RESEARCH ARTICLE

The effect of different storage period on some egg quality characteristics and ovalbumin levels in goose eggs

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ABSTRACT

The aim of the current study was to investigate effect of different storage period on some external and internal egg quality and ovalbumin levels of goose eggs. The alterations of these traits have not been examined sufficiently in goose eggs during the storage. For this purpose, a total of 150 eggs were examined from 50 weeks-old German origin Embden geese. The eggs were stored under constant temperature $(+4^{\circ}C)$ and $\sim 60-65\%$ relative humidity conditions for a period of 7, 14 and 21 days. The weights of 50 eggs were measured and analyzed at 7, 14, and 21 days, respectively. The weight loss, eggshell thickness, and the pH of the albumen and the yolk were different between the storage groups. Other parameters such as, shape index; weights and ratios of albumen, yolk, eggshell, and yolk color were similar between the groups. The pH values of the albumen and yolk, the weight loss, and ovalbumin levels of the albumen had increased during storage. In contrast, the eggshell thickness had declined. According to the results of this study, eggs stored under refrigeration had lower variability on quality parameters. Depending on the increase of ovalbumin with storage, it will be more appropriate to consume goose eggs by allergy-sensitive individuals freshly or after at least end of 1-week storage.

Keywords: Egg quality; Goose eggs; Ovalbumin; pH; Storage

INTRODUCTION

Hen eggs have a major place in the egg industry. The market of other avian species' eggs is limited due to the small number of eggs and seasonal egg production. Therefore, these eggs are defined as "non-table eggs". The eggs produced by ducks, geese, pheasants and turkeys are considered to be non-table. The eggs of these avian species are mainly used for reproduction, research purposes, and industry of decorative items (Tserveni-Goussi and Fortomaris 2011). However, consumption of edible goose eggs is increasing in many countries (Kumbar et al., 2016). In addition, there is not enough data on the quality characteristics of goose eggs. Studies involving quality assessments focused more on hen and quail eggs because of commercial importance. The storage of the eggs is crucial to preserve quality and maintain an appropriate food process (Tabidi 2011). Different practices in the storage of the eggs have also created a handicap for egg trading between the countries (Jones et al., 2018). As it is known, some alteration occurs in eggs during storage and, this affects the quality of the eggs. The albumen is the most affected part of the egg during the storage. The vitelline membrane quality also decreases so the yolk becomes more susceptible to an eruption with absorbing the water from albumen (Berardinelli et al., 2008; Nadia et al., 2012). The loss of carbon dioxide and moisture from the eggshell pores increase the pH of the albumen and the yolk, decreases moisture percentage of egg albumen, and decrease the albumen weight (Eke et al., 2013). At the same time, the egg weight also decreases. It is suggested that the refrigeration of an egg will retain its nutritional value and wholesomeness for considerable shelf life. Eggs stored under refrigeration had lower variability on quality parameters than eggs stored under room temperature (Feddern et al., 2017). A small number of studies were conducted on the storage conditions of goose eggs (Tilki and Inal 2004; Kumbar et al., 2016). Besides pH values, albumen and yolk indexes, and eggshell parameters, the alterations of shape index, albumen and yolk ratio, yolk

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color have not been examined sufficiently in goose eggs during the storage. As mentioned above, several chemicalphysical modifications occur inside an egg during the storage. Moreover, the most essential factor that determines the quality of albumen is protein content except for physical quality characteristics. There is a protein named ovalbumin (OVA), which is highly effective on the foaming capacity of the albumen and which is the most abundant in the albumen. OVA is a globular, acidic protein that comprises a single polypeptide chain of 385 amino acid residues with a molecular weight of approximately 45-48 kDa. This protein has also an effect on the physical characteristics of albumen such as the foaming capacity which is important for the food sector (Alleoni 2006; Baykalir and Aslan 2020). OVA was not been investigated in point of food quality in goose eggs. Nevertheless, OVA had investigated in a case report related to the allergenic effect of albumen proteins while consumption of the goose egg. According to this case report, it was stated that OVA was responsible for the allergic condition caused by the consumption of the eggs of Anseriformes species (duck and goose) (Anibarro et al., 2000). The aim of the present study was to study the effect of different storage duration on the parameters describing some egg external and internal quality of goose eggs. The study also deals with the the effect of the different storage period on the OVA levels of the goose eggs.

