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Impacts of red pepper supplemented diets and different storage conditions on eggs obtained from free-range laying hens

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Abstract

Egg quality depends on many conditions, including diet and storage temperature. Consumers usually assess it by checking yolk colour. The aim of the study was to indicate the effects of storage periods (7, 14 and 21 days) and temperature (room and refrigerator) on egg quality parameters, especially yolk colour. The experiment was carried out with 150 eggs, which were collected from free-range Lohmann brown laying hens (42 weeks old) fed with or without 0.75% red pepper supplemented diets. The highest weight loss was observed in the eggs stored at room temperature (23 °C, 64% humidity) for 21 days. In addition, it was discovered that the highest air cell height (ACH) was detected in the eggs stored at room temperature for 14 and 21 days. Red pepper supplementation and storage time and temperature were found to have been effective on the Roche yolk colour fan values (RYCF), lightness (L*) and yellowness (b*). Additionally, red pepper supplementation had a significantly positive effect on the redness (a*) value of the yolk. However, no statistical difference in the a* value was determined between the fresh eggs and the stored eggs supplemented with red pepper. The supplementation of 0.75% red pepper as a natural colouring agent to the diets of free-range laying hens had a positive effect on the yolk colour, which is an important attribution for consumer perception. Furthermore, the colours of the yolks of eggs stored in the refrigerator for a week were not significantly different from the fresh ones. Consequently, the present study suggests feeding laying hens diets supplemented with 0.75% red pepper might be useful in improving yolk colour. The results indicate that the eggs stored in the refrigerator might be closer to the characteristics of fresh eggs. In contrast, eggs stored at room temperature showed more deterioration between treatments.

Keywords: Colour evaluation methods, egg quality, free-range, yolk colour
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Introduction

The colour of egg yolk is one of the main parameters for assessing egg quality besides shape, weight, eggshell strength and freshness (Beardsworth, 2004; Spasevski *et al*., 2018). In addition, the colour of the yolk is an essential criterion for consumer preferences and varies according to countries, culture and traditions. For example, on the RYCF scale, the preferred yolk colour in Ireland, North England and Sweden is 8–9, in Australia, Finland and France, it is about 11 and in Germany, Netherlands, Spain and Belgium it is about 13 (Grashorn & Steinberg, 2002; Roberts, 2004; Shahsavari, 2014; Spasevski *et al*., 2017). Yolk colour also has an important effect on industrial food products that contain egg (Shahsavari, 2014). Yolk colour might be affected and improved by carotenoids. However, animals can only convert or metabolize carotenoids; they are not able to synthesize them (Hencken, 1992; Spasevski *et al*., 2018). Because carotenoids have an essential role in the colouration of poultry meat, skin, fat and egg yolk, bird diets should contain carotenoids to meet this need. There are synthetic and natural sources for feed carotenoids (Grashorn & Steinberg, 2002; Beardsworth, 2004). However, there are limitations in the use of synthetic carotenoids, which differ among countries (Grashorn & Steinberg, 2002; Roberts, 2004; Zaheer, 2017; Spasevski *et al*., 2018). Naturally carotenoid-rich sources are yellow corn, corn gluten meal, alfalfa meal, marigold, algae and red pepper (Leeson & Summer, 1997; Kirkpinar & Erkek, 1999a; Gálvez *et al*., 2008; Kermanshahi *et al*., 2011; Zaheer, 2017). The carotenoids in red pepper *(Capsicum annuum)* consist of capsanthin and capsorubin at levels of 70% and 80%. These colouring agents form egg yolk colours from golden-yellow to orange (Kirkpinar & Erkek, 1999b). However, it was reported that the hens in organic

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systems and village-type production systems obtained the colouring agents they needed from green leafy vegetation, insects and excrement while grazing on pasture during the day (Kirkpinar & Erkek, 1999a; Zaheer, 2017). This advantage does not exist in the conventional systems, which constitute the overwhelming majority of poultry production systems. Nevertheless, free-range systems, with advantages similar to those of village-type rearing systems, have gained importance in the last decade.

Moreover, growing interest in "healthy" nutrition has led to an increase in the demand for natural and health-promoting food products. Because of these developments, studies on natural feed additives have gained importance (Ekiz & Acikgoz, 2016). It is essential that the feed additives used in diets should have no negative effects on the health of animals and consumers. Furthermore, with the awareness of environmental protection and increased income level, the importance attached to animal welfare is rising, along with consumer expectations of good quality food products. Thus, the emphasis was placed on the free-range system in the study. The aim of the study was to investigate the effects of a natural feed carotenoid (red pepper), storage temperature and time on the egg quality parameters and yolk colour change of eggs obtained from free-range laying hens.

