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Effect of Storage Time, Temperature and Hen Age on Egg Quality in Free-Range Layer Hens

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Abstract: A total of 360 eggs obtained from brown-nick hens at 22 and 50 weeks of age were used to investigate the effect of hen age, storage time and storage temperature on interior quality of eggs. The eggs were sampled fresh and after storage for 3, 7, 10 and 14 days at 4 and 20°C. At sampling, eggs were weighed and broken and albumen and yolk height and width, pH, Haugh Unit (HU), air cell size, weight loss and specific gravity were measured. There was a clearly negative effect of storage time and temperature on weight loss, specific gravity, yolk and albumen index and HU. Weight loss was significantly raised by increased hen age, storage time and temperature. Albumen and yolk pH were also significantly increased by storage time and temperature. As a result, the role of hen age, storage temperature and time were found to be important in keeping of the egg freshness. It has seemed that chilling storage was the only condition to guarantee a certain level of freshness.

Key words: Egg, shelf life, age of hens, storage time, storage temperature, brown-nick hens

INTRODUCTION

All food has a limited shelf life, which will vary depending on the type of food and storage conditions. The egg is very perishable food product, which could lose its quality rapidly during the period between storage and consumption. Egg quality can be affected by the environmental conditions such as temperature and humidity of storage, as well as the gaseous environment and storage time. Storage can modify some characteristics of the egg including loss of water, carbon dioxide and a subsequent increase in the pH of the albumen (Decuypere *et al*., 2001). The poultry industry measures albumen quality primarily to judge the freshness of an egg. The Haugh Unit (HU) is the measure used most commonly today (Williams, 1992). This is the logarithm of the height of the inner thick albumen adjusted for egg weight (Haugh, 1937). The higher value of HU corresponds to better quality of eggs if other characteristics are good (Adamiec *et al*., 2002). Albumen height is affected by the strain and age of the hen laying the egg and storage time and conditions (Silversides and Scott, 2001).

Young hens produce eggs with thicker shells and longer pores than older hens (Britton, 1977). The shell membranes function to retain the fluid of the albumen and to rest bacterial invasion (Burley and Vadehra, 1989). As an egg ages, it loses carbon dioxide and moisture through the shell pores. This causes the air cell within the egg to get larger. With this loss of carbon dioxide, the egg's pH becomes more basic and structural changes take place in the albumen. The mechanism involved is not completely understood, but the result is a thinning of the albumen. It is for this reason that fresher eggs fry better: the yolk is still well centered and protected by the thick albumen (Bradley and King, 2005). Furthermore, the age and production period of a hen affect shell structure and consequently, the rate of diffusion through the pores of the eggshell. Such changes may also contribute to the reduction in hatchability associated with egg storage (Etches, 1996).

Vitelline membrane strength has become increasingly important for food safety reason (Messens *et al*., 2005). The strength of the vitelline membrane decreases with increasing storage time. This may allow nutrients in the yolk to become available to any microorganism that are present in the albumen. Kirunda and McKee (2000) found that membrane strength values are significantly related to yolk index and HU.

As the egg ages, the perivitelline layer weakens and becomes more elastic and some components are altered or removed (Brake *et al*., 1997). Changes in perivitelline layer weight and in protein and hexosamine content are associated with an increase in albumen pH, which can be inhibited by oiling the eggshell or enhanced by increasing the rate of rise of albumen pH (Fromm, 1967). The yolk pH

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is about 6.0 and contains no carbon dioxide, but the addition of carbon dioxide to the storage environment retards the movement of water from the albumen to the yolk (Romanoff and Romanoff, 1949). Similarly, a decreased storage temperature causes a decrease in water movement from the albumen to yolk (Brake *et al*., 1997). Both temperature and pH affect albumen quality. The decrease in perivitelline layer strength observed during storage has been associated with the dissolution of the chalaziferous layer of the albumen, which occurs during long-term, but not short-term, storage (Fromm, 1967).

