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Original Article

Influences of Egg Washing and Storage Temperature on Quality and Shelf Life of Duck Eggs During Storage

■Author(s)

Tan FJ[I] https://orcid.org/0000-0002-2884-1093
Rungruengpet W[I] https://orcid.org/0000-0001-8611-2051
Simsiri U[I] https://orcid.org/0000-0003-3019-1656
Kaewkot C[I] https://orcid.org/0000-0001-8453-8905
Sun YM[II] https://orcid.org/0000-0003-0941-5678
Chumngoen W[III] https://orcid.org/0000-0003-4160-2027

[I] Department of Animal Science, National Chung Hsing University, Taichung, 402, Taiwan.
[II] National Animal Industry Foundation, Taipei, 100, Taiwan.
[III] Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, (KU KPS), Nakhon Pathom 73140, Thailand.

■Mail Address

Corresponding author e-mail address
Wanwisa Chumngoen
Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, (KU KPS), 1 Moo.6Malaiman Road, Kamphaeng Saen District, Nakhon Pathom, 73140, Thailand.
Phone: +866 3 4351892
Email: wanwisa.c@ku.th

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ABSTRACT

Studies on how washing and storage influence duck egg quality are scarce compared with those on chicken egg quality. The present study investigated the quality of washed and unwashed duck eggs stored at 7 °C and 25 °C for 8 weeks. Quality parameters, including Haugh unit (HU), yolk index, thick albumen ratio, albumen pH, and air cell size, indicated that egg quality deteriorated during prolonged storage, and cuticle staining confirmed that washing reduced cuticle coverage. Washed eggs stored at 7 °C maintained high quality (grade B; HU: 54) according to the United States Department of Agriculture (USDA, 2000) after storage for 8 weeks, whereas unwashed eggs stored at 25 °C exhibited a low but acceptable quality (grade B, but HU: 36) after 7 weeks. Strong correlations were observed between the quality parameters evaluated. In conclusion, duck eggs should be washed and then stored at 7 °C to enhance microbial safety and maintain quality to achieve a shelf life of at least 8 weeks.

INTRODUCTION

Duck eggs, an inexpensive source of essential nutrients, are commonly consumed in many Asian countries and account for 10%-30% of total global egg consumption (Quan & Benjakul, 2019). In addition to their consumption as processed eggs in forms such as salted eggs, pidan, and balut, consumption of fresh duck eggs has become more popular recently (Huang *et al.*, 2007; Tuyen, 2007; Jalaludeen *et al.*, 2009; Quan & Benjakul, 2019).Limited studies regarding the quality of duck eggs during storage are available (Lokaewmanee, 2017; Quan & Benjakul, 2018; Quan & Benjakul, 2019), whereas many researchers targeted on the quality changes of more commonly produced chicken eggs (Liu *et al.*, 2016). Nevertheless, some relevant quality changes which occur in chicken eggs could be applied to duck eggs. Duck egg quality is affected by many factors, including the duck's genetics and diet as well as the egg storage conditions, including storage duration, temperature, humidity, and handling processes (Lokaewmanee, 2017). During prolonged storage, the eggs lose water and carbon dioxide through the eggshell, which reduces moisture content and increases albumen pH, yolk pH, and air cell size (Liu *et al.*, 2016). Changes in the ovomucin-lysozyme complex might result in the deterioration of gelatinous structures of thick albumen and eventually albumen thinning (Liu *et al.*, 2016). Meanwhile, migration of water from the albumen through the weaker vitelline membrane causes chicken egg yolk flattening (Jones & Musgrove, 2005). Such quality changes and deterioration of duck eggs by loss of water and carbon dioxide, albumen thinning, and increased albumen pH occur as storage time increases (Quan & Benjakul, 2019). Decrease in duck egg quality can be slowed substantially by reducing the storage temperature and duration (Pandian *et al.*, 2012; Lokaewmanee, 2017; Quan & Benjakul, 2018). Many parameters, such as albumen and yolk pH, Haugh unit (HU), yolk index (YI), and air cell size, serve as quality indices for judging chicken egg freshness because they are considerably influenced by storage time and conditions (Liu *et al.*, 2016).