Materials and Methods

The eggs were obtained from 42-week-old Lohmann Brown laying hens that were reared free range on pasture at the farm of the Agricultural Faculty (Tekirdag Namık Kemal University, Turkey). The pasture consists of six parcels, each being 4 x 50 = 200 m^2 in size. The pasture parcels contained a ranging area and an indoor area for the hens, and 26 hens were placed in each parcel. A pasture area of 8 m^2 on average was allocated per animal. The plant composition of the pasture parcels consisted of 75% *Lolium perenne*, 20% *Festuca rubra* var. *rubra* and 5% *Trifolium repens*. However, a basal diet (16.75% crude protein and 2,795 kcal/kg of metabolic energy) was prepared according to NRC (1994) and Lohmann's (2012) free-range catalogue values. The basal diet was based on corn (59%), soybean (17%) and sunflower (13%) meals. Birds were fed either a basal diet or the basal diet supplemented with 0.75% red pepper. Poultry management and nutrition were carried out in accordance with the Turkish guidelines for experimental animal protection in line with the European regulations (Royal Decree 53/2013, 2013).

The eggs were collected and weighed two hours after they were laid. Meanwhile, 150 eggs were randomly separated to 14 treatments (2 w/wo red pepper–2 storage conditions–3 storage times–plus 1 group of fresh eggs w red pepper–1 group of fresh eggs without red pepper). The storage conditions in the study were determined as room (23 °C; 64% humidity) and refrigerator (3 °C; 45% humidity). The eggs were stored under these conditions for three periods (7, 14 and 21 days). In total, 30 fresh eggs (with/without red pepper) were analysed. The remaining 120 eggs were examined at the end of every storage period, with 10 eggs being examined for each treatment.

Internal and external egg quality parameters have been used for decades to determine egg quality. for example, egg weight, shape, specific gravity, eggshell deformation, eggshell colour, eggshell breaking strength, eggshell weight, eggshell thickness and eggshell percentage were used as the external quality parameters. For internal egg quality parameters, air cell height, yolk colour and index (yolk height/yolk diameter), albumen index, Haugh unit (HU) [HU =100 log (H+ 7.57- 1.7 x W x 0.37); H: albumen height (mm), W: egg weight (g)], yolk pH and albumen pH are used (Samli *et al*., 2005; Agma Okur & Samli, 2014).

The fresh and post-storage weights of all eggs were recorded. In this way, the weight loss was determined. An analysis of the internal and external quality of 40 eggs was made at the end of each storage period.

When determining the yolk colour as an internal quality parameter, two methods were used and the values were compared. First, the colours of the egg yolks were determined visiually using the Roche yolk colour fan (a practical tool that includies 1 to 15 tones of yellow) (Hoffmann-La Roche, Ltd, Basel, Switzerland). Second, the colour of whole egg yolks was measured from three parts of the sample according to the L* a* b* colour system. In this method, the values of L* (lightness) (which ranges from 0 to 100), a* (red and green) (positive values indicate red) and b* (yellow and blue) (positive values indicate yellow) were measured by HunterLab D25LT device (HunterLab, 1996; HunterLab Technical Manual, 2008).

Data of fresh and stored eggs were subjected to Duncan's multiple range test together. Also, statistical analyses of the data without fresh eggs were made with Statistica (1999) software. An ANOVA using a general linear model included the main effects of red pepper supplementation, storage time and storage temperature of eggs and the interactions between these factors.

Results

The effects of storage period, temperature and red pepper supplementation on the egg yolk colour are presented in Table 1. The colour measurements provided in the table were performed with the Roche yolk colour fan (RYCF) and HunterLab D25LT device.

While the values of L* and b* were found to be higher in the group without red pepper supplementation, the value of a* was higher in the group with red pepper supplementation (Table 1). This finding is consistent with the RYCF values, as an expected result.

Red pepper supplementation, storage period and storage temperature were found to have affected the Roche L* and b* values. The Roche fan value was 12.07 in the fresh eggs obtained from the birds fed with red pepper supplemented diet, whereas this value was determined as 9.60 without red pepper supplementation (*P* <0.001). Furthermore, the Roche value decreased in the group with no supplementation on increase in the storage temperature and the storage period. The value of L* was 46.88 in the fresh eggs without red pepper supplementation, while the highest values of L* were 51.81 and 52.97 in the eggs stored at room temperature for two and three weeks. In addition, no statistical difference was detected in the L* values of the eggs stored for two and three weeks, whereas L* value rose with the increases in the storage period and the storage temperature. The value of redness (a*) for the fresh eggs was 10.93 in the group with red pepper supplementation and increased with storage for a week, two weeks and three weeks (*P* <0.001) (Table 1). The highest value was recorded as 12.09 in the eggs stored in the refrigerator for three weeks. However there was no significant difference between weeks.

