

The effect of storage conditions and packing materials on the quality properties of chicken eggs

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Abstract. This study investigated how storing eggs in different packaging (unpacked eggs-control, cardboard, styrofoam, plastic) and temperatures (4–6, 20–22 °C) for 28 days affects their quality. Eight eggs were analysed on days 0, 7, 14, 21, and 28 for each treatment. Storage temperature–ST, packaging material–PM, storage duration–SD, and their interaction encouraged egg weight loss–EWL ($P < 0.05$). The interaction of the three factors showed no significant difference in the EWL. Storage temperature significantly influenced ($P < 0.05$), eggshell thickness–EST, albumen index–AI, albumen pH–ApH, Haugh unit–HU, yolk height–YH, and yolk index–YI. Some parameters like egg weight–EW g, shape index–SI and albumen weight–AW g, changed significantly ($P < 0.05$) according to storage in different PM. The SI, eggshell weight–ESW g, EST, eggshell index–ESI, albumen ratio–AR, AI, ApH, HU, yolk weight–YW, yolk height–YH, YI, yolk colour–YC, and yolk:albumen–Y:A ratio changed during storage. The ST×SD was significant for EW, ESW g, EST, AW g, albumen ratio–AR, AI, ApH, HU, YW g, YH, and YI ($P < 0.05$). As a result of the PM×SD interaction, significant differences ($P < 0.05$) were observed in EW g, ESW g, EST, AW g, and YI. A significant interaction effect of ST×PM×SD was found on AI, YH, and yolk pH–YpH ($P < 0.05$). The purpose of this research is to give an overview of the storage conditions in order to have good quality eggs.

Key words: chicken eggs, quality characteristics, packing material, storage duration and temperature.

INTRODUCTION

There is no doubt that eggs are an ideal food and an excellent source of high-quality protein, as well as essential vitamins and minerals. Eggs are also an affordable source of nutrients for a healthy diet and life. A previous study has shown that eggs play an important role in human nutrition, particularly for certain population such as the elderly,

pregnant woman, children, convalescents, and athletes (Miranda et al., 2015). On the other hand, if they are not stored in proper conditions, they can spoil and become a health hazard. Ensuring high egg quality is crucial for consumer satisfaction and the overall success of the egg industry. Today this industry faces several challenges related to ensuring and maintaining egg quality. Thus, Preisinger (2018) in his research described that one of the challenges of the poultry industry is to provide the consumer with eggs of the best possible quality.

Several factors influence the quality of table eggs, including the flock's age (Freitas et al., 2017), genotype, and raising system (Sokolowicz et al., 2018), methods of hen feeding (Anene et al., 2023), diet composition (Díaz-Echeverría et al., 2023) or the storage temperature and storage duration (Akyurek & Okur, 2009; Madrigal-Portilla et al., 2023; Murshed et al., 2023). Storage conditions play an important role in preserving the freshness and quality of eggs by influencing their appearance, weight, structure, taste, color, and nutritional value. Grashorn et al. (2016), also demonstrated that the main factors influencing internal egg quality are duration and temperature of storage, highlighting an important interaction between these two factors. Several authors have studied the effect of storage time and temperature on chicken egg quality. During storage, the egg begins to lose weight due to water evaporation through the membrane and pores of the shell. Based on previous studies (Wickramasinghe et al., 2013; Akter et al., 2014; Feddern et al., 2017; Martinez et al., 2021), egg weight loss increased, significantly. This decrease in weight is more pronounced in eggs stored at room temperature, achieving ~ 9% and for refrigerated eggs, this loss was only < 6% at the end of 9 weeks (Feddern et al., 2017). Storage temperature and storage duration affect the reduction of the albumen quality. This was found in the research of Jones et al. (2018) who recommended storing eggs at low temperatures to avoid problems of loss in albumen quality. Other significant ($P < 0.05$) changes that could be observed during storage time and temperature were demonstrated by Akter et al. (2014), for egg weight loss, specific gravity, Haugh Unit, pH of the albumen, albumen weight loss, increased pH of the yolk, yolk weight and percentage of yolk weight. A significant decrease in albumen, yolk, and shell weight was observed also by Murshed et al. (2023).

On the other hand, Wickramasinghe et al. (2013) studied the effect of packaging material that could be used with minimal changes in egg quality during storage at room temperature in Sri Lanka. In this case, the packaging material did not show significant changes for Haugh Unit, albumen pH, yolk pH, yolk color, and yolk index, while the effect was significant for weight loss (%) and air cell size at 32 °C.

In this regard, only a few studies have been published that have analysed egg quality during different egg storage conditions. Therefore, to further clarify this, our study aimed to determine the effects of storage temperature and duration, and packaging material on the quality of eggs during storage, until it reaches our table.

MATERIALS AND METHODS

Egg collection and preparation for storage

Three Hundred Twenty chicken eggs, collected on the day of laying, were obtained from laying hens kept in cage system, from Gjakova region and have been sent to the laboratory of the Faculty of Agriculture and Veterinary, University of Prishtina 'Hasan Prishtina', Prishtina, Kosovo. Table 1 gives the design of the experiment.

Egg storage methods description

The eggs storage methods consist of using three packing materials: cardboard, styrofoam, plastic boxes, and control (unpacked eggs stored in glass shelf of refrigerator). Eggs packaged in each packaging material, which was closed, and stored in a refrigerator (Gorenje, Slovenia) at temperature 4–6 and at the counter at 20–22 °C. The eggs were weighed and analyzed at both storage temperatures: at the start (day 0), 7, 14, 21, and 28 days. The storage temperature was monitored daily through a digital thermometer (CombiSteel LCD Multi thermometer with ± 1 °C).

Table 1. Design of the experiment

Storage temperature, °C	Packing material	Storage duration, days				
		0	7	14	21	28
4–6 °C	Control	Eight eggs for each treatment				
	Cardboard					
	Styrofoam					
	Plastic					
20–22 °C	Control	Eight eggs for each treatment				
	Cardboard					
	Styrofoam					
	Plastic					

Each egg was weighed with a digital analytical scale (KERN ALS 120-4N) with an accuracy of 0.01 g and the weight of each egg was recorded.