The albumen pH at oviposition is about 7.6, which is slightly more basic than the uterine fluid (Arad *et al*., 1989) and rises to about 9.0 during storage as the dissolved carbon dioxide diffuses out (Keener *et al*., 2001). The buffering capacity of albumen is weakest between 7.5 and 8.5 (Brake *et al*., 1997), which accounts for the rapid increase as carbon dioxide is lost. The characteristics of albumen are not only measures of egg quality. The advent of the egg breaking industry has greatly increased the importance of the relative proportion of the egg components (Ahn *et al*., 1997). The proportion of yolk and albumen is largely determined by the age and strain of the hen.

A number of studies have been conducted concerning the effects of storage time on egg quality. However, the interaction of hen age, storage time and temperature is not fully known. Therefore, the aim of this study was to determine the effects of hen age, storage time and storage temperature on interior egg quality.

MATERIALS AND METHODS

Eggs were obtained from brown-nick layer hybrids that were reared at a commercial egg farm in Pinarhisar, Kirklareli. Eggs were collected over two-age periods when the hens were 22 and 50 weeks old. The flocks were reared in floor pens and kept during lay under free-range management conditions. Hens were provided an organic layer diet in mash form according to NRC (1994) recommendations. Water was available for *ad libitum* consumption and natural daylight was supplemented with artificial light to give a 16 h photoperiod.

A total of 360 eggs were analyzed when the hens were at 22 and 50 weeks old. Fresh eggs were collected and measured within 2 h of being laid. Each of 20 sampled eggs was stored in chambers for 3, 7, 10 or 14 days in refrigerator (4°C) and at room temperature (20°C). Humidity was 55% for all treatments. Thus, 360 eggs were collected and used in 18 treatments (2 different ages ×4 storage periods × 2 storage temperatures plus 2 groups of fresh eggs) with 20 eggs examined in each.

At sampling, eggs were weighed and broken onto a flat surface, where the height of the thick albumen and egg yolk were measured within a tripod micrometer. The yolk was separated from the albumen and weighed. The pH of the albumen and yolk was measured immediately using a pH meter (pH meter, Inolab level 1, WTW GmbH, Weilheim, Germany). Haugh units were calculated from the HU formula:

$$HU = 100 \log (H - 1.7 W^{0.37} + 7.57)$$

Albumen and egg yolk width were measured by using a compass. The albumen indices were then calculated as follows:

$$\text{Albumen index} = \left(\frac{\text{Albumen height}}{\text{Albumen length} + \text{Albumen width/2}} \right) \times 100$$

and the yolk indices were then calculated as follows:

$$\text{Yolk index} = \frac{\text{Yolk height}}{\text{Yolk width}}$$

Air cell (distance between eggshell and membrane (mm)) and eggshell thickness (mean of 3 different sides of eggs (μm)) were measured with same micrometer:

(Type D/06 20717 (0.01-0.25 mm)
Fabrikat Mauser, Germany)

To measure the Specific Gravity (SG) of the egg, saline solutions used varied in SG from 1.060-1.100 in increments of 0.005.

Data for fresh and stored eggs together were subjected to Duncan's multiple range test. The data without fresh eggs were analyzed using the SAS (1994) statistical package. An ANOVA using a general linear model included the main effects of age, storage time and storage temperature of eggs and the two-three way interactions between these factors. Although, all interactions were significant a further ANOVA used only main effects.