External quality parameters of the eggs are presented in Table 2. As expected, the egg weight decreased, depending on the storage period and temperature, with the highest weight loss being observed in the eggs stored at room temperature for three weeks (*P* <0.001). The weight loss in the eggs stored for three weeks was 1.33 and 1.22 in the groups with and without red pepper supplementation, respectively. Additionally, storage period and temperature affected the ACH, too. An increase in the ACH occurred with longer storage time.

In the internal egg quality results, a decrease in yolk height values was observed with an increase in the storage time, whereas the highest yolk height values were seen in the eggs stored in the refrigerator (Table 3). Haugh unit, yolk and albumen heights were significantly decreased by the storage period and the storage temperature (*P* <0.001). The lowest values were seen in the eggs stored at room temperature for two and three weeks, while no significant effect were observed from red pepper supplementation. Additionally, albumen pH values tended to increase with storage time and temperature (*P* <0.001; Table 3). When albumen pH levels were compared among treatments, eggs stored in room conditions were highest. Yolk height, albumen height and Haugh units decreased with storage time and this decrease was quicker at higher temperatures (Roberts, 2004).

In the study, it was discovered that the yolk diameter increased with a longer storage period and at room temperature conditions (*P* <0.001), with the highest values being observed in the eggs stored at room temperature for three weeks (Table 3). In addition, storage period and storage temperature affected the yolk index. Moreover, the highest values were observed in the fresh eggs and in the eggs stored in the refrigerator for a week, whereas the lowest yolk index value was recorded in the eggs stored at room temperature for three weeks (*P* <0.001).

The HU (*P* <0.001) was affected by the interactions among albumen pH, yolk diameter, storage period and storage temperature (*P* <0.01). Furthermore, the values of albumen weight and yolk viscosity were affected by the interaction between red pepper supplementation and storage temperature (*P* <0.01). Accordingly, the lowest yolk viscosity value was observed in the eggs from groups fed the diet without red pepper, which had been stored at room temperature for three weeks.

Discussion

Laying hens are able to transfer only 20% to 60% of colouring agents from feed to the egg yolk, depending on the levels of the colouring agents in their diet. The effects of colouring agents on the egg yolk colour were reported to hinge on many factors, namely the source of the colouring agent, the components making up the diet, ages of birds, hen strain, disease and environmental conditions (Gokmen, 2006; Spasevski *et al*., 2017).

The effect of the colouring agents in the diet was observed in the second egg after consuming the diet and it was reported to have reached the maximum level in 9–12 days. However, the effect of removing a colouring agent from the diet was slower and a change in the egg yolk colour was observed in 9–10 days (Gokmen, 2006).