Egg weight loss

Egg weight loss was determined by weighing eggs individually at each storage time interval using a digital analytical scale with an accuracy of 0.01 g. All weights were expressed in grams and weight loss was determined as a percentage considering the difference between the initial egg weight and weight obtained at each time interval of storage, at both temperatures (4–6 °C and 20–22 °C) and in all types of packaging (cardboard, styrofoam, plastic boxes, and control). The egg weight losses (%) were calculated using the formula:

$$\text{Weight loss (\%)} = [(Initial\ weight - Final\ weight)/Initial\ weight] \times 100. \quad (1)$$

External quality of chicken eggs

All external egg quality parameters (egg weight, shape index, eggshell weight, eggshell thickness, eggshell index, eggshell ratio, egg surface area and specific gravity) were evaluated on the collected day and weekly periods. The length and width of the egg was measured through a digital calliper. From these measurements was calculated egg shape index, according to the following equation:

$$\text{Shape index} = (\text{egg width}/\text{egg length}) \times 100. \quad (2)$$

The surface area of the egg was calculated according to the following equation:

$$\text{Egg surface area} = 3.9782 \times EW^{0.7056} \text{ (Carter, 1975), EW-Egg weight.} \quad (3)$$

To calculate the specific gravity of the egg, we used the formula:

$$\text{Specific gravity} = \text{Egg weight}/\text{Egg volume.} \quad (4)$$

The weight of the shell was measured using a digital analytical scale with an accuracy of 0.01 g. Meanwhile, the thickness of the shell was measured using a digital Verner calliper with an accuracy of 0.01 mm. The shell ratio was calculated by dividing the weight of the shell by the weight of the egg and multiplying by 100. The eggshell index was calculated according to the following equation:

$$\text{Eggshell index} = (\text{eggshell weight}/\text{eggshell surface}) \times 100 \text{ (Ahmed et al., 2005)} \quad (5)$$

Internal quality of chicken eggs

To assess the effect of different storing condition on the internal quality of eggs (albumen weight, albumen ratio, yolk weight, yolk ratio, albumen, index, yolk index, albumen pH, yolk pH, Haugh unit, yolk colour and yolk:albumen ratio) each eggs were weightened and broken. After breaking the egg on the flat glass platform, the height of the dense albumen, the width and length of the albumen, the height of the yolk, and the width and length of the yolk were measured using a digital Vernier calliper. The intensity of the yellow colour was measured using the Roche paper color scale (1–15). In addition, the albumen was separated from the yolk, and their weights were measured. The weights of the albumen and yolk were recorded as absolute values, while the relative weights were calculated by dividing their absolute weight by the weight of the egg and expressed as a percentage. Through the pH meter (pH-mètre crison glp 21, developed and manufactured in Spain by Crison Instruments, S.A), was determined pH of the egg albumen and egg yolk. The Haugh unit was calculated after determining the egg weight and the height of the dense albumen. The following equation was used to calculate the Haugh unit:

$$\text{Haugh Unit (HU)} = 100 \log [h - 1.7W^{0.37} + 7.6] \text{ (Raymond Haugh, 1937)} \quad (6)$$

Statistical analysis

All data are presented as the mean of eight eggs and \pm standard error of the mean (SEM). The collected data on various egg quality parameters were statistically analysed using JMP IN 7, statistical software (business unit of SAS). Tukey-Kramer HSD post hoc test was used to compare mean group differences. Differences in the mean were considered significant $P < 0.05$.

RESULTS AND DISCUSSION

The effect of storage temperature, packing material, storage duration and their interaction on egg weight and egg weight loss %

The effect of storage condition on the egg weight and egg weight loss are given in Table 2. The results of this research show that the no significant difference ($P > 0.05$) of storage temperature and storage duration was observed on egg weight. However, the type of packaging material has shown a significant influence ($P < 0.05$) on egg weight (g). This observation can be explained by the fact that the cardboard material serves as a favor for moisture absorption resulting in lower egg weight. The present finding is in line with the work of Akyurek & Okur (2009) and Martinez et al. (2021) who found that egg weight not affected ($P > 0.05$) by storage temperature, storage duration and their interaction, during 14, 10 days, respectively. Similar to our value was also reported Jin et al. (2011), who concluded that the storage duration for ten days did not affect the egg weight ($P > 0.05$) but observed significant difference ($P < 0.05$) of storage temperature on egg weight. Furthermore, the results obtained by Jin et al. (2011) showed that the interaction between storage temperature and storage duration did not affect egg weight. Yildirim (2017) studied the changes in quality characteristics during storage time of egg from layer hens fed diet supplemented with Panaxginseng Meyer leaf extract and found that the egg weight was not affected ($P > 0.05$) by storage times (28 days). When considering the interaction between $ST \times SD$ and $PM \times SD$ there were observed significant differences ($P = 0.0002$, $P = 0.0068$) in egg weight. While, the interaction

between ST × PM, as well as ST × PM × SD did not show any significant difference on egg weight ($P > 0.05$).

The mean values of EWL (%) were significantly influenced ($P < 0.05$) by storage temperature, storage duration, and packaging material (Table 2). The loss of weight and the expansion of the air cell occur due to water diffusion through the eggshell. The permeability of the eggshell is influenced by factors such as shell thickness, pore count, and the quality of the cuticle (Grashorn, et al., 2016). In our study the refrigerator demonstrated greater effectiveness in preserving the egg weight compared to ambient conditions. Significantly higher weight loss (1.98%) was observed in eggs stored at 20–22 °C than in eggs (0.93%) stored at 4–6 °C. This agrees with past works (Akyurek & Okur, 2009; Akter et al., 2014) that reported the room temperatures negatively affect ($P < 0.001$) EWL (%). Moreover, the decrease of egg weight in different storage temperature was also reported by Grashorn et al. (2016); Drabik et al. (2021); Martinez et al. (2021). These authors in their study concluded that the EWL (%) was significantly affected ($P < 0.001$) by storage temperature.

