 ± 0.00 ^{Ba}	1.57 ± 0.05 ^{Eb}	S
RHC	2.03 ± 0.02 ^{Da}	1.79 ± 0.08 ^{Db}	1.00 ± 0.00 ^{Dc}
RHR	2.44 ± 0.28 ^{Cb}	2.84 ± 0.06 ^{Aa}	2.95 ± 0.00 ^{Aa}
RHH	3.34 ± 0.03 ^{Aa}	2.10 ± 0.17 ^{Cb}	S

Values are indicated as mean ± SD, n=4.

Abbreviations are the same used in Table 1.

^{A-F} Means in the same column with different lowercase letters are significantly different ($p < 0.05$).

^{a-b} Means in the same row with different capital letters are significantly different ($p < 0.05$).

*S: Not determined due to egg being spoiled

three groups according to the storage conditions in step 3. The eggs stored at 7°C had decreased TPCs, which reached approximately 1 log CFU/mL in week 4, whereas the eggshell TPCs of the eggs stored at 25°C increased. For the eggs stored at 30°C in step 3, the eggshell TPCs decreased from week 0 to week 2, and the eggs were spoiled by week 4, probably because bacteria and mold may have grown on the surface of eggs under high humidity (Erkmen & Bozoglu, 2016). Aygun & Sert (2013) reported that the eggshell TPC of washed eggs stored at 22°C gradually increased, whereas that of eggs stored at 5°C gradually decreased during 6-week storage. Park *et al.* (2003) reported that the total viable cell counts of the eggshell of washed eggs held at 30°C were markedly decreased on day 10; they explained that this decrease in the microbial number was probably because of the residual protection from washing, sanitation, and coating blocking microbial growth during the initial storage. In the current study, no *Salmonella* or total aerobic bacteria were found in the egg contents for all treatments during the 4-week storage. Mokhtari *et al.* (2006) correlated salmonellosis and consumers' egg handling and consumption behaviors in their epidemiological study. Gole *et al.* (2014) indicated that eggs should be dried after washing and stored appropriately to prevent salmonellosis. Clauer (2009) suggested that consumers should always keep eggs in a refrigerator and eggs should not be left at room temperature before cooking for more than 20 min.



CONCLUSION

The present study showed that washed eggs had superior quality when kept at 7°C throughout the entire cold chain in a simulation of the post-washing processing. Washed egg quality is highly dependent on the completeness of the cold chain. After purchasing, chilled storage of washed eggs at the consumer stage should be considered a critical handling practice and a critical component of a complete cold chain. In order to maintain the quality and safety of eggs, producers and consumers should refrigerate washed eggs from post-washing until consumption. Thus, consumers should play a crucial role in reducing the risk of egg-borne illness.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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