In some areas, laying ducks are frequently raised in backyards and herds, although some are raised in confined cages (Quan & Benjakul, 2019). Their eggs are at risk of contamination with pathogens, particularly *Salmonella*, from feces and other environmental sources (Huang & Lin, 2011). The benefits of duck egg washing, particularly with the aid of some chlorine sanitizers to reduce microbial loads on eggshells, have been well documented (Huang & Lin, 2011). However, mechanical damage to eggshell surface microstructure during improper washing may increase the risk of *Salmonella* penetration of chicken eggs (Gole *et al.*, 2014; Liu *et al.*, 2016). USDA (2000) states that eggs washed in accordance with US voluntary egg grading standards and requirements must be refrigerated after washing. Furthermore, US producers with > 3000 hens on site must refrigerate eggs within 36 h of lay (FDA, 2009). The National Bureau of Agricultural Commodity and Food Standards (ACFS, 2012) recommends that duck eggs that require storage for >1 week should be stored in a refrigerator or in a room with a controlled temperature between 10 °C and 13 °C and relative humidity of 70%-85%.

Many researchers have analyzed the influence of washing and storage conditions on chicken egg quality, but few studies have conducted such analyses on duck eggs (Lokaewmanee, 2017; Quan & Benjakul, 2018). Shelf life of a food, as defined as "the time span under defined storage conditions within which a food remains acceptable for human consumption in terms of its safety, nutritional attributes, and sensory characteristics" by Corradini (2018) is crucial to both consumers and producers. To the best of our knowledge, only the Thai Agricultural Standard, which uses HU as a quality index, is available for determining the freshness and classification of duck egg quality. Therefore, this study evaluated (1) the influence of egg washing and storage temperature on duck egg quality during storage, (2) the relationship among the quality parameters evaluated, and (3) the suggested shelf life of duck eggs based on the physical and microbial qualities.

MATERIALS AND METHODS

Egg resources and sampling

A total of 720 freshly laid eggs which originated from a flock of 50-week aged Brown Tsaiya Duck (Taiwan No. 1) with a density of 5,000 ducks/1,160 m^2/duck house were obtained from a local farm (Changhua, Taiwan). Half of the eggs were washed commercially (Sin Chao Fa Enterprise Co., Tainan, Taiwan). In brief, the eggs were loaded onto the conveyor belt of the egg washer (Sin Chao Fa, Tainan, Taiwan). After rinsing, eggs were sprayed of a mix of potable water and a chlorine-based washing agent at the manufacturer's recommended concentrations required to maintain a pH level between 10 and 11.5 at a temperature between 42 and 46 °C and brushed. After rinsing, the eggs were then subsequently forced-air dried. The eggs were packaged in commercial 10-piece egg plastic boxes and delivered to our laboratory within 30 min and assigned to one of the following four groups: washed and unwashed eggs stored at 7 °C in a refrigerator (WC and UC, respectively) and washed and unwashed eggs stored at 25 °C in an incubator (WR and UR, respectively). The eggs were stored for 8 weeks and analyzed every week.

Egg quality assessment

The extent of cuticle coverage was determined according to the method introduced by Liu *et al.* (2016). In brief, eggs were immersed in an aqueous solution containing 7.2 g of tartrazine and 2.8 g of Green S in 1 L of water for 1 min to stain the cuticle, rinsed in water to remove excess dye, and finally air dried for approximately 5 min prior to determining cuticle coverage. Cuticle coverage was quantified by measuring the colour difference of the eggshell before and after staining at four points around the equator by using a colourimeter (NR-3000, Nippon Denshoku, Japan). ΔE^*ab was calculated as $\Delta E^*ab = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$, where L^*, a^*, and b^* represent the lightness, redness, and yellowness of the samples, respectively; a higher ΔE^*ab indicates more cuticle coverage. Air cell size was determined using a tripod according to the method used by Samli *et al.* (2005). The cumulative weight loss of eggs was determined according to the method used by Caner & Yuceer (2015). During storage, each egg was weighed using a scale (YJ-GS-600 g, Shanghai Yajin Electronic Technology Co., Ltd., Shanghai, China). After weighing, the cumulative weight loss (%) was calculated using the following formula: [(start weight

- current weight)/start weight]×100. Eggs were then manually shelled and placed on a flat surface on an egg quality measurement stand (FHK NFN-381, Ozaki Manufacturing, Japan). The height of thick albumen was determined using an egg quality gauge (FHK NFR3, Ozaki Manufacturing, Japan) to calculate HU using the formula 100 log ($h - 1.7w^{0.37} + 7.6$), where h is the height of the albumen (mm) and w is the weight of the egg (g). The width and height of the yolk, which were measured using the same gauge, were used to calculate YI according to the method used by Liu *et al.* (2016) as follows:

YI = (yolk height) / (yolk width)

After pouring albumen through a 2.0 mm mesh nylon sieve, the weights of filtrate (thin albumen) and residue (thick albumen) were recorded to calculate the thick-to-thin albumen ratio (Wan *et al.* 2019) by using the formula:

Thick albumen ratio (%) = 100 × (thick albumen weight / egg weight)

Thin albumen ratio (%) = 100 × (thin albumen weight / egg weight)

Thick-to-thin albumen ratio = (thick albumen %) / (thin albumen %).

The albumen moisture content was determined according to the Association of Analytical Communities (1990) method, and the pH of homogenized (BagMixer, InterScience, France; for 30 s) albumen and yolk was measured using a pH meter (PHM 210 Standard, Radiometer, France).

Microbiological analysis

Changes of microorganisms on the eggshell surface and egg content during storage were determined according to the method used by Cader *et al.* (2014). Each egg was aseptically placed in a sterile plastic bag containing 10 mL of 0.1% peptone solution and was gently shaken by hand for 1 min to release bacteria from the eggshell surface. After 75% ethanol was sprayed on the eggshell, the egg was manually cracked. Egg content in a 1:10 dilution of 0.1% peptone water was homogenized using a Stomacher (BagMixer, InterScience, France) for 1 min. Twenty-five mL of the egg was placed in a sterile stomacher bag to which 225 ml of buffered peptone water was added. The sample was homogenized in the stomacher for one minute to obtain a homogeneous primary sample (Cader *et al.*, 2014). Serial dilutions were performed in 0.1% peptone water. For the total plate count (TPC), viable cells of the eggshell and egg content (log CFU/mL) were enumerated on plate count agar by using the pour plate method and then incubated at 35 °C for 48 h. The presence of *Salmonella* spp. in each egg was determined using the 3M Petrifilm *Salmonella* Express system according to the manufacturer's instructions.

Statistical analysis

The means of the data were compared using one-way analysis of variance with a 5% level of significance. Means were compared using the Scheffé test. The Chi-square analysis was used to assess the prevalence of *Salmonella* spp. (Djeffal *et al.*, 2018). Pearson correlation coefficients were evaluated using the Proc Corr procedure to determine the relationships between the various egg quality parameters of duck eggs washed and stored at the various temperatures. All statistical analyses were performed using Statistical Analysis System software (Version 9.4; 2014, SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

In the current study, egg quality was determined on the basis of the ACFS (2012): duck eggs with HUs of >72 and 71-60 were classified into quality grades AA and A, respectively, which is the same standard applied by the USDA (2000) for evaluating chicken eggs. To classify quality more precisely, duck eggs with HUs of 59-31 and <31 were further classified into grades B and C, respectively, on the basis of Caner and Yuceer (2015) chicken egg standard, whereas duck eggs with HUs of <60 were all categorized as grade B according to the ACFS (2012) duck egg standard.