Table 1 Effects of red pepper supplementation and storage on the egg yolk colour

Storage period	Red pepper	Storage temperature	Colour, RYCF$^{\varphi}$	L*	a*	b*	Yellowness Index
Fresh	-	-	9.60 ± 1.35^{de}	46.88 ± 1.48^{efg}	5.36 ± 2.25^{bc}	31.00 ± 0.68^{e}	94.49 ± 1.29^{a}
	+	-	12.07 ± 0.80^{a}	44.80 ± 1.28^{h}	10.93 ± 2.02^{a}	29.51 ± 0.65^{f}	94.10 ± 0.98^{ab}
1 week	-	Refrigerator	9.10 ± 0.99^{ef}	47.22 ± 1.89^{defg}	4.51 ± 1.58^{c}	31.18 ± 0.94^{e}	94.35 ± 1.21^{ab}
	+	Refrigerator	12.22 ± 0.42^{a}	45.69 ± 1.06^{fgh}	11.24 ± 1.79^{a}	30.00 ± 0.63^{f}	93.79 ± 0.82^{ab}
	-	Room	9.00 ± 0.71^{ef}	49.46 ± 1.12^{b}	6.88 ± 1.73^{b}	32.75 ± 0.52^{b}	94.61 ± 0.94^{a}
	+	Room	10.75 ± 1.48^{bc}	47.30 ± 2.37^{def}	10.84 ± 3.78^{a}	30.96 ± 0.97^{e}	93.61 ± 1.96^{ab}
2 weeks	-	Refrigerator	8.44 ± 0.70^{f}	47.66 ± 1.26^{cde}	5.48 ± 1.27^{bc}	31.52 ± 0.61^{de}	91.17 ± 9.12^{b}
	+	Refrigerator	10.88 ± 0.82^{bc}	45.63 ± 1.46^{gh}	11.54 ± 1.38^{a}	29.87 ± 0.32^{f}	93.58 ± 2.41^{ab}
	-	Room	7.00 ± 0.94^{g}	51.81 ± 1.37^{a}	4.61 ± 2.25^{bc}	34.01 ± 0.56^{a}	93.81 ± 1.48^{ab}
	+	Room	9.89 ± 0.57^{cde}	48.13 ± 1.47^{bcde}	10.77 ± 2.05^{a}	31.63 ± 0.68^{cde}	93.91 ± 1.41^{ab}
3 weeks	-	Refrigerator	8.33 ± 1.49^{f}	48.69 ± 1.58^{bcd}	6.86 ± 3.68^{b}	32.01 ± 0.93^{cd}	93.93 ± 0.81^{ab}
	+	Refrigerator	11.50 ± 0.85^{ab}	45.78 ± 1.32^{fgh}	12.09 ± 1.61^{a}	30.00 ± 0.82^{f}	93.63 ± 1.09^{ab}
	-	Room	6.80 ± 1.23^{g}	52.97 ± 1.59^{a}	4.43 ± 2.22^{c}	34.48 ± 0.49^{a}	93.05 ± 1.83^{ab}
	+	Room	10.33 ± 0.74^{cd}	49.20 ± 1.14b^{c}	11.85 ± 1.38^{a}	32.31 ± 0.65^{bc}	93.82 ± 0.83^{ab}
				P levels			
Storage period (1)			0.786	0.000	0.000	0.000	0.444
Red pepper supplementation (2)			0.309	0.420	0.000	0.000	0.000
Storage temperature (3)			0.224	0.000	0.000	0.000	0.383
1 x 2			0.492	0.648	0.140	0.130	0.613
1 x 3			0.647	0.008	0.436	0.031	0.075
2 x 3			0.008	0.316	0.653	0.092	0.871
1 x 2 x 3			0.615	0.369	0.085	0.798	0.070

$^{\varphi}$ Colour score range from 1(i.e. yellow) to 15 (i.e. orange)
HunterLab colourimeter model DP-9000 D25A (Hunter Associates Laboratory, Reston, VA, USA) Hunter L (lightness), a* (redness) and b* (yellowness).
RYCF: Roche yolk colour fan values
The values with different superscripts (a–h) in the same column are significantly (P <0.01) different