MATERIALS AND METHODS

Egg sampling and experimental design

The eggs were collected from 50 weeks-old German origin Embden geese flock in Turkey, Elazig province. The birds were reared under semi-extensive conditions. In addition to pasture, the commercial mixture feed (16% crude protein and 2900 ME kcal/kg) and freshwater were provided ad libitum. A total of 150 eggs were used in the study. The eggs were stored under constant temperature (+4 °C) and ~60-65% relative 40% humidity conditions for a period of 7, 14 and 21 days in a refrigerator.

Egg quality measurements

At first, the fresh eggs were weighed for weight loss and relevant values (egg width-mm and length-mm) were determined for the change in shape index values during the storage. At the end of each experimental storage day, the 50 eggs were weighed and the shape index determined again. After these processes, all eggs were broken carefully. Prior to the weighing of both albumen and yolk, the yolks were separated from the albumen and the yolks were rolled on a filter paper for removing any albumen residues. The pH measurement (Hanna Instruments, HI99163, USA) was taken individually in both albumen and yolk. The yolk color was determined visually by using Roche yolk color

700

fan. The eggshells with membranes were cleaned and dried on air for 24 h under room temperature and weighed. The eggshell thicknesses were determined with the membranes by a digital micrometer (Mitutuyo, 0-25 mm, Japan) from the mid-point of the eggs according to Zhang et al. (2017). The weight loss was calculated according to this formula:

Weight loss (%) = [(initial egg weight-egg weight after storage)/(initial egg weight)] \times 100 (Feddern et al. 2017). Shape index, albumen and yolk ratios were determined according to Panda (1996), Baykalir and Simsek (2018).

SDS-PAGE analysis of OVA

The 30 albumens from each storage group (total of 90 albumens) were analyzed with SDS-PAGE. SDS-PAGE procedure was applied according to Baykalir and Aslan (2020). Each albumen was homogenized 1/10 (w/v) with 25 mM Tris (pH: 7.4). The total protein concentration of albumen was measured with a nano drop spectrophotometer (Thermo Fisher, NanoDrop 2000c, USA). The protein samples were mixed with 4X sample buffer (1 M Tris-HCl pH: 6.8, SDS, 0.1% Bromophenol blue, glycerol and 14.3 M β mercaptoethanol) and boiled at 97°C for 5 min. The $30\mu g/30\mu l$ from each protein samples were run on discontinuous polyacrylamide gel (4% stacking and 10% separating gel) and stained with the 1% coomassie brilliant blue dye solution for 1 hour. Then after destaining of Gel, each band was analyzed with image processing software (NIH Image) after grayscale calibration for obtaining relative density values (RD, %).

Statistical analysis

The sample size was determined by GPower 3.1 power analysis software (Faul et al., 2007). After normality check of all data, the egg weights, weight loss, shape index, albumen and yolk weights, albumen, yolk, and eggshell ratios, the pH of the albumen and yolk, eggshell weights, thicknesses and, OVA levels of albumens were compared with the analysis of variance (One-Way ANOVA) test. Tukey post hoc test was performed to determine the differences within the groups. The yolk color was compared with the Kruskal Wallis H test because of the yolk color values are discrete data. The data were processed with IBM *SPSS 22.0 statistical package program (IBM, New York, USA). The statistical significance was considered when $P \leq 0.05$ (Petrie and Watson 2013).