Table 2. Effect of storage temperature, packing material, storage duration and their interaction on egg weight loss, g and % (Mean ± SEM)

Treatments	Parameters		
	EW, g	EWL, g	EWL, %
Storage temperature, °C			
20–22 °C	61.64 ± 0.47	1.25 ± 0.10 ^a	1.98 ± 0.16 ^a
4–6 °C	61.81 ± 0.59	0.58 ± 0.05 ^b	0.93 ± 0.08 ^b
Packing material, box			
Control	62.50 ± 0.78 ^a	1.10 ± 0.15 ^a	1.69 ± 0.22 ^a
Cardbox	59.93 ± 0.76 ^b	1.03 ± 0.13 ^a	1.70 ± 0.21 ^a
Styrofoam	62.54 ± 0.75 ^a	0.86 ± 0.10 ^b	1.36 ± 0.16 ^b
Plastic	61.94 ± 0.70 ^a	0.66 ± 0.10 ^c	1.06 ± 0.16 ^c
Storage duration, day			
7	62.07 ± 0.88	0.44 ± 0.03 ^d	0.72 ± 0.05 ^d
14	62.61 ± 0.90	0.89 ± 0.09 ^c	1.38 ± 0.13 ^c
21	62.24 ± 0.76	1.29 ± 0.09 ^b	2.07 ± 0.14 ^b
28	60.67 ± 0.82	1.94 ± 0.15 ^a	3.09 ± 0.23 ^a
<i>P</i> value			
ST	ns	< .0001	< .0001
PM	0.0264	< .0001	< .0001
SD	ns	< .0001	< .0001
ST × PM	ns	0.0019	0.0018
ST × SD	0.0002	< .0001	< .0001
PM × SD	0.0068	< .0001	< .0001
ST × PM × SD	ns	ns	ns

EW – Egg weight; EWL – Egg weight loss; ST – Storage temperature; PM – Packing material; SD – Storage duration, ns – non significant. ^{abcd}Means with different superscripts within the same column are significantly different at $P < 0.05$.

Table 2 shows that the type of packaging material has a significant effect on EWL percent ($P < 0.05$). The maximum EWL (%) were recorded at cardboard boxes (1.70%) and at control group (1.69%), compared to eggs stored in styrofoam and plastic packaging. This can happen because cardboard box has a higher potential to absorb

moisture. Also, this observation can be explained by the fact that the plastic and styrofoam material served as a barrier to moisture loss, resulting in lower EWL percentage (1.36% and 1.06%, respectively). Our results are somewhat in agreement with previous study reports in which significant changes ($P < 0.05$) of EWL percentage have also occurred on eggs stored as uncoated (control) (Jariyapamornkoon et al., 2023) and cardboard material (Wickramasinghe et al., 2013). In case of uncoated eggs showed significantly higher weight loss compared to coated eggs. It is important to note that similar results to ours, regarding the influence of packaging material on egg weight loss percentage ($P < 0.05$) have also been reported by Drabik et al. (2021). According to them the lowest percentage of egg weight losses were recorded at eggs stored in plastic boxes.

The study revealed that the EWL (%) was ($P < 0.05$) affected by storage duration. Similarly, to previous statement, interaction between ST \times PM, ST \times SD and PM \times SD did affect ($P > 0.05$) the EWL percentage. There was no significant difference ($P > 0.05$) in EWL percentage when the three factors interacted (storage temperature, packing material and storage duration). Our study showed, as the storage time increases, changes occur in the weight and inside of the egg. Greatest weight loss was observed from day 21 to day 28 (1.02%). From the day 7–14, the egg had smaller weight loss by 0.66%. On the other hand, between days 14 and 21, the eggs experienced a 0.69% weight loss. This was due to the release of water from the egg albumen through the pores of the eggshell. This reality is supported by other researcher (Jin et al., 2011; Wickramasinghe et al., 2013; Grashorn, 2016; Martinez et al., 2021; Murshed et al., 2023; Jariyapamornkoon et al., 2023) who found that storage time significantly affected egg weight loss. Martinez et al. (2021) and Jin et al. 2011 reported similar results ($P < 0.05$) to our findings for the effect of interaction between storage duration and storage time in EWL (%). The results reported by Drabik et al. (2021) and Martinez et al. (2021) for the effect of interaction between ST \times SD on egg weight losses is also similar to those derived from our research.

The effect of storage temperature, packing material, storage duration and their interaction on the external quality of eggs

The external quality of the egg and in particular the shell is very important for the processing industry and food safety. The results of external quality of chicken eggs were summarized in Table 3. The storage temperature, packing material, storage duration and interaction between them, had statistically no significant ($P > 0.05$) influence for shape index. Similar observation was also reported by Akyurek & Okur (2009); Yildirim (2017); Uyanga et al. (2020) and Murshed et al. (2023). They stated that storage duration for 14, 28, 10 and 30 days, respectively, did not significantly ($P > 0.05$) affected shape index. The results are in conformity also with the findings of Akter et al. (2014) who reported that shape index was not affected ($P > 0.05$) by storage temperature and storage duration. No significant difference ($P > 0.05$) of interaction between ST \times SD and storage duration in shape index was also observed by other researcher (Akyurek & Okur, 2009). The changes of egg surface area (cm^2) at eggs stored in refrigerator and those stored at room temperature was non-significantly ($P > 0.05$). Also, there were no significant changes to the ESA (cm^2) even during storage and interaction of ST \times PM, ST \times SD, PM \times SD and ST \times PM \times SD. Another researcher (Yildirim, 2017) was noticed similar trend in ESA (cm^2) after storage duration. Meanwhile, the results of the packing material on the ESA (cm^2) are at the limit of statistical significance ($P = 0.0452$). Our research shows (Table 3) that ST, PM and SD significantly influenced

($P < 0.05$) the specific gravity of eggs (ESG, g cm^{-3}). A slight decrease in ESG was recorded with increasing storage temperature from 4–6 to 20–22 °C (1.080 g cm^{-3} and 1.076 g cm^{-3} respectively). The egg specific gravity is a physical parameter related to the moisture loss from the egg or the weight of the egg. The higher ESG was recorded at eggs stored in plastic box (0.028, 0.005 and 0.003 score, respectively) compared with control, cardboard and styrofoam box. On the other hand, no similar finding obtained by Drabik et al. (2021), who explained that the packing material did not cause changes in egg specific gravity. A significant decrease of ESG ($P < 0.05$) was recorded with increasing in storage duration. The specific gravity of eggs was decreased from maximum of 1.104 g cm^{-3} recorded on the day of eggs collection to a minimum of 1.054 g cm^{-3} observed after 28 days' storage (Table 3). The interaction of ST \times PM, ST \times SD, PM \times SD and ST \times PM \times SD on the specific gravity of the eggs was also significant ($P < 0.05$). Overall, the interaction between storage temperature, packaging material and storage duration creates a complex but crucial factor in maintaining egg quality. By understanding these interactions, we can ensure that optimal storage conditions positively affect specific gravity, thereby providing fresher and higher quality eggs for a longer period.