RESULTS AND DISCUSSION

Table 1 and 2 display the results for this study. Age of hens, storage time and storage temperature significantly affected to egg quality parameters. Table 1 and 2 revealed that there was a negative effect of storage time and storage temperature on egg weight loss, specific gravity, yolk index, albumen index and HU ($p<0.001$). Egg weight loss was raised by increased hen age, storage time

Table 1: Effects of storage conditions and hen age on egg quality

Age (weeks)	Storage Temp. (°C)	Storage Time (day)	N	Egg weight (g)	Loss (g)	Air cell (mm)	Specific gravity (g cm^{-3})	Shell weight (g)	Shell tickness (mm)	Shape index
22	4	3	20	60.94bcd	0.15i	3.65gh	1.090cd	6.76bcd	0.307ef	78.04ab
22	4	7	20	60.41bcd	0.35hi	5.14ef	1.090cd	6.89bcd	0.317cdef	79.03ab
22	4	10	20	60.43bcd	0.43gh	5.02ef	1.089cd	7.00abcd	0.329abcde	77.36ab
22	4	14	20	59.55d	0.56efgh	5.10ef	1.087de	6.76bcd	0.319cdef	78.15ab
22	20	3	20	59.83cd	0.31hi	4.40fg	1.088de	6.66cd	0.310def	77.80ab
22	20	7	20	61.37bcd	0.68efg	5.77de	1.083ef	6.93abcd	0.310def	74.59b
22	20	10	20	59.58d	0.99cd	6.69bc	1.078fg	6.68bcd	0.312def	79.00ab
22	20	14	20	58.98d	1.37b	7.04abc	1.068hi	6.71bcd	0.318cdef	79.39ab
50	4	3	20	65.39ab	0.16ij	5.55de	1.098a	6.94abcd	0.348ab	76.54ab
50	4	7	20	63.56abcd	0.32hi	4.63f	1.090bcd	7.01abcd	0.336abcd	76.48ab
50	4	10	20	62.67abcd	0.37hi	5.06ef	1.090cd	7.18abcd	0.345ab	76.93ab
50	4	14	20	64.68abc	0.70ef	6.37bcd	1.075g	6.95abcd	0.323bcde	76.12ab
50	20	3	20	65.08ab	0.50fgh	5.67de	1.095abc	6.80bcd	0.349ab	75.86ab
50	20	7	20	63.82abcd	0.86de	6.21cd	1.084de	7.26abc	0.354a	75.71ab
50	20	10	20	66.52a	1.19bc	7.21ab	1.073gh	7.32ab	0.340abc	76.65ab
50	20	14	20	62.93abcd	1.97a	7.72a	1.064i	6.58d	0.303ef	76.34ab
22	Fresh	Fresh	20	61.31bcd	-	3.14h	1.097ab	7.55a	0.307ef	80.36a
50	Fresh	Fresh	20	65.02ab	-	3.34h	1.098a	6.98abcd	0.296f	80.36a
SEM				0.261	0.030	0.079	0.059	0.033	0.001	0.272
Source of variation										
Age				<0.001	<0.001	<0.001	NS	<0.001	<0.001	NS
Storage temperature				NS	<0.001	<0.001	<0.001	NS	NS	NS
Storage time				NS	<0.001	<0.001	<0.001	<0.001	NS	NS
Age×storage Temp.				NS	<0.001	NS	NS	NS	NS	NS
Age×storage time				NS	NS	<0.001	<0.001	NS	NS	NS
Storage Temp.×storage time				NS	<0.001	<0.001	<0.001	NS	NS	NS
Age×storage Temp.×storage time				NS	NS	<0.001	<0.001	NS	NS	NS

[a-i]Within columns, values with no common superscripts are significantly different (p<0.05)

