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Research Article

Physical changes in hen eggs stored at different temperatures

Haldun Hüseyin ÖZDEMİR, Sevim KAYA, Burcu GÖKKAYA ERDEM

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Gaziantep University, Faculty of Engineering, Department of Food Engineering, 27310, Gaziantep, Türkiye

ORCID IDs of the authors:
H.H.Ö. 0009-0005-5562-8174
S.K. 0000-0003-4790-7630
B.G.E. 0000-0002-4112-5729

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Correspondence:
Sevim KAYA
E-mail: skaya@gantep.edu.tr

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ABSTRACT

Eggs are among the most nutritious foods, but they are perishable. Immediately after they are laid, ageing processes begin in shell eggs, altering their chemical, physical and functional properties. This study aimed to determine the effect of storage temperature on the shelf life of hen eggs according to changes in some important physical properties (weight, pH, Haugh Unit, Albumen Index, and Yolk Index). The studied temperatures were selected as possible refrigerator and room temperatures (10, 20 and 30°C). It was revealed that temperature significantly influenced egg quality, with the most significant effects observed in the Haugh unit and pH due to weight changes. The highest quality loss occurred at 30°C. The activation energies for each quality parameter were determined using the Arrhenius equation. The pH of the fresh egg was 7.6 ± 0.1 and increased to over 8.5 – 9.0 during storage, depending on the temperature. Haugh unit, albumen index, and yolk index decreased, and the area of both yolk and albumen increased during storage.

Keywords: Hen egg, Temperature, Physical properties, Shelf life, Egg quality

Introduction

The egg is a kind of important animal protein resource obtained from fowl. There are different egg types, such as duck, bird and turkey. However, in Codex Alimentarius, egg refers to common hen eggs, which are easier to produce and more nutritious. Hen eggs contain high-quality protein, important vitamins, and minerals (Afacan, 2023). The product comprises 65.6% water, 12.1% proteins, 10.5% lipid, 0.9% carbohydrates and 10.9% minerals. Chicken eggs are used as the basic ingredient in many products due to their high nutritional value and multifunctional properties, such as emulsification, foaming, gelling, thickening and flavouring, which improve the textural and sensory development of foodstuffs (Rossi et al., 2010). Egg composition may vary, not only in terms of genetic heritage but also in the chicken's feeding method. Housing conditions are another factor that affects this. Egg composition may vary depending on the hen's feeding method and genetic heritage. Housing condition is also another factor that affects the egg structure. The determination of shelf life and its relation to storage temperature is important. In order to determine shelf-life, the most important parameter has to be selected. There are some useful methods to determine the change in the quality of eggs' external and internal qualities. Chemical (protein content, lipid content, and solid content) and physical (colour, weight of egg, and height of albumen) are the most commonly used. In addition, the age and breeding environment of the hen have an important impact on the level and quality of egg components (Suk & Park, 2001). Traditional cages, free range and barns are examples of breeding methods for chicken animals. Instead of using standard feed for their chickens, some egg producers prefer to supplement the feed with chia seeds, fish oil, etc., to increase their nutritional value. In addition to this housing system, they have been feeding supplemented with several nutrients (such as chia, fish oil, flaxseed, and grape pomace) to improve the nutrition level (such as omega 3 fatty acids) of the egg (Sherwin et al., 2010; Kara et al., 2016). Also, egg quality can be affected by environmental conditions such as humidity, temperature and storage times (Akyurek & Okur, 2009). The good quality of the egg still corresponds to the consumer's expectation. Knowledge of typical egg structure, good quality characteristics, and deterioration stages is necessary for effective egg quality parameter testing. On the other hand, knowing the effect of shelf life and temperature on food structure is necessary. Environmental conditions on the farm, in the warehouse and at home become important in deciding which process features to apply (Feddem et al., 2017).

Studies in the literature have examined the impact of storage duration on hen egg quality. However, the other most important parameter, storage temperature efficiency, has not been examined in detail. Therefore, the main objective of this research was to investigate the effect of three different storage temperatures on the shelf life of the egg according to changes in physical properties.