Table 3. Effect of storage temperature, packing material, storage duration and their interaction on external egg parameters (Mean \pm SEM)

Treatments	Parameters		
	SI, %	ESA, cm^{-2}	ESG, g cm^{-3}
Storage temperature, °C			
20–22 °C	79.09 \pm 0.51	72.84 \pm 0.40	1.076 \pm 0.02 ^b
4–6 °C	78.37 \pm 0.24	72.95 \pm 0.49	1.080 \pm 0.02 ^a
Packing material, box			
Control	78.46 \pm 0.35	73.54 \pm 0.65	1.064 \pm 0.02 ^b
Cardbox	77.89 \pm 0.40	71.39 \pm 0.64	1.087 \pm 0.01 ^{ab}
Styrofoam	78.47 \pm 0.41	73.58 \pm 0.62	1.089 \pm 0.02 ^{ab}
Plastic	80.08 \pm 0.88	73.08 \pm 0.58	1.092 \pm 0.01 ^a
Storage duration, day			
0	80.30 \pm 1.10	73.62 \pm 0.74	1.104 \pm 0.10 ^a
7	77.56 \pm 1.10	73.18 \pm 0.70	1.078 \pm 0.02 ^b
14	79.05 \pm 0.34	73.62 \pm 0.74	1.061 \pm 0.01 ^c
21	78.37 \pm 0.43	73.34 \pm 0.66	1.055 \pm 0.01 ^d
28	78.36 \pm 0.43	72.00 \pm 0.69	1.054 \pm 0.00 ^d
<i>P</i> value			
ST	ns	ns	< .0001
PM	ns	0.0452	0.0073
SD	ns	ns	< .0001
ST \times PM	ns	ns	0.0065
ST \times SD	ns	ns	< .0001
PM \times SD	ns	ns	0.0033
ST \times PM \times SD	ns	ns	< .0001

SI – Shape index; ESA – Egg surface area; ESG – Egg specific gravity; ST – Storage temperature; PM – Packing material; SD – Storage duration. ^{abcd}Means with different superscripts within the same column are significantly different at ($P < 0.05$).

External parameters of eggs, such as shape, surface area, thickness, etc. play a decisive role not only in determining the egg quality but also in consumer preference. Therefore, storage conditions, such as: temperature, packaging and storage duration can affect the external parameters of the eggs.

Besides the storage duration, the interaction between ST × SD, PM × SD and ST × PM × SD affected the ESW, g. Other factors (ST, PM and ST × PM) and their interaction did not affect the ESW, g. The results of our research, presented in Table 4, show that the SD has also affected EST and ESI. Our results are not in line with those of other authors (Murshed et al., 2023) who observed that the storage duration did not affect significantly ($P > 0.05$) eggshell weight. The longer eggs are stored the integrity of the eggshell may deteriorate slightly. This could happen because of the weakening of the eggshell structure or microcracks that develop over time, leading to a decrease in eggshell weight. Therefore, in present research, the decreasing of eggshell thickness is associated with changes in the eggshell weight during storage and with a decrease of the eggshell index.

Table 4. Effect of storage temperature, packing material, storage duration and their interaction on eggshell parameters (Mean ± SEM)

Treatments	Parameters			
	ESW, g	ESW, %	EST, mm	ESI
Storage Temperature (ST), °C				
20–22 °C	7.46 ± 0.06	12.13 ± 0.07	0.44 ± 0.01 ^a	10.24 ± 0.06
4–6 °C	7.52 ± 0.08	12.21 ± 0.11	0.51 ± 0.01 ^b	10.32 ± 0.08
Packing Material (PM), box				
Control	7.63 ± 0.08	12.25±0.11	0.49±0.02	10.38 ± 0.08
Cardbox	7.37 ± 0.10	12.31±0.11	0.47±0.02	10.32 ± 0.09
Styrofoam	7.39 ± 0.08	11.87±0.15	0.48±0.02	10.06 ± 0.11
Plastic	7.57 ± 0.10	12.24±0.13	0.46±0.02	10.36 ± 0.11
Storage duration (SD), day				
0	7.73 ± 0.13 ^a	12.48 ± 0.18	0.57 ± 0.02 ^a	10.56 ± 0.014 ^a
7	7.47 ± 0.09 ^{ab}	11.96 ± 0.12	0.59 ± 0.01 ^a	10.14 ± 0.09 ^b
14	7.54 ± 0.10 ^{ab}	12.16 ± 0.18	0.31 ± 0.02 ^d	10.30 ± 0.13 ^{ab}
21	7.28 ± 0.09 ^b	12.03 ± 0.12	0.51 ± 0.01 ^b	10.11 ± 0.09 ^b
28	7.44 ± 0.09 ^{ab}	12.21 ± 0.11	0.40 ± 0.01 ^c	10.27 ± 0.08 ^{ab}
<i>P</i> value				
ST	ns	ns	< .0001	ns
PM	ns	ns	ns	ns
SD	0.0203	ns	< .0001	0.0397
ST × PM	ns	ns	0.0092	ns
ST × SD	0.0378	ns	0.0013	ns
PM × SD	0.0038	ns	0.0062	ns
ST × PM × SD	0.0401	ns	ns	ns

ESW – Egg shell weight; EST – Egg shell thickness; ESI – Egg shell index; ST – Storage temperature; PM – Packing material; SD – Storage duration. ^{abcd}Means with different superscripts within the same column are significantly different at ($P < 0.05$).