Table 2: Effects of storage conditions and hen age on interior quality of eggs

Age (weeks)	Storage Temp. (°C)	Storage Time (day)	N	Weight (g) Yolk	Weight (g) Albumen	pH Yolk	pH Albumen	Index Yolk	Index Albumen	Haugh units
22	4	3	20	14.89fg	36.84	6.189ab	8.876de	47.43ab	9.19b	81.88ab
22	4	7	20	15.02efg	35.93	6.128abc	8.962cd	47.43a	8.43bcd	79.21b
22	4	10	20	15.35defg	35.74	6.105abcd	8.968cd	46.29ab	7.43bcdef	74.81bcd
22	4	14	20	14.98efg	35.56	6.160ab	8.876bc	46.48ab	9.19bcde	76.48bcd
22	20	3	20	14.81fg	35.42	6.133abc	8.876cde	45.31bc	6.77defg	72.68bcde
22	20	7	20	15.40cdefg	36.08	6.124abc	9.142ab	44.68bcd	5.72fgh	65.36def
22	20	10	20	15.39cdefg	34.30	6.062cde	9.183a	42.16def	4.14hij	56.31fg
22	20	14	20	15.22efg	34.08	6.198a	9.217a	39.78f	3.70ij	52.11gh
50	4	3	20	17.20ab	38.37	6.027def	8.649f	45.84bc	8.85bc	78.16bc
50	4	7	20	16.90abc	35.34	5.974ef	8.807e	43.30cde	7.09cdef	73.72bcd
50	4	10	20	16.49abcdef	36.66	6.028def	8.793e	44.55bcd	7.64bcdef	76.18bcd
50	4	14	20	16.84abcd	37.60	6.096bcd	8.977cd	47.36ab	6.01efgh	66.81cdef
50	20	3	20	17.00ab	38.05	5.946fg	9.019bcd	41.32ef	9.19ghi	61.89efg
50	20	7	20	17.34ab	34.83	6.012def	9.027bc	36.38g	3.11ijk	44.36hi
50	20	10	20	17.93a	37.53	6.040cdefg	9.139ab	35.03g	2.49jk	35.92i
50	20	14	20	17.28ab	35.35	6.134abc	9.252a	30.92h	1.60k	32.55i
22	Fresh	Fresh	20	14.11g	36.470	6.052cde	7.945h	46.12abc	11.38a	91.48a
50	Fresh	Fresh	20	15.99bcdef	37.29	5.874g	8.216g	45.76bc	9.35b	81.53ab
SEM				0.092	0.220	0.006	0.019	0.285	0.165	0.983
Source of variation										
Age				<0.001	<0.017	<0.001	<0.001	<0.001	<0.001	<0.001
Storage temperature				NS	NS	NS	<0.001	<0.001	<0.001	<0.001
Storage time				NS	NS	<0.001	<0.001	<0.001	<0.001	<0.001
Age×storage Temp.				NS	NS	NS	<0.001	<0.001	<0.001	<0.001
Age×storage time				NS	NS	<0.001	NS	<0.001	NS	NS
Storage Temp.×storage time				NS	NS	<0.001	NS	<0.001	NS	<0.001
Age×storage Temp.×storage time				NS	NS	NS	<0.001	<0.001	NS	NS

[a-k]Within columns, values with no common superscripts are significantly different (p<0.05)

and temperature (p<0.001). Albumen pH and yolk pH were also increased by storage time and storage temperature (p<0.001). However, increasing of hen age 20-50 weeks albumen and yolk pH decreased (p<0.001).

Egg weight was increased by hen age (p<0.001), but was not affected storage time and temperature. However, during storage at 4°C egg weight loss significantly increased to 0.15 and 0.56 g at 3 and 14 days of storage