RESULTS AND DISCUSSION

The quality characteristics of the stored eggs are presented in Table 1. The egg weights were 125.65 g, 126.13 g, and 127.54 g. Egg weight varies between 130-210 g in geese (Onk and Kirmizibayrak 2019). In this study, the egg

Table 1: E	Table 1: Egg quality characteristics of the stored eggs	aracteristic	s of the store	d eggs								
Groups	Egg weight (g)	Weight loss (%)	Shape index (%)	Albumen weight (g)	Yolk weight (g)	Albumen ratio (%)	Yolk ratio (%)	Eggshell weight (g)		Eggshell Eggshell ratio Albumen pH thickness (%) (mm)	Albumen pH	Yolk pH
7 days	' days 127.54±1.67 0.53±0.02 ^a 65.52±0.33	0.53±0.02ª	65.52±0.33	69.05±0.98	39.61±0.75	54.17±0.41	31.07±0.37	14.66±0.23	0.56±0.01ª	31.07±0.37 14.66±0.23 0.56±0.01 ^a 11.51±0.12	8.59±0.02ª	6.14±0.30ª
14 days	14 days 126.13±1.81 1.17±0.05 ^b 64.22±0.58	1.17±0.05 ^b	64.22±0.58	66.88±1.00	40.82±0.97	53.08±0.40	32.27±0.50	32.27±0.50 14.21±0.21 0.53±0.01ª	0.53±0.01ª	11.30±0.13	8.64±0.02ª	6.30±0.50 ^b
21 days	21 days 125.65±2.20 1.77±0.05° 64.67±0.49	1.77±0.05°	64.67±0.49	66.32±0.90	39.55±0.66	52.97±0.41	31.53±0.29	31.53±0.29 14.52±0.24 0.49±0.01 ^b	0.49±0.01 ^b	11.62±0.17	8.74±0.01 ^b	6.36±0.30 ^b
P values	0.770	<0.001 0.160	0.160	0.111	0.456	0.076	0.106	0.369	<0.001	0.301	<0.001	<0.001
The data w	ere presented as	mean ± stand	lard error. ^{a,b,c} : St	The data were presented as mean \pm standard error. ^{abo} : Superscripts in the same column show the difference between the droups. P ≤ 0.05 is statistically significant	column show t	the difference betwee	an the groups. P	0 ≤ 0.05 is stati	stically significe	ant		

weights varied between 125.65 g and 127.54 g. On the other hand, the shape index values resulted in 64.22%, 64.67% and 65.52% in the storage groups (Table 1). The weight loss, eggshell thickness, and the pH of the albumen and the yolk were different between groups (P < 0.001). It was observed that the pH in both albumen and yolk increased with storage. There was no statistical difference between the groups regarding the albumen pH on 7th (8.59) and 14th (8.64) days. The lowest yolk pH value was obtained on the 7th day (6.14). On the other hand, the volk pH was similar between on 14th (6.30) and 21st (6.36) days. It is reported that genotype, age and rearing systems have an impact on egg quality in poultry (Scheideler et al., 1998; Holt et al., 2011). Beyond these, storage conditions also have a significant impact on egg quality traits. Studies conducted on storage conditions focus more on hen egg. However, it is insufficient data on the storage conditions of goose eggs on different days. These values were lower than Turkish native goose breeds those previously reported. Onk and Kirmizibayrak (2019) reported that the mean value of the shape index of 1-year-old native Turkish geese was 66.27%. The diversity in the results of the different studies with respect to some external egg traits might be due to the different breeds used. According to this study, the weight loss, eggshell thickness, and the pH of albumen and yolk exhibited significant alterations between the storage groups. The weight loss of the eggs and pH values were observed to have tendency to increase as the storage duration increases. In contrast, the shell thickness declined while storage. It is stated that the storage period affects the eggshell quality (de Abreu Fernandes and Litz 2017). The eggshell thickness was reduced during storage in current study. This result agrees with the findings of Alsobayel and Albadry (2011). In addition, the important relationship between eggshell thickness and other eggshell parameters such as eggshell strength has been determined (Ketta and Tumova 2018). During the storage, the shell membrane is separated and the rupture of the inner membrane occurs (Jan et al., 2018). Consequently, the eggshell thickness and eggshell strength also declines during storage (de Abreu Fernandes and Litz 2017). On the other hand, diameters of eggshell pore located on the egg surface increase when eggs are kept under low ambient temperature e.g. in a refrigerator. This is because the cuticle sealing the air pores of the eggshell of eggs dried faster and began to shrink (Stadelman and Cotterill 2007; Eke et al., 2013). Therefore, increased pore diameter may have caused thinning of the eggshell. Immediately after oviposition, albumen pH is around 7.6 and 8.5; pH of yolk is 6.0. Increases in albumen pH are due to CO₂ loss during storage and depend on dissolved CO₂, bicarbonate ions, carbonate ions, and protein equilibrium (de Oliveira and de Oliveira 2013). According to Silversides and Scott (2001), the albumen pH was not influenced by the age and genotype; however, they stated that only storage had a significant impact on the albumen pH. The pH of egg yolk increases during storage as well as in albumin. Egg yolk pH increases to 6.4-6.9 during storage (Belitz et al. 2009). In the current study, the pH values of the albumen and yolk significantly increased while storage similar to other studies (Tilki and Inal 2004; Kumbar et al., 2016). The weight loss increased also in stored eggs similarly to the pH. In contrast, the shell thickness reduced during storage. The lowest eggshell thickness was found to be on 21st (0.49 mm) day. The weight loss interchange of the eggs was 0.64%, 0.60%, and 1.24% from 7-14 days, 14-21 days, and 7-21 days, respectively (Table 1). Although the albumen weight decreased during the storage, there was no statistical difference regarding the albumen weights (P > 0.05). The yolk color of the stored eggs is presented in Table 2. According to the findings of the current study, the median of the yolk color of the goose egg was 9 and 10, the minimum value resulted in 4 and 6, the maximum color was 13. The yolk color was also not changed during the storage (P > 0.05). This result had a disagreement with the findings of dos Santos et al. (2009) who reported that the yolk color decreased during storage under both room and refrigerator temperature. The OVA levels of the eggs are shown in Fig. 1. The OVA levels were 28.20%, 34.19%, 37.61% on 7th, 14th and 21st day, respectively. The OVA levels on 14th and 21st days were similar. OVA is responsible for IgE-mediated allergic reactions. Therefore, ovalbumin is known as a primary allergen of the egg (Caubet and Wang 2011). When an egg is aging, OVA converts into