At the end of the storage period, the eggshell thickness decreased by 29.82%. Similarly, Alshaikhi et al. (2020), Grashorn et al. (2016) and Sekeroglu et al. (2016) in their research found that the eggshell thickness was negatively influenced ($P < 0.05$) by storage duration. On the other hand, Murshed et al. (2023) reported that the storage

duration did not affect eggshell thickness ($P > 0.05$). Our results are in contrast with the findings of Lee et al. (2016), who reported that storage duration showed no significant effect on eggshell thickness. Our findings are in line with Martinez et al. (2021) and Camargo et al. (2021), who found significant difference for eggshell thickness during storage ($P < 0.05$). The decrease in eggshell index during storage (2.75%) may have been due to changes in ESW (g) and EST. The combined effects of ST \times SD, PM \times SD and ST \times PM \times SD (as shown in Table 4), significantly affected ($P < 0.05$) ESW (g). At the end of the storage period, the eggs stored at 4–6 °C, recorded a greater decrease of ESW (5.12%), compared to those that were stored for the same period of storage but at 20–22 °C (2.10%), respectively. Eggs stored in plastic box at the end of the storage period recorded a greater decrease in ESW (9.46%) compared to those stored in cardboard, styrofoam boxes and the control group, which recorded a decrease of ESW for 8.30, 3.24 and 6.81%. At the end of the storage period, the results of the present study showed (Table 4) that the eggs stored at 4–6 °C and packed in cardboard boxes recorded a greater decrease of ESW (19.08%), while the smallest decrease of ESW during storage (7.24%) was recorded in eggs stored at 20–22 °C and packed in plastic boxes. Moreover, EST was found to be statistically influenced ($P < 0.05$) by the interaction of ST \times PM, ST \times SD and PM \times SD. Eggs of the control group, those in styrofoam, plastic and cardboard boxes, stored at 4–6 °C recorded 21.52, 10.00, 14.29 and 6.12% thicker eggshells compared to those stored at 20–22 °C. Eggs stored at 20–22 °C at the end of the storage period had 9.20% thinner shells compared to those stored at 4–6 °C. Martinez et al. (2021) have found a significant difference of interactions between ST \times SD on eggshell thickness ($P < 0.001$). Our results are contrary to those of Grashorn et al. (2016) and Lee et al. (2016), who found that the eggshell thickness was not affected ($P > 0.05$) by interaction between ST \times SD. At the end of the storage period (28 days), the eggs of the control group, those stored in styrofoam, plastic and cardboard boxes, recorded a decrease in eggshell thickness for 26.79, 36.80, 25.14 and 24.96%, respectively. In the case of our study, it can be assumed that eggshell weight can be an indicator of shell thickness and vice versa. The weight of the eggshell can influence the eggshell index because ESI is a calculated value that considers the ratio between shell weight and egg surface area, expressed as a percentage. A heavier eggshell generally contributes to a higher ESI, indicating a greater amount of eggshell material present. Kibala et al. (2018), in their study showed that eggshell thickness is closely related and is an indicator of other eggshell quality traits.

The effect of storage temperature, packing material, storage duration and their interaction on the internal quality of eggs

Inappropriate egg storage conditions can have negative effects on the freshness, structure and safety of chicken eggs, compromising their internal quality. Internal quality is assessed by albumen and yolk quality.

The present study (Table 5) shows that the storage temperature did not affect ($P > 0.05$) albumen weight and ratio. The AW, g from eggs stored at 20–22 °C decreased slightly (0.29%), but not significantly. This is not consistent with finding of Kim et al. (2024), who claimed that the storage temperature does affect albumen weight significantly ($P < 0.01$). Our results are in harmony with the Addo (2016), who presented the fact that the storage temperature did not affect significantly albumen ratio ($P > 0.05$) but interaction of different storage temperature and different storage duration significantly influenced

($P < 0.05$) albumen ratio. The albumen index was significantly ($P < 0.05$) affected by storage temperature. The eggs stored at 4–6 °C had a higher albumen index (41.37%) than those stored at 20–22 °C. Similar to our study, some researchers (Altunatmaz et al., 2020) found that the storage temperature negatively affects the albumen index ($P < 0.05$).

Table 5. Effect of storage temperature, packing material, storage duration and their interaction on egg albumen parameters (Mean \pm SEM)

Treatments	Parameters				
	AW, g	AR, %	AI, %	ApH	HU
Storage Temperature, °C					
20–22 °C	38.35 \pm 0.39	62.12 \pm 0.25	2.65 \pm 0.08 ^b	9.30 \pm 0.02 ^a	59.48 \pm 1.01 ^b
4–6 °C	38.46 \pm 0.49	62.09 \pm 0.29	4.52 \pm 0.13 ^a	8.97 \pm 0.03 ^b	74.46 \pm 1.00 ^a
Packing Material, box					
Control	39.15 \pm 0.63 ^a	62.54 \pm 0.37	3.55 \pm 0.19	9.14 \pm 0.04	69.10 \pm 1.52
Cardbox	36.83 \pm 0.59 ^b	61.36 \pm 0.34	3.53 \pm 0.19	9.17 \pm 0.03	69.96 \pm 1.41
Styrofoam	39.17 \pm 0.67 ^a	62.48 \pm 0.48	3.36 \pm 0.20	9.10 \pm 0.07	67.75 \pm 1.56
Plastic	38.48 \pm 0.56 ^{ab}	62.04 \pm 0.29	3.88 \pm 0.22	9.13 \pm 0.03	70.16 \pm 1.54
Storage Duration, day					
0	39.21 \pm 0.67	63.10 \pm 0.46 ^a	2.78 \pm 0.11 ^c	8.91 \pm 0.04 ^b	72.47 \pm 0.40 ^a
7	39.03 \pm 0.75	62.20 \pm 0.40 ^{ab}	3.87 \pm 0.20 ^a	9.13 \pm 0.08 ^a	72.11 \pm 0.57 ^a
14	38.76 \pm 0.68	62.15 \pm 0.46 ^{ab}	4.23 \pm 0.22 ^a	9.20 \pm 0.04 ^a	71.36 \pm 1.50 ^a
21	37.67 \pm 0.69	61.97 \pm 0.38 ^{ab}	3.78 \pm 0.24 ^{ab}	9.22 \pm 0.04 ^a	69.84 \pm 1.97 ^a
28	37.36 \pm 0.68	61.10 \pm 0.37 ^b	3.25 \pm 0.26 ^{bc}	9.21 \pm 0.03 ^a	60.43 \pm 2.28 ^b
<i>P</i> value					
ST	ns	ns	< 0.0001	< 0.0001	< 0.0001
PM	0.0242	ns	ns	ns	ns
SD	ns	0.0173	< 0.0001	< 0.0001	< 0.0001
ST \times PM	ns	ns	ns	ns	ns
ST \times SD	0.0004	0.0340	< 0.0001	0.0003	< 0.0001
PM \times SD	0.0109	ns	ns	ns	ns
ST \times PM \times SD	ns	ns	0.0313	ns	ns

AW – Albumen Weight; AR – Albumen Ratio; AI – Albumen Index; ApH – Albumen pH; HU – Haugh Unit; ST – Storage temperature; PM – Packing material; SD – Storage duration. ^{abcd^{gh}}Means with different superscripts within the same column are significantly different at ($P < .05$).