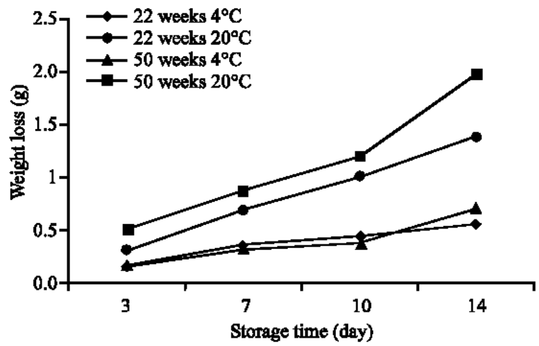

Fig. 1: Effects of storage conditions and hen age on egg weight loss

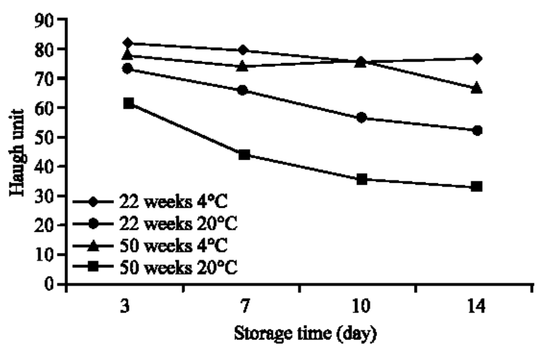

Fig. 2: Effects of storage conditions and hen age on Haugh units

time, respectively at the age of 22 weeks. Analogously, egg weight loss was significantly increased to 0.16 and 0.70 g at 3 and 14 days of storage time, respectively at the same temperature condition at the age of 50 weeks. When storage temperature increased to 20°C, loss of egg weight dramatically increased to 0.31 and 1.37 g at the age of 22 weeks and to 0.50 and 1.97 g at the age of 50 weeks, respectively (Fig. 1).

Albumen, yolk and shell weight were not changed by storage time and temperature. These results are in agreement with those of Ahn *et al.* (1999) who have found that shell weight does not change with storage. However, Walsh *et al.* (1995) who stated egg weight ($p<0.001$) decreases of 0.36 and 0.57 g, respectively, within 7 and 14 days of storage.

Egg weight increased with the increasing age of the hen. Yolk and albumen weight increased to 50 weeks. The shell weight increased to 22 weeks and declined slightly to 50 weeks, because of the increasing egg size, the egg shell weight declined with increasing age of the hen. These changes are in agreement with published reports (Ahn *et al.*, 1997).

Albumen height, HU and yolk index were also decreased with hen age, storage time and temperature.

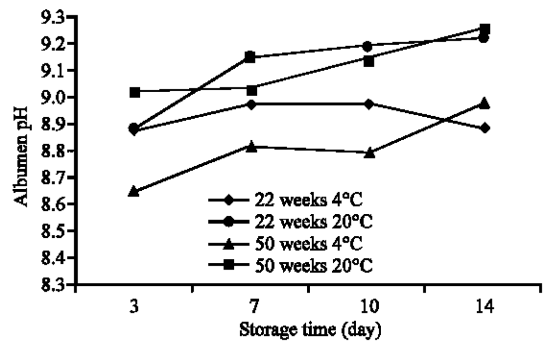

Fig. 3: Effects of storage conditions and hen age on albumen pH

Scott and Silversides (2000), who reported a significant decrease from 9.16-4.75 mm in albumen height ($p<0.05$) in stored eggs at 10 days. Haugh unit decreased in 22 weeks of age from 91.48-52.11 at 20°C during 14 days of storage, whereas at 50 weeks of age this decline from 81.53-32.55 at same temperature and storage time. These results agree with reports on Haugh units (Silversides and Scott, 2001). HU was not significantly decreased by storage for 3-14 days at 4°C. However, during storage at 20°C HU decreased from 72.68-52.11 and from 61.89-32.55 at the age of 22 and 50 weeks, respectively ($p<0.001$). These results are in agreement with Tona *et al.* (2004), who reported storage adversely affected HU ($p<0.001$). Figure 2 shows the changes in Haugh unit with storage time, temperature and increasing hen age.

Yolk and albumen index were considerable decreased by during storage at 20°C. At 20°C the yolk index decreased from 45.31-39.78 and from 41.32-30.92 at the age of 22 and 50 weeks, respectively during 14 days of storage. These results are in agreement with those of Kirunda and McKee (2000), who have found that decreases in yolk index and Haugh units were observed in aged whole and yolk only eggs compared to fresh whole and yolk only eggs.