Materials and Methods

One of the most important preference parameters when choosing the eggs used in this study was their freshness. Therefore, 150 freshly laid chicken eggs were supplied simultaneously from a previously informed local farm in Gaziantep. The laying hen was kept constant to ensure the homogeneity of the eggs used in the study (*Gallus gallus var. domesticus)*. Bought eggs were rested in the room until they reached room temperature. After the suitable temperature was reached, the surface of the egg samples was checked for cracks, contamination or foreign matter and the defective ones were separated. Intact eggs were grouped according to weight and size differences. As a result, 130 eggs were used as the representative samples for analyses. The selected temperatures were possible for refrigerator and room temperatures, such as 10, 20, and 30 °C. At each temperature condition, samples were divided into two lots (30 eggs for each lot); weight and colour changes of the same eggs in the first lot were followed periodically. The five eggs from the second lot determined the other physical characteristics for each test time. All chemicals used were reagent grade.

Weight Change Determination

Firstly, the egg samples were equilibrated at room conditions. After that, their initial weight values were recorded. Ten eggs were numbered and placed at three instead four different temperatures (refrigerator's door temperature (10°C), incubator (20°C), and incubator (30°C)) to test their weight changes (Feddern, 2017). The selected storage temperatures were determined by considering the conditions of commercial sales places. A precision balance (Precisa, 163 XB 220A, Dietikon, Switzerland) was used to weigh the egg samples periodically.

Colour Determination

The eggshell colour was measured from the same side of the samples, and a Hunter Lab Color Flex (A60-1010-615 Model Colorimeter, Hunter Lab, Reston VA) was used for detection. The L (Lightness), a (redness) and b (yellowness) values of the samples were obtained with the CIE Lab colour scale, and the average value of three readings was reported.

Changes in pH, Weight, Area and Height Determination

Five trials were conducted for each temperature group. An apparatus was set up using a precision balance to determine the weight change of shell eggs, whole eggs, whites and yolks. A glass material (190 x 190 mm) was put on the balance and calibrated with a spirit level. The board was placed in front of the balance to stabilize the height and distance of the photo taken. Lastly, the mechanism consisted of a small box that displayed the height during the image analysis process. At first, glass was put on the precision balance and tare weight was taken. Once the shell was weighed, it was carefully broken on plate glass, preserving the membranes between the yolk and white. After five minutes, a picture of the eggs was taken using a digital camera (Kodak, EasyShare, C713). The weight of the whole egg was then recorded. Both front and top photographs of the whole egg were taken to measure the height of the yolk and the area of the yolk and white. A pH meter (Eutech, EcoScan) was used to test the pH of the whole egg (Afacan, 2023). Before pH measurement, the egg yolk and white were mixed with a magnetic stirrer until they became completely homogeneous. The area and height of the yolk, and also an area of the albumin values, were detected by image analysis software (UTHSCSA Image Tool for Windows, Version: 3.00). A pin box was placed near the whole egg and photographs were taken to obtain the height of the egg yolk. System calibration measurement (from pixels to millimetres) was made by taking the initial height value of the box. The defined program determined the yolk height. A similar system was utilized to measure egg yolk and albumen areas. In this stage, the flat glass, which the egg was broken, was the material and its length was measured. Calibration from pixel to millimetre was done using the predetermined value. Then, the albumen surface, which spread on the flat glass, was pointed. When marking was made to create a closure, the program automatically calculated the albumen area. The same steps were applied to yolk area calculations.

Haugh Unit and Other Parameters

The Haugh Unit shows the internal quality of the egg. This approach was a correlation of albumen quality between the height of the thick albumen (in millimetres) and the weight of the egg (in grams) (Akyurek, 2009; Kirikçi et al., 2003). The Haugh Unit equation was given as follows:

Haugh Unit (HU) = $(100 \log (h - 1.7w^{0.37} + 7.6))$ (1)

Where h shows the height of the albumen (mm), and w is the weight of the egg (grams).