Table 2 Results of the external egg quality analyses

Storage period	Red pepper	Storage temperature	Fresh egg weight g	Weight loss g	ACH mm	Shell weight g	Shell thickness mm	Shape index %	Eggshell ratio %
Fresh	-	-	65.95 ± 3.05[c]	0.00 ± 0.00[f]	3.30 ± 0.45[d]	7.54 ± 0.40[ab]	33.93 ± 1.31[d]	82.33 ± 7.28[a]	11.43 ± 0.44[bc]
	+	-	69.56 ± 4.83[abc]	0.00 ± 0.00[f]	3.07 ± 0.47[d]	7.56 ± 0.44[ab]	33.62 ± 2.23[d]	77.70 ± 2.53[b]	11.06 ± 0.96[bc]
1 week	-	Refrigerator	66.45 ± 2.25[b]	0.29 ± 0.14[e]	4.24 ± 0.46[c]	7.18 ± 0.84[b]	36.67 ± 1.96[ab]	79.01 ± 2.74[b]	10.85 ± 1.16[bc]
	+	Refrigerator	69.82 ± 5.70[abc]	0.30 ± 0.14[e]	3.56 ± 0.72[d]	7.23 ± 0.62[ab]	36.19 ± 2.30[abc]	77.60 ± 2.09[b]	10.42 ± 0.71[c]
	-	Room	67.96 ± 5.31[abc]	0.49 ± 0.15[d]	5.35 ± 0.52[b]	7.36 ± 1.21[ab]	36.93 ± 2.41[a]	78.02 ± 2.17[b]	10.87 ± 1.24[bc]
	+	Room	68.55 ± 4.60[abc]	0.55 ± 0.12[d]	5.48 ± 0.57[b]	7.81 ± 1.05[ab]	37.25 ± 1.87[a]	79.36 ± 3.77[b]	11.45 ± 1.15[bc]
2 weeks	-	Refrigerator	68.21 ± 2.83[abc]	0.43 ± 0.07[de]	4.93 ± 0.66[b]	7.70 ± 0.35[ab]	33.74 ± 1.46[d]	78.37 ± 3.29[b]	11.38 ± 0.64[bc]
	+	Refrigerator	71.43 ± 4.91[a]	0.55 ± 0.19[d]	4.92 ± 0.74[b]	7.74 ± 0.57[ab]	32.63 ± 1.98[d]	79.22 ± 2.19[b]	10.92 ± 0.62[bc]
	-	Room	69.74 ± 4.06[abc]	0.97 ± 0.16[b]	6.56 ± 0.45[a]	7.58 ± 0.63[ab]	34.03 ± 2.39[d]	77.67 ± 1.77[b]	11.02 ± 0.56[bc]
	+	Room	65.44 ± 4.88[c]	1.00 ± 0.20[b]	6.57 ± 0.71[a]	7.49 ± 0.78[ab]	34.67 ± 2.36[bcd]	77.49 ± 1.41[b]	11.62 ± 0.64[bc]
3 weeks	-	Refrigerator	68.18 ± 2.62[abc]	0.78 ± 0.21[bc]	5.48 ± 0.43[b]	7.68 ± 0.38[ab]	33.72 ± 2.00[d]	77.00 ± 2.09[b]	12.83 ± 0.93[a]
	+	Refrigerator	70.85 ± 3.18[ab]	0.87 ± 0.16[bc]	5.27 ± 0.77[b]	7.94 ± 0.41[a]	34.07 ± 2.14[d]	77.41 ± 1.65[b]	11.35 ± 0.53[bc]
	-	Room	68.98 ± 4.93[abc]	1.33 ± 0.37[a]	6.62 ± 1.04[a]	7.67 ± 0.30[ab]	37.90 ± 1.83[a]	77.45 ± 1.41[b]	11.85 ± 0.93[b]
	+	Room	67.75 ± 3.35[abc]	1.22 ± 0.20[a]	6.49 ± 0.92[a]	7.53 ± 0.58[ab]	34.44 ± 2.81[cd]	80.04 ± 3.77[ab]	10.86 ± 1.22[bc]
					P levels				
Storage period (1)			0.786	0.000	0.000	0.172	0.000	0.626	0.025
Red pepper supplementation (2)			0.309	0.420	0.319	0.402	0.127	0.202	0.131
Storage temperature (3)			0.224	0.000	0.000	0.980	0.001	0.694	0.887
1 x 2			0.492	0.648	0.758	0.714	0.302	0.461	0.045
1 x 3			0.647	0.008	0.399	0.191	0.299	0.076	0.093
2 x 3			0.008	0.316	0.310	0.939	0.656	0.213	0.073
1 x 2 x 3			0.615	0.369	0.483	0.449	0.017	0.237	0.938

ACH: air cell height
The values with different superscripts (a–f) in the same column are significantly (P <0.05) different