Statistical analysis identified additional significant differences ($P < 0.05$) in eggs stored at different temperatures, specifically in albumen pH and Haugh Unit values. The ApH for eggs stored at higher temperatures was 0.33 unit higher or more alkaline than those stored at lower temperatures. Previous studies (Luo et al., 2020) also reported that albumen pH was significantly ($P < 0.05$) affected by storage temperature. After the laying of the egg, carbon dioxide begins to be released from the white, and when the eggs are stored at a higher temperature, the CO₂ release becomes more active. In this case, the egg albumen becomes more alkaline and can provide an antibacterial defence system (Guyot et al., 2016). The present study showed that the HU value was lower by 14.98 units or 20.12% in eggs stored at refrigerator temperature compared with those stored at room temperature. Similarly, Wlaźlak et al. (2024) observed significant influence ($P < 0.001$) of storage temperature on HU of duck eggs. They reported that the storing eggs at a higher (17 °C) temperature resulted in lower height of thick albumen a lower HU value (60.87) than the other stored in 7 °C (72.08). Although the weight of

albumen changed significantly ($P < 0.05$) with the change of packaging material, the changes of AR, AI, ApH and HU were not so large ($P > 0.05$). This is in line with the work of Drabik et al. (2021) who found that the type of packing material significantly ($P < 0.05$) contributed to the changes of albumen weight, g. It was also observed that the HU was better at eggs stored at plastic material (70.16), and the lowest value of HU registered at eggs of control group (69.10) but these changes were not significant ($P > 0.05$). A similar observation ($P > 0.05$) was also reported by another researcher (Drabik et al., 2021).

Our results regarding the influence of storage duration on albumen parameters are presented in Table 5. The main effect of storage duration on the AR, AI, ApH and HU were highly significant ($P < 0.05$), while on the AW were non-significant ($P > 0.05$). As the storage period changed, the albumen parameters also changed. Results showed that albumen ratio decreased by 1.44, 1.52, 1.81 and 3.22% after the first, second, third and fourth week, respectively. These differences were more noticeable after the first week, and between the third and fourth weeks (1.44 and 1.41%, respectively). Findings in our study showed that the albumen index changed significantly as a storage duration increased ($P < 0.05$). The AI in fresh eggs was 2.78%, increasing it to 3.25% at the end of storage period. This trend of increasing albumen index during the storage period in this study aligns with the findings of Ondrušíková et al. (2018), who also reported an increase in albumen index at the end of the fourth week (from 9.37 at fresh eggs to 10.47% at fourth week), at quail eggs. In their research the albumen index in the first, second and fourth weeks was higher (11.35, 10.19 and 10.47%), than at the beginning of the experiment (9.37%). The data regarding albumen pH presented in Table 5 revealed significant differences ($P < 0.05$) in pH with the storage duration. The pH value of the egg albumen increased from 8.91 to 9.21. These results agree with reports of Kocetkovs et al. (2022), who found the significant effect of storage duration on albumen pH. Egg albumen naturally becomes more alkaline (higher pH) as they age due to the loss of carbon dioxide and changes in the way proteins interact (Kocetkovs et al., 2022). Our data clearly demonstrates that the Haugh unit was influenced ($P < 0.05$) by storage duration. The results showed that the freshness of the eggs decreased drastically at the end of the storage period, resulting in a drop in HU from 72.47 to 60.43, making them unusable for the consumer. This is attributed to the continuous reduction of egg weight and albumen height. Our findings were also confirmed by Martinez et al. (2021), who reported that the storage duration significantly affected ($P < 0.05$) the HU, decreasing it from 101.63 to 79.17 after 10 days.

Our results show that the egg albumen parameters included in our research were not affected by the interaction between $ST \times PM$. On this case, results recorded from our study are in contrast with the findings of Drabik et al. (2021), who reported significant influence ($P < 0.05$) of interaction between $ST \times PM$ on albumen weight and ratio and Haugh unit. But same as our results they did not find significant differences ($P = 0.136$) of these interaction on albumen pH. On the other hand, the interaction between $ST \times SD$ caused significant ($P < 0.05$) changes in all albumen parameters. At the end of the storage period (28 days), the albumen weight of eggs stored at 20–22 °C decreased progressively by 10.76%, while the albumen weight of eggs stored at 4–6 °C at the end of this period decreased by 5.59%. Albumen weight also decreased due to the interaction effects of $PM \times SD$ ($P < 0.05$). At the end of the storage period (28 days), greatest loss was recorded for eggs stored in cardboard boxes (14.29%). While eggs stored in plastic

boxes recorded lower weight loss of albumen (1.94%). The interaction between three factors (ST × PM × SD) changed the albumen index and these changes are significantly different ($P < 0.05$). The lower albumen index (1.91) observed at eggs stored in cardboard box, at 20–22 °C for 28 days.

The results of our research presented in Table 6 showed that the yolk height and yolk index were significantly influenced by storage temperature ($P < 0.05$). The yolk height of eggs stored at 20–22 °C was 17.18% lower compared to those stored at 4–6 °C.