Equations 2 and 3 were used to calculate albumen and yolk indexes, respectively:

Albumen index (%) =

((height of the albumen / diameter of the albumen) x 100) (2)

Yolk index (%) =

((height of the yolk / diameter of the yolk) x 100) (3)

The Shelf Life and Activation Energy

By definition, a product's shelf life is the amount of time it can remain on the store shelf without any negative reaction from the consumer (Labuza, 1982). Egg quality parameter changes were found to obey zero order rate expression:

$$A = A_0 - kt \qquad (4)$$

Where "A" refers to the amount left after the time of t, "A_o" refers to initial quality, k is the rate constant and " t" refers to time. If the A is selected as the endpoint, t is to be shelf-life. It is explained in the literature that the steeper the slope, the more sensitive it will be to temperature fluctuations.

Arrhenius type equation can be used to determine temperature-dependent degradation reactions, given as:

$$k = k_o e^{-EA/RT} \qquad (5)$$

Where ko is the pre-exponent constant, EA is the activation energy (cal/mole), R refers to the gas constant (1.987 kcal / (mole) (K)), and T refers to temperature (K). ln (k) values of each parameter determined were drawn concerning the inverse of absolute temperature (1/T); the activation energies were calculated from the slopes of these lines since the slope is equal to $-E_A/R$.

Statistical Calculation

Analysis of variance (ANOVA) was performed using SPSS software (v.15.0.0) to investigate some parameter effects (temperature and time) on the Haugh unit, weight change, and pH of the egg. A univariate linear model was utilized, and the Tukey multiple comparison test was selected as a post hoc test.

Results and Discussion

The Results of Egg Weight Change

Figure 1 shows the weight change determinations of the analyzed samples at three different storage temperatures. According to the results, it was determined that there was an inverse proportion between egg quality and egg weight. This means that the rate of weight loss increases with the increase in storage temperature. In the study, it was understood that the highest weight loss was detected in egg samples placed at the highest temperature (30°C), as seen in Figure 1. The main reason for the loss of not only weight but also quality of eggs is the loss of CO_2 and H_2O through the shell pores (Suk & Park, 2001). The weight change continues on the market shelf, and after a while, the egg becomes stale, and the product reaches the end of its shelf life. Therefore, one of the most important quality parameters determining eggs' shelf life can be expressed as weight loss.

Colour Change of the Egg Shell

This research investigated changes in colour properties during storage to determine whether there is a relationship between eggshell colour and the shelf life of hen eggs. The L (Lightness), a (redness) and b (yellowness) values of the studied egg samples stored at 10, 20 and 30°C are given in Table 1. It was observed that the "L value" had a decreasing tendency during the storage period. This behaviour caused the final colour of the eggshells to be darker than the initial colour. Similarly, the other colour parameters of "a" showed a decreasing trend with increasing storage time. This means that the last colour of the egg changes from reddish to greenish. However, the "b value" showed an unstable change during the storage period.

Determination of Haugh Unit

Haugh unit (HU) is the most useful data for estimating egg freshness (Ahmadi & Rahimi, 2011). The variation of HU at 10°C, 20°C and 30°C was determined and given in Figure 2. The observations showed that a steady decrease in HU occurred during storage. HU is the correlation between egg weight and the height of the albumin. Therefore, this situation can be expected. In this study, it was understood that if the eggs were to be stored for a longer period of time, they would continue to lose weight. Similar trends were observed in the literature (Kahraman Dogan and Bayindirli, 1996). The decomposition rate constants (k) for each storage temperature were calculated from the line slopes and demonstrated in Table 2. The highest storage temperature (30°C) was found to have the highest k value in direct proportion. After 20 days of storage at 30°C, the acceptability of egg samples was lost due to the highest rates of white height and weight decrease. A decrease in the strength of the yolk vitelline membrane and the inner shell membrane can relate to this decreasing trend in white height. Another reason was the increase in the viscosity of the egg yolk (Karoui et al., 2006). The increase in the moisture content of the yolk explained these situations.