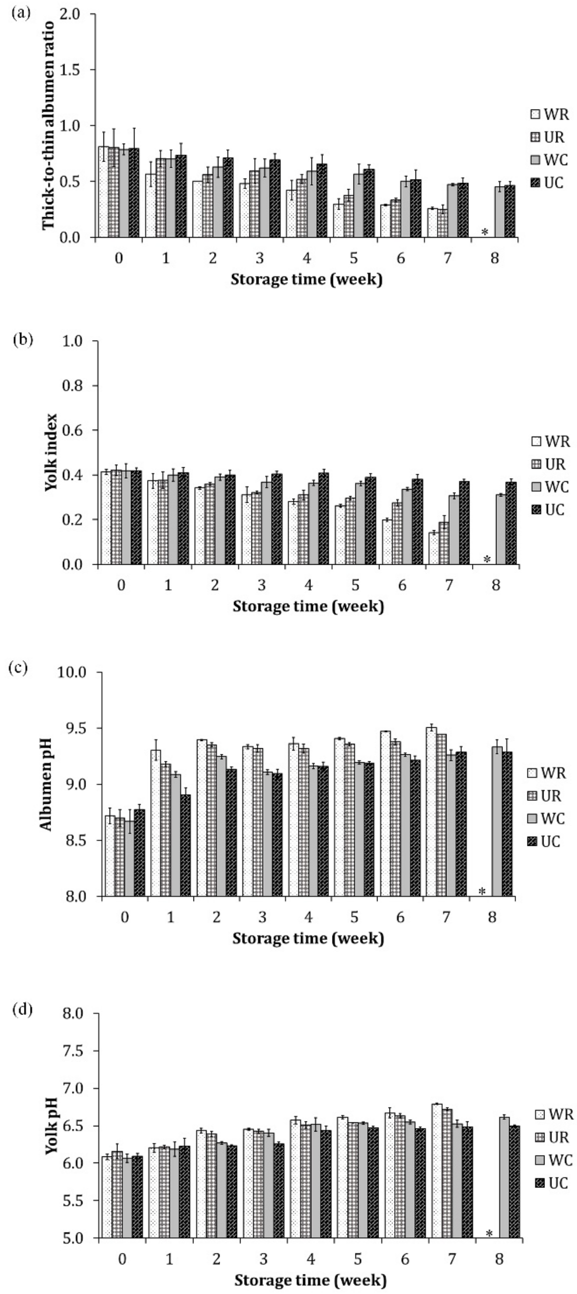
At week 0, eggs in all treatments had HUs of approximately 80, which was recognized as AA quality, without significant differences among treatments ($p>0.05$) (Table 1). As expected, Hus decreased in all groups during extended storage. HUs of the eggs stored at 25 ºC (WR and UR) were significantly lower and decreased more rapidly than those of the eggs stored at 7 ºC (WC and UC) at each week of storage ($p<0.05$). At week 7, UC and WC eggs had grades A and B, respectively (HUs of 61.2 and 56.7), whereas the quality of UR and WR eggs deteriorated to grades B (36.1) and C (27.8), respectively. After 8 weeks of storage, quality of UC and WC eggs remained at high B, whereas HUs of WR and UR eggs could not be determined because of broken vitelline membranes. Similar changing patterns were observed in the thick-

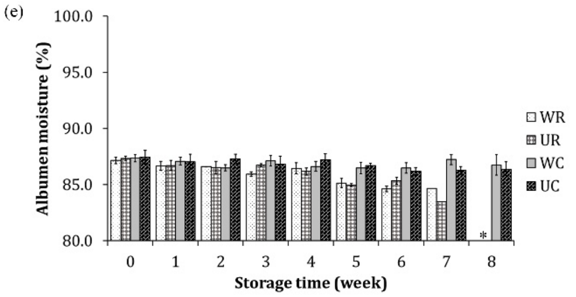
Table 1 – Effect of egg washing and storage temperature on Haugh unit (HU) and egg grade during storage.

Treatment[1]	Haugh unitEgg grade[2]								
	week 0	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8
WR	$80.79 \pm 1.62^{A,a}$	$65.48 \pm 2.56^{B,b}$	$58.98 \pm 4.74^{B,b}$	$45.40 \pm 4.97^{C,c}$	$40.10 \pm 4.98^{CD,c}$	$37.81 \pm 6.32^{CD,d}$	$33.91 \pm 2.07^{DE,d}$	$27.83 \pm 3.44^{E,c}$	ND
	AA	A	B	B	B	B	B	B (C)[3]	
UR	$80.91 \pm 3.90^{A,a}$	$69.24 \pm 3.11^{B,b}$	$64.07 \pm 4.82^{B,b}$	$53.46 \pm 3.82^{C,b}$	$46.92 \pm 5.33^{CD,c}$	$45.55 \pm 5.49^{D,c}$	$39.78 \pm 5.52^{DE,c}$	$36.12 \pm 5.08^{E,b}$	ND
	AA	A	A	B	B	B	B	B	
WC	$80.84 \pm 1.00^{A,a}$	$74.75 \pm 3.55^{B,b}$	$71.02 \pm 1.65^{BC,a}$	$69.13 \pm 3.29^{C,a}$	$62.59 \pm 1.62^{D,b}$	$60.03 \pm 1.95^{DE,b}$	$58.61 \pm 3.66^{DEF,b}$	$56.66 \pm 5.32^{EF,a}$	$54.47 \pm 4.60^{F,a}$
	AA	AA	A	A	A	A	B	B	B
UC	$82.34 \pm 2.06^{A,a}$	$77.19 \pm 6.26^{AB,a}$	$72.96 \pm 2.64^{BC,a}$	$71.20 \pm 1.52^{BC,a}$	$70.07 \pm 4.81^{C,a}$	$68.62 \pm 3.42^{C,a}$	$66.62 \pm 2.27^{CD,a}$	$61.23 \pm 3.99^{DE,a}$	$58.21 \pm 5.86^{E,a}$
	AA	AA	AA	A	A	A	A	A	B