Table 6. Effect of storage temperature, packing material, storage duration and their interaction on egg yolk parameters (Mean ± SEM)

Treatments	Parameters			
	YW, g	YW, %	YH, mm	YI, %
Storage Temperature, °C				
20–22 °C	15.83 ± 0.15	25.75 ± 0.23	13.84 ± 0.23	32.27 ± 0.68 ^b
4–6 °C	15.82 ± 0.16	25.70 ± 0.25	16.71 ± 0.19	42.26 ± 0.56 ^a
Packing Material, box				
Control	15.72 ± 0.24	25.21 ± 0.35	15.40 ± 0.32	37.54 ± 1.02
Cardbox	15.73 ± 0.22	26.32 ± 0.31	15.10 ± 0.34	36.78 ± 1.06
Styrofoam	15.98 ± 0.25	25.65 ± 0.40	15.04 ± 0.37	36.48 ± 1.13
Plastic	15.88 ± 0.16	25.72 ± 0.26	15.56 ± 0.42	38.27 ± 1.30
Storage Duration, day				
0	15.13 ± 0.28 ^b	24.42 ± 0.41 ^b	14.68 ± 0.26 ^c	36.08 ± 0.79 ^{bc}
7	16.11 ± 0.22 ^a	25.84 ± 0.36 ^a	16.72 ± 0.26 ^a	40.10 ± 0.77 ^a
14	15.94 ± 0.23 ^a	25.68 ± 0.36 ^a	16.18 ± 0.34 ^{ab}	39.57 ± 1.12 ^a
21	15.70 ± 0.19 ^{ab}	26.00 ± 0.35 ^a	15.60 ± 0.34 ^{bc}	37.83 ± 1.26 ^{ab}
28	16.26 ± 0.25 ^a	26.68 ± 0.33 ^a	13.20 ± 0.52 ^d	32.77 ± 1.77 ^c
<i>P</i> value				
ST	ns	ns	< 0.0001	< 0.0001
PM	ns	ns	ns	ns
SD	0.0094	0.0006	< 0.0001	< 0.0001
ST × PM	ns	ns	ns	0.0470
ST × SD	0.0360	ns	< 0.0001	< 0.0001
PM × SD	ns	ns	ns	0.0262
ST × PM × SD	ns	ns	0.0166	ns

YW – Yolk Weight; YH – Yolk Height; YI – Yolk Index. ST – Storage temperature; PM – Packing material; SD – Storage duration. ^{abcd^{efgh}}Means with different superscripts within the same column are significantly different at ($P < 0.05$).

The eggs stored at a higher temperature resulted in a lower yolk index (32.27). The changes in YH and YI observed in our study are similar those noticed in previous studies (Feddern et al., 2017; Drabik et al., 2021). They reported that the storage temperature significantly affects yolk index ($P < 0.05$). The effect of storage temperature on YW, yolk pH, YC and Y:A ratio was found to be statistically insignificant ($P > 0.05$). In contrast to our results, Kim et al. (2024) reported that yolk weight, yolk ratio and yolk pH were affected by changing storage temperature ($P < 0.05$). Similar to our results they reported that yolk colour was not affected by storage temperature ($P > 0.05$). Also, Drabik et al. (2021) reported that the yolk weight was affected ($P < 0.05$) by storage temperature. No significant differences ($P > 0.05$) were observed in egg yolk parameters when considering the effect of storing in different packing material. Similarly, Drabik et

al. (2021) found a non-significant effect of packing in the YW, YR, YC and YI. Egg yolk parameters (except pH) changed significantly ($P < 0.05$) with increasing storage time. Yolk weight (g) has increased by 7.47% during the storage period (28 days), while the yolk ratio also increased from 24.42 to 26.68%. This effect has been previously discussed by Murshed et al. (2023), Wengerska et al. (2023) and Jin et al. (2011). In their research they found that the storage duration significantly affected ($P < 0.05$) yolk weight. Carvalho et al. (2023) and Adamski et al. (2017) reported that the yolk weight increased during storage because of water diffusion from albumen through the vitelline membrane into the yolk. But the storage period in their study did not affect ($P > 0.05$) yolk weight. The value of yolk height was increased by 13.90, 10.22 and 6.27% at day 7, 14 and 21, respectively. But at the end of storage period (day 28) the yolk height decreases by 10.08%. Some similar changes were found in the YI. The yolk index was increased at the end of the first week of storage (11.14%), while after the first week it again began to decrease. So, from 7–14, 14–24 and 21–28 the YI decrease by 1.32%, 4.40% and 13.32%. Moreover, this index recorded a decrease from 36.08 to 32.77 or 9.17% during all storage period (0–28 days). Other authors (Carvalho et al. 2023) also confirmed the influence of storage duration on yolk height and yolk index. In our case, a reduction in yolk height (from 14.68 to 13.20 mm) contributed to a lower yolk index (from 36.08 to 32.77%), and these changes were significant ($P < 0.05$).

The colour is an important sensory index of preserved eggs (Luo et al., 2020). The yolk colour was adversely affected by the length of storage, decreasing by 6.03%. Similar results were presented by other researcher (Murshed et al. 2023, Wengerska et al., 2023 and Lee et al., 2016) who showed significant changes in yolk colour depending of storage duration.

As observed in our research (Table 7) the duration of storage has an effect on Y:A ratio. This ratio increased by 12.58%. While not a definitive indicator, this is because albumen loses moisture more easily than yolk during storage, which can potentially affect the ratio. A higher Y:A ratio can generally indicate a higher concentration of nutrients in the egg yolk and making it more flavourful.

Interaction effects between ST \times PM were significantly lower ($P < 0.05$) in terms of egg yolk index. The yolk index in the eggs of the control group, those packed in plastic, cardboard and styrofoam boxes and stored at a temperature of 20–22 °C was lower (33.16, 31.82, 32.21 and 31.89%) compared to the eggs stored at temperature 4–6 °C and that had the same storage packages (41.92, 44.72, 41.35 and 41.06%), respectively. There was no effect ($P > 0.05$) of ST \times PM interaction on the YW (g and %), YH, YpH, YC and Y:A ratio. The same trend as our findings (except yolk index) were reported by Drabik et al. (2021), who confirm that the interaction between the ST \times PM were not affected ($P < 0.05$) the change in the YW (g), YC and YpH.

In terms of egg yolk quality parameters (Table 6), it was found that the YW, YH and YI were significantly influenced ($P < 0.05$) by the interaction of ST \times SD, while the other parameters (Table 6 and 7) yolk ratio, YpH, YC and Y:A ratio did not differ significantly ($P > 0.05$).