Table 1. Colour change of egg samples at 10, 20 and 30°C

Time (day)	10°C			20°C			30°C		
	L	a	b	L	a	b	L	a	b
0	46.67	0.20	1.25	50.87	0.30	2.13	49.06	0.20	1.76
4	47.57	0.23	1.26	50.94	0.24	2.65	49.45	0.10	1.78
6	47.52	0.22	1.81	51.07	0.19	1.90	50.10	0.03	1.87
9	47.57	0.24	2.86	50.99	0.20	2.31	49.55	0.06	3.14
12	47.98	0.17	1.28	51.31	0.15	2.18	49.89	0.01	1.59
14	47.99	0.20	1.14	51.66	0.17	2.44	50.12	0.02	2.48
16	48.02	0.19	1.32	51.23	0.16	2.77	50.18	0.02	2.27
19	47.79	0.16	1.32	51.16	0.15	1.81	50.07	-0.01	1.98
21	48.02	0.13	2.32	50.93	0.13	2.06	50.12	-0.03	2.05
23	47.71	0.15	1.63	51.28	0.13	1.93	49.83	-0.03	2.00

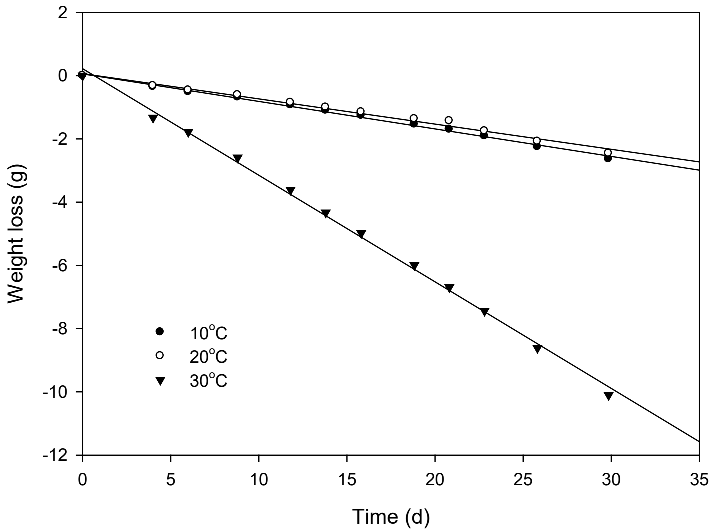

Figure 1. Weight loss of egg samples stored at 10, 20 and 30°C

Table 2. The deterioration rate constants (k) for quality parameter change at the studied temperature range

Quality parameters	Storage temperature°C		
	10	20	30
Weight Change (gr)	0.086	0.199	0.335
Haugh Unit	0.076	0.533	2.201
pH	0.019	0.030	0.048
Albumen Area (mm^2)	159.433	297.060	465.456
Yolk Area (mm^2)	5.334	12.697	99.340
Albumen Index	0.105	0.416	0.487
Yolk Index	0.074	0.512	1.508

Determination of pH

The pH results of the studied egg specimens are illustrated in Figure 3. The pH value of fresh eggs was 7.6. During storage of shell eggs, pH increased to a maximum of around 8.2. Close values were reported by Karauri et al (2006), but the authors only investigated the pH of egg white in their study (Karoui et al., 2006). The eggshell can breathe, meaning this material is moisture and gas (carbon dioxide) permeable (Caner, 2005). When carbon dioxide gas is released from the egg, the acidity in the egg matrix decreases. Additionally, the evaporation of water from the eggshell surface due to pores reduces acidity. Deterioration rate constants according to pH changes are shown in Table 2. As the storage temperature increased, gas solubility decreased, and the water evaporation rate increased. Increasing constants with increasing storage temperature indicate an increase in quality loss. It was seen that deterioration rate and quality loss reached the highest values at 30°C (Table 2). However, a non-linear pH increase was detected. Albumin buffering ability against pH changes is the weakest and appears to rapidly increase in the first few days of storage, as reported by other researchers (Karoui et al., 2006).