Data are expressed as means ± standard deviations.
Means within a same row with different superscripts $^{(A-F)}$ differ significantly at ($p<0.05$).
Means within a same column in each week with different superscripts $^{(a-d)}$ differ significantly at ($p<0.05$).
ND: not detectable due to weakening or broken of vitelline membrane.
[1]WR: washed and storedat 25°C; UR: unwashed and stored at 25°C; WC: washed and stored at 7°C; UC: unwashed and stored at 7°C.
[2] determined according to the Thai Agricultural Standard (duck egg): AA, HU ≥ 72; A, HU = 71- 60; B, HU < 60.
[3] determined according to Caner and Yuceer (2015): AA, HU ≥ 72; A, HU = 71- 60; B, HU = 59 - 31; C, HU < 31.

to-thin albumen ratio (Figure 1a) and YI (Figure 1b). During extended storage, progressive weakening of vitelline membranes and osmotic diffusion of water from the albumen resulted in flattening of the yolk and decreased YI values (Ragni *et al.*, 2007; Caner & Yuceer, 2015). Such water movement was influenced by the storage temperature Keener *et al.* (2006) and Shin *et al.* (2012) suggested that vitelline membrane was highly sensitive to temperature changes during storage. Figures 1c and 1d illustrate that albumen and yolk pH values increased significantly with storage temperature and time, whereas the albumen moisture content of WR and UR eggs decreased considerably, particularly after >5 weeks of storage (Figure 1e).

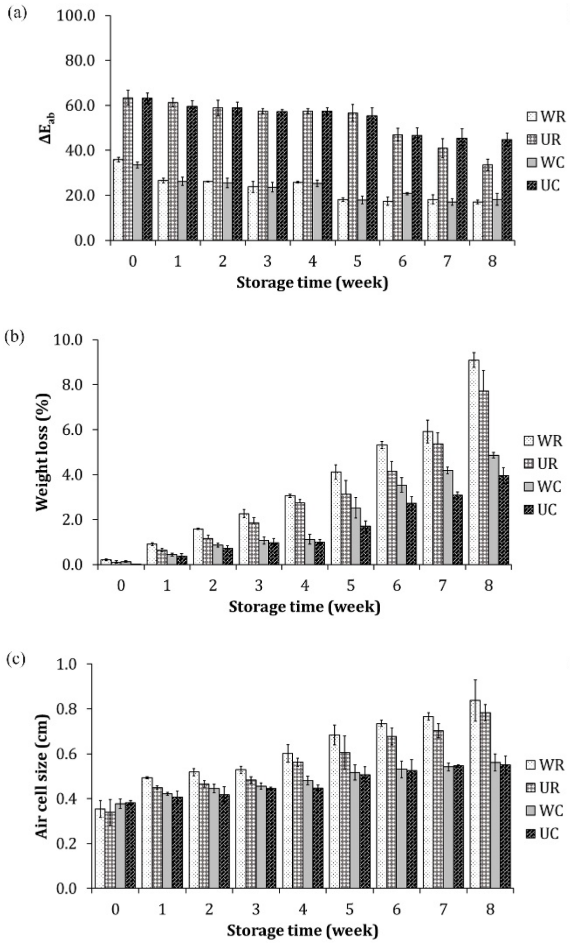




Prolonged storage duration and increased storage temperatures considerably reduced egg quality (Huang & Lin, 2011; Pandian *et al.*, 2012; Lokaewmanee, 2017; Quan & Benjakul, 2018; Quan & Benjakul, 2019). The release of carbon dioxide through eggshell pores during the extended storage resulted in a breakdown of the carbonic acid-bicarbonate buffer system in albumen, thus increasing albumen pH (Samli *et al.*, 2005; Ragni *et al.*, 2007; Shin *et al.*, 2012). Yuceer & Caner (2014) explained that protease enzymes, which are depolymerized by hydroxyl ions at increased pH, destabilize the ovomucin-lysozyme complex, causing thick albumen to lose its gelatinous structure and become thinner, eventually reducing the HUs of eggs. Higher storage temperatures might increase protein changes and moisture exchange from albumen to yolk, thus reducing the Hus of stored eggs (Brake *et al.*, 1997).