Groups	Min	Max	Median	P value
7 days	4	13	9	0.458
14 days	4	13	10	
21 days	6	13	10	

 $P \le 0.05$ is statistically significant

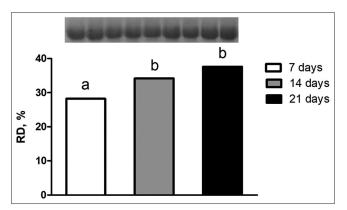


Fig 1. Ovalbumin levels of the goose eggs under different storage duration.

RD, % Relative density of the bands. a,b: Different letters on the bars show the statistical differences between the groups. \Box : 7 days kept in the refrigerator, \blacksquare : 21 days kept in the refrigerator. Every 3 bands belong to each column of the groups.

S-ovalbumin form which is thermally stable to protect itself from deterioration (Pelegrine and Gasparetto 2006). Huang et al. (2012) demonstrated that there is a positive correlation between ovalbumin and albumen pH during the storage. The OVA levels of the albumen increased with storage as also the pH. It is suggested to consider that the time factor must be linked to the temperature factor to achieve effective quality control since the timetemperature interaction directly affects reactions' kinetics (Guerrini et al., 2021). Moreover, increasing OVA levels of the albumen might be related to a rational increase in the protein content of the albumen due to water loss during storage (Brumshtein et al., 2008).

CONCLUSION

According to the present study, egg quality characteristics have not considerable changed with the storage in the refrigerator except for egg weight, the pH of the albumen and yolk and, the eggshell thicknesses. Depending on the increase of ovalbumin with storage, it will be more appropriate to consume goose eggs by allergy sensitive individuals freshly or after at least end of 1-week storage. Advanced protein studies such as proteomics should be conducted on S-ovalbumin in order to determine the foaming capacity of goose eggs during the storage. Such studies may be beneficial to provide an opportunity for using of goose eggs in the food industry.

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Authors' contrubituons

Yasin Baykalir: Conceptualization; Experimental design; Experiment performer; Drafted the manuscript; Laboratory and Data analysis; Writing - review and editing. Mehmet Eroglu: Laboratory Analysis. Zeki Erisir: Intermediator for material supplying.

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