Eggs stored at 20–22 °C for 28 days showed a significant (8.38%) increase in yolk weight (from 14.79 to 16.03 g). While eggs stored at a temperature of 4–6 °C for the same storage period showed a 6.53% increase in yolk weight (from an initial value of 15.47 to 16.48 g). This indicates greater albumen degradation at higher temperatures, resulting in a higher capacity for the yolk to absorb moisture from the albumen. The

interaction between storage temperature and storage duration affected also yolk height, which is directly related to yolk index. In this study, eggs stored at a lower temperature at the end of the storage period resulted in an increase in yolk height from 14.65 to 16.25 mm (10.92%), while those stored for 28 days at a higher temperature, the height was reduced from 14.70 to 10.15 mm (30.95%). The same trend has occurred in the yolk index. After 28 days of storage at 4–6 °C, the eggs showed an increase (19.80%) of egg yolk index, while those stored at 20–22 °C resulted in a high decrease of this index by 33.22%, which directly related with their height and diameter. The results of the study conducted by Madrigal-Portilla et al. (2023), also proved that the interaction between storage duration and storage temperature does not lead to a significant change ($P > 0.05$) in the colour of the yolk.

Table 7. Effect of storage temperature, packing material, storage duration and their interaction on egg yolk pH, colour and yolk:albumen ratio (Mean \pm SEM)

Treatments	Parameters		
	YpH	YC, Roche	Y:A
Storage Temperature, °C			
20–22 °C	6.29 \pm 0.02	12.22 \pm 0.06	41.65 \pm 0.53
4–6 °C	6.36 \pm 0.06	12.24 \pm 0.05	41.65 \pm 0.59
Packing Material, box			
Control	6.34 \pm 0.08	12.18 \pm 0.08	40.54 \pm 0.81
Cardbox	6.32 \pm 0.04	12.24 \pm 0.09	43.10 \pm 0.74
Styrofoam	6.38 \pm 0.08	12.16 \pm 0.07	41.39 \pm 0.93
Plastic	6.25 \pm 0.03	12.34 \pm 0.09	41.59 \pm 0.61
Storage Duration, day			
0	6.21 \pm 0.10	12.77 \pm 0.12 ^a	38.95 \pm 0.91 ^b
7	6.41 \pm 0.09	12.05 \pm 0.03 ^b	41.74 \pm 0.83 ^a
14	6.38 \pm 0.03	12.17 \pm 0.07 ^b	41.58 \pm 0.89 ^a
21	6.28 \pm 0.04	12.15 \pm 0.07 ^b	42.15 \pm 0.82 ^a
28	6.34 \pm 0.03	12.00 \pm 0.05 ^b	43.85 \pm 0.81 ^a
<i>P</i> value			
ST	ns	ns	ns
PM	ns	ns	ns
SD	ns	< 0.0001	0.0020
ST \times PM	ns	ns	ns
ST \times SD	ns	ns	ns
PM \times SD	ns	ns	ns
ST \times PM \times SD	0.0072	ns	ns

YpH – Yolk pH; YC – Yolk color; Y:A – Yolk:Albumen ratio; ST – Storage temperature; PM – Packing material; SD – Storage duration. ^{abcd^{efgh}}Means with different superscripts within the same column are significantly different at ($P < 0.05$).

No significant differences ($P > 0.05$) were determined in YW (g and %), YH, YpH, YC and Y:A ratio, because of the interaction effect between PM \times SD. This interaction showed its effect ($P < 0.05$) on the yolk index. On the seventh day of storage, the eggs of the control group recorded an increase in the yolk index from 38.56–42.48%, but at the end of the storage period YI decreased to 33.41%. The same trend has also occurred in the eggs placed in plastic box, where the yolk index on the seventh day marked an increase from 37.68 to 41.53%, while at the end of the storage period (28 days) the yolk

index decreased to 33.65%. In eggs stored in cardboard boxes at the end of the storage period YI decreased from 35.45 to 31.80%. The yolk index of eggs stored in styrofoam box, at the end of the storage period, recorded a decrease from 32.62 to 32.22% or 1.23%, respectively.

The yolk height of the eggs of the control group, those packed in cardboard, styrofoam and plastic boxes increased significantly by 7.89, 2.67, 18.48 and 15.58% during the storage periods at 4–6 °C. On the other hand, eggs stored at 20–22 °C resulted in a significant decrease in yolk height, with 30.26, 29.17, 26.39 and 15.00%, respectively. This decrease in the height of the yolk may occur due to the storage of eggs at high temperature, which affects the deterioration of the structure of the vitelline membrane (Bulut & Aygun, 2023).

This study discovered that the interaction between ST × PM × SD significantly affect ($P < 0.05$) YH and YpH. At the end of the storage period, eggs of the control group stored at 4–6 °C recorded a significant decrease in yolk pH from 7.23 to 6.19. Also, eggs placed in styrofoam boxes and stored at 4–6 °C, at the end of the storage period, showed a significant decrease in yolk pH from 7.06 to 6.49. While the eggs placed in plastic and cardboard boxes and stored at a temperature of 4–6 °C, did not record significant changes in yolk pH at the end of storage period. Regarding the eggs stored at 20–22 °C, in all forms of packaging (cardboard, styrofoam and plastic), including those of the control group, at the end of the storage period, the pH of the yolk increased from 6.04 to 6.43; 6.10 to 6.48; 6.14 to 6.24 and 6.13 to 6.28, respectively, but this increase was not significant. No recent data was found regarding the effect of storage temperature, storage duration and packing material on yolk height and yolk pH.

CONCLUSION

Based on the results reported herein, the main conclusion is that most influential factor is storage duration followed by storage temperature. The effect of storage duration is observed on almost all internal egg quality parameters, being most expressed after 14 days of storage. The study reconfirmed results of other studies related to negative effect of high storage temperature (20–22 °C), on critical egg quality parameters, such as egg weight loss, albumen index, pH, Haugh unit, yolk height, and yolk index. Packing material affected few external parameters and just albumen weight. However, further studies on the effects of storage condition (temperature and duration), different packing material and their interaction on egg quality are recommended.

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