Regarding the effects of washing, washed eggs tended to have lower quality than unwashed eggs at the same storage temperature and time, probably because of the loss protection provided by the cuticle layers of the eggshell (Liu *et al.*, 2016). Nevertheless, after 7 weeks of storage, washing and storage at 25 °C resulted in unacceptable quality grades in WR eggs (grade C by the Caner & Yuceer (2015) standards), whereas storage at 7 °C maintained the quality of UC and WC eggs. This result is in agreement with that reported by Jones *et al.* (2018), who found that refrigeration could maintain better egg quality than washing and oiling could.

A calcified eggshell protects the egg from physical damage and possible microbial contamination (Hincke *et al.*, 2011). Acting as a natural barrier, eggshell cuticles not only reduce moisture loss, thus maintaining egg freshness, but also prevent microbial invasion (Liu *et al.*, 2016). Some cuticle coverage might be depleted during egg washing, as illustrated in Figure 2a; this indicates that washed eggs (WR and WC) have significantly lower cuticle coverage than unwashed eggs (UR and UC). Liu *et al.* (2016) observed through scanning electron microscopy the loss of cuticle layers in eggshells caused by washing and extended storage and reported that the cuticle deterioration rate of eggs stored at 25 °C was higher than that of eggs stored at 7 °C. Similarly, Gole *et al.* (2014) found that washed chicken eggs had a significantly higher cuticle score (i.e., poor cuticle quality) as compared to unwashed eggs. Consistent with these findings, UC eggs in the current study had greater cuticle coverage than UR eggs after storage for >7 weeks. Chen *et al.* (2019) indicated that cuticle quality was closely related to the bird species. Nevertheless, cuticle quality might be influenced by many factors such as genetics, ages of animals, egg freshness, processing, and storage (Leleu *et al.*, 2011). As expected, egg weight loss (Figure 2b) and air cell size (Figure 2c) increased during storage, mainly due to moisture evaporation and loss of carbon dioxide through the porous shell (Samli *et al.*, 2005; Ragni *et al.*, 2007). After storage for 8 weeks, the cumulative weight loss of WR and UR eggs (9.09% and 7.71%) was significantly higher than that of WC and UC eggs (4.86% and 3.95%). The washed groups showed greater weight loss than the unwashed groups (WR vs. UR; WC vs. UC), probably due to the depletion of the cuticle layer functioning as a barrier against moisture loss.



Because of the unique raising systems of duck herds, fecal and microbial contamination of duck eggs is more common among ducks raised in ponds or backyards than among those raised in confining hen batteries, mainly because of the high density of feces and other environmental contaminants, and detection and prevention measures should be implemented (Quan & Benjakul, 2019). Saitanu *et al.* (1994) found that 12.4% of duck eggs collected from open markets in Thailand had *Salmonella* on the eggshells; the authors suggested that to reduce the risk of salmonellosis, duck eggs should be cleaned properly for the elimination of contamination on the eggshells, stored in a refrigerator for the prevention of *Salmonella* propagation, and completely cooked before eating. Washing, commonly consisted of wetting, washing, rinsing, and drying, is considered a useful practice to decontaminate eggs in some countries (Messens *et al.*, 2011). Figure 2d shows changes in the TPCs of eggshell during storage. At week 0, significantly lower TPCs of WR and WC eggs (3.37 and 3.12 log CFU/mL) than those of UR and UC eggs (5.08 and 4.94 log CFU/mL) demonstrated that washing considerably reduced the microbial load on eggshells. The eggshell TPCs of refrigerated eggs (WC and UC) decreased gradually during storage, whereas those of eggs stored at ambient temperature (WR and UR) increased. These changes in microbial patterns due to the effect of storage temperature were in agreement with the findings of a study conducted by Aygun & Sert (2013) and underscore the importance of storage temperature. Notably, even with lower initial microbial counts immediately after washing, the eggshell TPCs of washed WR eggs increased after prolonged storage. The viable cell count for the egg content was $<1\times10^1$ CFU/mL, and no *Salmonella* was detected during storage in eggs under any of the treatment conditions in the current study. Table 2 shows the correlations between the quality parameters of WC eggs determined in the current study. The correlations between the various quality parameters (i.e., HU, albumen pH, thick-to-thin albumen ratio, YI, yolk pH, weight loss, and air cell size) of duck eggs were high, and similarly high correlations were also observed in other groups (data not shown) and previous studies (Liu *et al.*, 2016).