Table 3 Results of the internal egg quality analyses

Storage period	Red pepper	Storage temperature	Yolk height mm	Albumen height mm	Haugh unit	Yolk weight g	Albumen weight g	Yolk pH
Fresh	-	-	21.04 ± 0.77[abc]	11.65 ± 1.46[a]	104.61 ± 6.55[a]	16.47 ± 1.06	38.32 ± 2.61[bc]	6.22 ± 0.07[e]
	+	-	21.20 ± 0.57[ab]	10.07 ± 1.81[b]	96.96 ± 9.34[b]	17.13 ± 1.22[bc]	40.61 ± 3.62[ab]	6.26 ± 0.06[de]
1 week	-	Refrigerator	22.51 ± 4.50[a]	10.86 ± 1.77[ab]	101.18 ± 7.51[ab]	17.15 ± 1.24[bc]	38.05 ± 1.43[bc]	6.32 ± 0.08[abcd]
	+	Refrigerator	22.84 ± 4.37[a]	9.83 ± 1.43[b]	95.98 ± 7.07[b]	18.64 ± 1.76[a]	39.97 ± 5.53[abc]	6.30 ± 0.10[bcde]
	-	Room	19.08 ± 0.65[bcd]	7.79 ± 1.09[c]	85.64 ± 7.90[c]	17.51 ± 1.02[abc]	39.71 ± 4.62[bc]	6.33 ± 0.07[abcd]
	+	Room	19.83 ± 0.59[bcd]	7.80 ± 0.83[c]	85.89 ± 5.03[c]	18.65 ± 2.06[a]	38.36 ± 4.00[bc]	6.34 ± 0.05[abcd]
2 weeks	-	Refrigerator	18.82 ± 1.12[d]	7.09 ± 1.22[cd]	81.34 ± 7.28[c]	17.47 ± 0.99[abc]	39.73 ± 2.59[abc]	6.32 ± 0.06[abcd]
	+	Refrigerator	19.43 ± 0.86[bcd]	7.13 ± 0.94[cd]	80.78 ± 6.08[cd]	17.99 ± 1.30[ab]	42.42 ± 3.94[a]	6.29 ± 0.10[cde]
	-	Room	15.65 ± 1.11[e]	4.38 ± 1.13[e]	56.73 ± 11.57[e]	17.64 ± 0.70[abc]	40.59 ± 3.59[ab]	6.35 ± 0.07[abcd]
	+	Room	15.63 ± 0.93[e]	4.09 ± 0.41[e]	56.61 ± 6.76[e]	17.78 ± 1.41[abc]	36.56 ± 3.26[bc]	6.37 ± 0.06[abc]
3 weeks	-	Refrigerator	19.02 ± 1.11[cd]	5.89 ± 1.35[d]	73.54 ± 11.56[d]	17.47 ± 1.01[abc]	39.70 ± 2.49[abc]	6.33 ± 0.07[abcd]
	+	Refrigerator	18.53 ± 0.71[d]	6.02 ± 0.47[d]	73.16 ± 3.35[d]	18.06 ± 1.34[ab]	41.76 ± 2.50[ab]	6.38 ± 0.08[ab]
	-	Room	13.69 ± 0.72[e]	3.73 ± 0.38[e]	52.12 ± 5.24[e]	17.80 ± 1.28[bc]	39.50 ± 4.65[abc]	6.40 ± 0.12[a]
	+	Room	14.10 ± 2.94[e]	3.30 ± 0.45[e]	42.88 ± 6.11[f]	17.78 ± 1.07[abc]	38.10 ± 2.33[bc]	6.33 ± 0.62[abcd]
					P levels			
Storage period (1)			0.000	0.000	0.000	0.701	0.833	0.071
Red pepper supplementation (2)			0.365	0.207	0.126	0.032	0.959	0.708
Storage temperature (3)			0.000	0.000	0.000	0.801	0.098	0.091
1 x 2			0.977	0.822	0.486	0.164	0.608	0.898
1 x 3			0.440	0.381	0.0002	0.098	0.313	0.605
2 x 3			0.682	0.622	0.933	0.536	0.002	0.619
1 x 2 x 3			0.626	0.209	0.228	0.873	0.542	0.154

The values with different superscripts (a–g) in the same column are significantly (P <0.05) different

Table 3 Results of the internal egg quality analyses (continued)