Significant increases in pH of albumen and yolk were also observed with increased storage time and temperature. Albumen and yolk pH were more increased at 20°C than 4°C. Albumen pH was increased by storage time from 7.945-9.217 at the age of 22 weeks. The effects of age and storage temperature on albumen pH are presented in Table 2. A highly significant interaction between hen age and storage temperature was observed for albumen pH ($p<0.001$). At two ages, albumen pH increased with storage time and temperature. This effect was pronounced in eggs from all hens. Overall, albumen pH increased at 22 and 50 weeks of age, from 7.945-9.217 and from 8.216-9.252 at 0 and 14 days stored eggs, respectively. But most of this increase occurred during the first 3 days

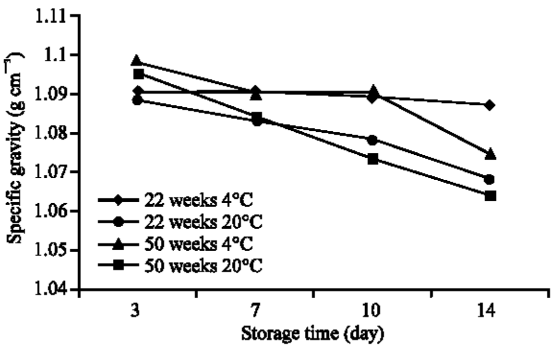

Fig. 4: Effects of storage conditions and hen age on egg specific gravity

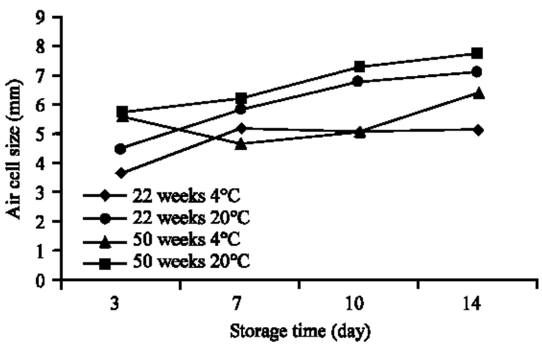

Fig. 5: Effects of storage conditions and hen age on egg air cell size

of storage. This rapid increase was followed by progressively slower rate of increase throughout the remainder of the storage period. At fresh eggs, pH increased ($p<0.001$) by hen age (Fig. 3). This might be explained by a higher egg shell conductance from older hens, which would allow for a more rapid release of carbon dioxide from the eggs (Brake *et al*., 1997).

The increase in yolk pH was not significantly affected by storage temperature. A highly significant interaction between hen age and storage time was observed for yolk pH ($p<0.001$).

Significant changes occurred in specific gravity (Fig. 4) and size of the air cell (Fig. 5) depending upon the increased temperature and storage time ($p<0.001$). Dramatic decreases were observed in specific gravity at the 20°C of storage temperature. Specific gravity of the fresh eggs at the age of 22 and 50 weeks old was 1.097 and 1.098, respectively. Whereas, specific gravity of the eggs declined to 1.068 and 1.064 due to increased storage time, at the age of 22 and 50 weeks, respectively. In addition size of the air cell increased with storage time and storage temperature. Size of the air cell exceeded 5 mm in 7 days at all storage temperatures.

CONCLUSION

This study has demonstrated that increasing of hen age can rapid deterioration of egg quality with increased storage time and temperature. There were significant interaction between egg quality and both storage time and storage temperature as well as the age of laying hens. Haugh unit was adversely affected by length of storage, increased storage temperature and age of hens. There was a clearly negative effect of storage time and storage temperature on egg weight loss, specific gravity, yolk index, albumen index and Haugh unit.

Egg weight loss was significantly raised by increasing of hen age. Albumen and yolk pH were significant increased by storage time, storage temperature and increasing of hen age. Significant increases in pH of albumen and yolk were also observed with increased storage time and storage temperature. Albumen and yolk pH were more increased at 20°C than 4°C.

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