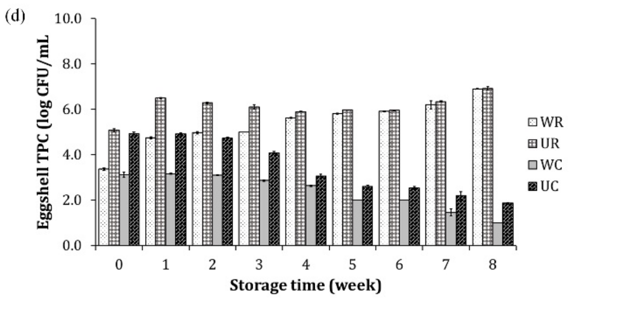


Table 2 – Pearson correlations between various egg quality parameters of duck eggs washed and stored at 7 °C.

	HU[1]	AP	AM	TAR	YI	YP	WL	ACS
HU	1.00	-0.94**	0.84**	0.96**	0.94**	-0.90**	-0.95**	-0.95**
AP		1.00	-0.74*	-0.87**	-0.86**	0.90**	0.83**	0.88**
AM			1.00	0.92**	0.92**	-0.78*	-0.90**	-0.93**
TAR				1.00	0.96**	-0.90**	-0.98**	-0.98**
YI					1.00	-0.80**	-0.96**	-0.96**
YP						1.00	0.83**	0.92**
WL							1.00	0.96**
ACS								1.00

*$p<0.05$, **$p<0.01$.

[1] HU: Haugh unit; AP: albumen pH; AM: albumen moisture; TAR: thick-to-thin albumen ratio; YI: yolk index; YP: yolk pH; WL: weight loss; ACS: air cell size.

According to the quality judgment criteria that a B grade (HU>31) or higher based on the Caner & Yuceer (2015) standards is required for acceptable quality, UR and WC eggs had shelf lives of approximately 7 weeks and >8 weeks (8-week was the maximum test period in the current study), respectively. Notably, our previous study revealed a more rapid decrease in chicken egg HU (Liu *et al.*, 2016). For example, during the 4-week storage period at 25 °C, the HU of UR chicken eggs decreased by 67.6%, whereas that of UR duck eggs decreased by only 42.0%. However, approximately 20% decreases in HU were observed in both chicken and duck WC eggs. This result agrees with that reported by Jalaludeen *et al.* (2009) indicating that the quality of duck eggs, particularly those that were unwashed and stored at 25 °C, was not severely affected by long storage times, unlike the quality of chicken eggs. Thus, different characteristics and compositions might influence duck egg storage compared with chicken egg storage, although these might vary further depending on the breed. A higher calcium shell content and more compact palisade layer of eggshells may function as physical and bacterial barriers, making duck eggshells thicker and stronger than chicken eggshells (Arthur, 2017). Some proteins extracted from the eggshell and cuticle of different domestic bird species demonstrated antimicrobial activities and revealed that duck eggs are more resistant to an aggressive external environment than chicken eggs (Wellman-Labadie *et al.*, 2008). Moreover, duck eggs' higher ability to resist bacterial spoilage than chicken eggs results in less loss of interior quality and better stability during storage at an ambient temperature (Quan & Benjakul, 2019). In practice, duck eggs should be refrigerated to maintain acceptable quality during storage (Quan & Benjakul, 2019).

CONCLUSION

The quality parameters, including HU, of duck eggs were remarkably affected by storage duration and temperature, and washing inevitably reduced cuticle coverage. Our findings emphasize the importance of washing duck eggs and storing them at low temperatures (refrigeration) to enhance microbial safety and egg quality. WC eggs (washed eggs stored at 7 °C) maintained a high acceptable quality (grade B, based on the USDA (2000) standard) after storage for 8 weeks, whereas UR eggs (unwashed eggs stored at 25 °C) exhibited a low but acceptable quality (grade B) after storage for 7 weeks. Washed eggs exhibit less deteriorations on both physical and microbial qualities when storage at low-temperature. A better understanding of duck egg quality changes during storage encourages the food industry and consumers to store, handle, and use eggs more efficiently.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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