Storage period	Red pepper	Storage temperature	Albumen pH	Yolk diameter cm	Yolk index	Yolk viscosity mPa.s	Albumen viscosity mPa.s
Fresh	-	-	8.35 ± 0.12^{g}	4.18 ± 0.14^{d}	0.50 ± 0.02^{a}	12.40 ± 5.19^{ab}	5.95 ± 4.11^{a}
	+	-	8.34 ± 0.10^{g}	4.17 ± 0.15^{d}	0.51 ± 0.01^{a}	15.89 ± 6.35^{a}	3.57 ± 2.57^{bc}
1 week	-	Refrigerator	9.02 ± 0.06^{f}	4.24 ± 0.16^{cd}	0.53 ± 0.09^{a}	12.45 ± 5.77^{ab}	3.70 ± 2.89^{b}
	+	Refrigerator	9.01 ± 0.06^{f}	4.23 ± 0.13^{cd}	0.54 ± 0.03^{a}	9.06 ± 4.45^{bcd}	2.17 ± 0.83^{bcd}
	-	Room	9.42 ± 0.08c^{d}	4.38 ± 0.16^{bc}	0.44 ± 0.02^{b}	5.61 ± 3.09^{cde}	1.33 ± 0.35^{d}
	+	Room	9.49 ± 0.07^{bc}	4.39 ± 0.18^{bc}	0.45 ± 0.02^{b}	6.00 ± 2.11^{cde}	1.50 ± 0.46^{cd}
2 weeks	-	Refrigerator	9.16 ± 0.25^{e}	4.34 ± 0.07^{bcd}	0.43 ± 0.03^{b}	11.33 ± 4.62^{b}	2.72 ± 1.65^{bcd}
	+	Refrigerator	9.16 ± 0.07^{e}	4.36 ± 0.07^{bcd}	0.45 ± 0.02^{b}	11.63 ± 3.53^{b}	2.94 ± 1.51^{bcd}
	-	Room	9.55 ± 0.14^{ab}	4.48 ± 0.14^{b}	0.35 ± 0.03^{c}	5.20 ± 1.25d^{e}	1.55 ± 0.44^{cd}
	+	Room	9.58 ± 0.10^{ab}	4.47 ± 0.23^{b}	0.35 ± 0.02^{c}	5.39 ± 1.27^{de}	1.33 ± 0.24^{d}
3 weeks	-	Refrigerator	9.33 ± 0.07^{d}	4.33 ± 0.16^{bcd}	0.44 ± 0.03^{b}	10.25 ± 2.32^{b}	1.58 ± 2.20^{cd}
	+	Refrigerator	9.31 ± 0.06^{d}	4.45 ± 0.15^{b}	0.42 ± 0.02^{b}	8.60 ± 2.72^{bcd}	1.80 ± 0.75^{bcd}
	-	Room	9.61 ± 0.03^{a}	4.83 ± 0.37^{a}	0.29 ± 0.03^{d}	3.00 ± 0.82^{e}	1.25 ± 0.35^{d}
	+	Room	9.53 ± 0.11^{bc}	4.70 ± 0.16^{a}	0.30 ± 0.06^{d}	9.53 ± 0.11^{bc}	3.25 ± 0.90^{bcd}
			P levels				
Storage period (1)			0.000	0.000	0.000	0.747	0.914
Red pepper supplementation (2)			0.822	0.812	0.362	0.687	0.782
Storage temperature (3)			0.000	0.000	0.000	0.000	0.0003
1 x 2			0.346	0.969	0.904	0.013	0.113
1 x 3			0.001	0.006	0.198	0.102	0.029
2 x 3			0.632	0.244	0.509	0.001	0.011
1 x 2 x 3			0.712	0.552	0.506	0.045	0.047

The values with different superscripts (a–g) in the same column are significantly (P <0.05) different

With their universal usage as colouring agents in laying hen diets, carotenoids can be synthesized only by plants, algae, bacteria and fungi, and not by birds (Nys, 2000; Gurbuz *et al.*, 2015). Therefore, the desired colour uniformity in the egg yolk depends on the colouration capacity and stability of the carotenoids in the diet (Nys, 2000).

Yellow corn, corn gluten meal, marigold, clover, algae and red pepper are widely used natural colouring agents in diets. In addition, colouring agents such as dried tomato pulp, watercress, safflower petals and henna (*Lawsonia inermis*) have been used. In these studies, it was observed that they affected the colour (Leeson & Summer, 1997; Kirkpinar & Erkek, 1999b; Baytok *et al*., 1999; Ayhan & Aktan, 2004) positively.

The ACH values were highest in the eggs stored at room temperature for two and three weeks. It was seen that the value of eggshell thickness was also affected by the storage period and temperature. However, this result is thought to have been due to the randomly selected eggs, for in the previous studies it was revealed that red pepper supplementation to the diet had no effect on the eggshell thickness (Shahsavari, 2014).

Yolk index (YI) value is related to yolk height and yolk diameter. Because of that, increasing the storage time and temperature affected YI and YI might be a indicator of deterioration. Feddern *et al.* (2017) reported that YI has to be between 0.39 and 0.45. The current results showed that YI values of eggs stored at room temperatures for two and three weeks are lower than 0.39.

Albumen height and Haugh unit values tend to decrease during storage, which concurs with the findings of Feddern *et al.* (2017). According to egg quality grades of USDA (2000) manual, a higher HU means higher egg quality. Eggs could be graded as follows;

- A firm albumen has a HU value of 72 or higher when measured at a temperature between 7.2 and 15.6 °C (AA quality).
- Less thick than firm albumen has a HU value of 60 up to but not including 72 when measured at a temperature between 7.2 and 15.6 °C (A quality).
- A thin and watery albumen has a HU value lower than 60 when measured at a temperature between 7.2 and 15.6 °C (B quality).

In the current research, only HU values of eggs stored at room conditions for two and three weeks were lower than 60.

Feddern *et al.* (2017) stored eggs for nine weeks under room and refrigerator temperatures in summer and autumn. According to their results, eggs kept at room temperature should be consumed in two weeks, while refrigerator-stored eggs should be consumed within eight weeks, preserving inner quality from farm to retail. By the end of the nine-week storage period, eggs kept under refrigeration presented similar quality parameters to eggs stored at room temperature for only three weeks. In contrast, eggs kept at room temperature presented faster spoilage from week 1 to 5. Similarly to the current research, the inner quality of eggs kept at room temperature deteriorated more at two and three weeks than the refrigerated ones.

Gurbuz *et al.* (2003) determined the optimum RYCF value for consumer preference as 9.55 in the group with a diet supplemented with 0.5% red pepper. They reported that the Roche value rose to 11.45 in the group they had supplemented with 1% red pepper and to 12.55 in the group with 2% red pepper supplementation. Shahsavari (2014) determined the highest egg yolk colour value (14.33) in the groups with 2% red pepper supplementation, while the lowest value was observed in the groups that had consumed the barley- and wheat-based diet (1.58) (P <0.05). However, Gurbuz *et al.* (2003) observed the highest RYCF values (14.30 and 14.45) in the groups in which they had supplemented the yellow corn-based diet with 3% and 4% red pepper. According to the results of the current study, the value of 12.07 was obtained with 0.75% red pepper supplementation.

The reason that the RYCF value of the animals fed with a diet without red pepper supplementation turned out to be as high as 9.60 might be that the animals were reared on pasture (8 m^2/hen) in the free-range system in spring with good pasture and the diets were based on corn. In their study, Mugnai *et al.* (2014) reported that the egg yolk α-tocopherol and carotenoid levels of the eggs of the animals fed green grass in different seasons increased significantly compared with those that had not been fed this grass. Some researchers (Lordelo *et al*., 2017; Yilmaz Dikmen *et al*., 2017) found that organic and free-range reared hens had lighter yolk colour than other systems. But pasture quality is an important factor for pigmenting egg yolk and might be affected by season. Campbell *et al.* (2017) reported that stocking density significantly affected egg yolk colour. The experimental range areas were 2 000 hens/ha (5 m^2/hen), 10 000 hens/ha (1 m^2/hen), 20 000 hens/ha (0.5 m^2/hen). The highest yolk colour was observed in the eggs laid by hens housed at the lowest stocking density (P <0.0001). This might be associated with stress and the time that the hen spends grazing.

In their study, Samli *et al.* (2005) used red pepper (*Capsicum annuum*) and corn gluten meal as natural colouring agent sources in the diets of old laying hens reared in cages. As a result of the study, the

RYCF values of the egg yolks in the groups with 0.5% red pepper and 0.3% red pepper + 1% corn gluten meal supplementation were the highest, namely 13.0 and 12.5, respectively (P <0.001). Besides, they stated that there were no statistically significant differences among the values of albumen height, albumen weight, yolk weight, and HU in terms of the effect of the natural colouring agents (P >0.05). These results resemble those in the current study.

Rowghanni *et al.* (2006) found the highest RYCF value was 12.55 in the group with a diet supplemented with 3% red pepper, while 0.5% red pepper supplementation ensured an egg yolk colour more preferred by consumers. Additionally, they determined the RYCF values for 0.5% red pepper supplementation and the supplementation of 0.6% commercial pigments as 9.67 and 9.57, respectively, and stated that there were no statistical differences between them. In conclusion, they recommended that red pepper could be used as a natural colouring agent instead of synthetic pigments.

RYCF is used in the most researches on egg yolk colour. There are few studies that evaluate yolk colour with RYCF and a device. RCYF is a simple and quick method, but results depend on the human eye and light. However, measurement with devices is more objective and reliable.

Spasevski *et al.* (2017) found that the supplementation of natural pigments to Lohmann Brown layer diet significantly (P <0.05) affected yolk redness. Paprika (1.5%) supplementation provided a higher value of yolk redness than the addition of marigold alone (1.5%), being in accord with the results of β-carotene content and RYCF. However, yolk L* and b* decreased significantly (P <0.05) when paprika was added alone and 1% paprika + 0.5% marigold. Their results are in agreement with RYCF scale and L*, a*, b* results of the current study.

Conclusions

The present study has shown that red pepper supplementation had a positive effect on the colour of the yolk of eggs obtained from free-range grazing laying hens. The colours of the yolks of eggs stored in the refrigerator for a week were found to be statistically no different from those of fresh ones. According to the results, as the storage time at room temperature increases, the internal egg characteristics are adversely influenced. On the other hand, the eggs stored in the refrigerator are less affected by the prolongation of the storage period than those stored at room temperature.

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Authors' contributions

AAO planned the experiment design, did the internal and external analysis of eggs, analysed the data statistically and wrote this manuscript. EUK did the internal and external analysis of eggs and wrote this manuscript. Both authors read and approved the final manuscript.

Conflict of Interest Declaration

None

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