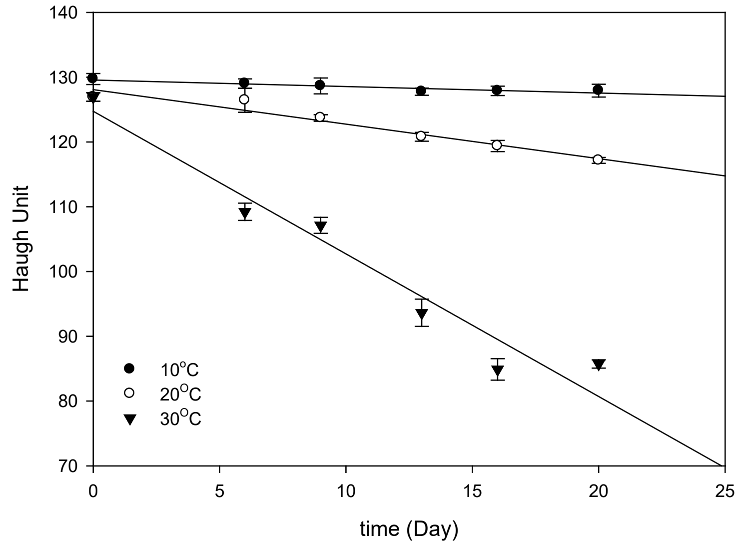

Figure 2. Haugh unit results of the egg samples stored at 10, 20 and 30°C

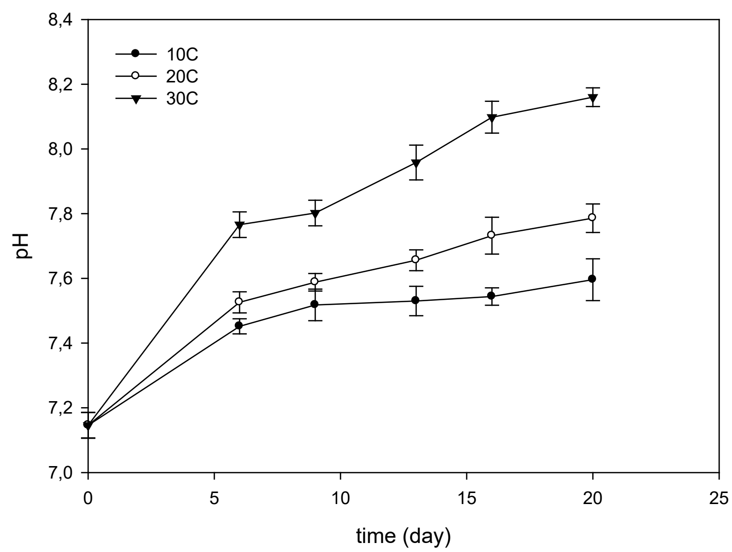

Figure 3. pH changes of egg samples stored at 10, 20 and 30°C

Area Change Results of the Egg Albumen and Yolk

When a stale egg is cracked, the yolk flattens out, usually gathering on one side, and the thick albumin around it thins out, causing a large area of albumin to collapse and flatten, forming a wider arc of liquid (Egg, 2000). Area variations of egg white and egg yolk samples were examined and shown in Figures 4 and 5, respectively. Degradation rate constants for albumin and yolk areas, determined using the zero-order rate equation, are tabulated in Table 2. Another study also detected the same increasing results for albumin (Karoui et al., 2006). The rate of increase was directly proportional to the temperature change. The highest degradation rate was recorded at a storage temperature of 30°C in both the albumin and yolk areas.

Albumen Index and Yolk Index Determination

After the egg was broken on a flat surface, the albumin and yolk index were determined and graphed using the height and diameter of the albumen and yolk (Figure 6 - 7). Both white and yolk indexes had a decreasing pattern. This may be attributed to the inverse relationship between index values and yolk and albumin area during storage. However, there was a direct proportionality between yolk and albumin height and index values. Similar observations were also stated by some other authors (Kahraman Dogan and Bayindirli, 1996).

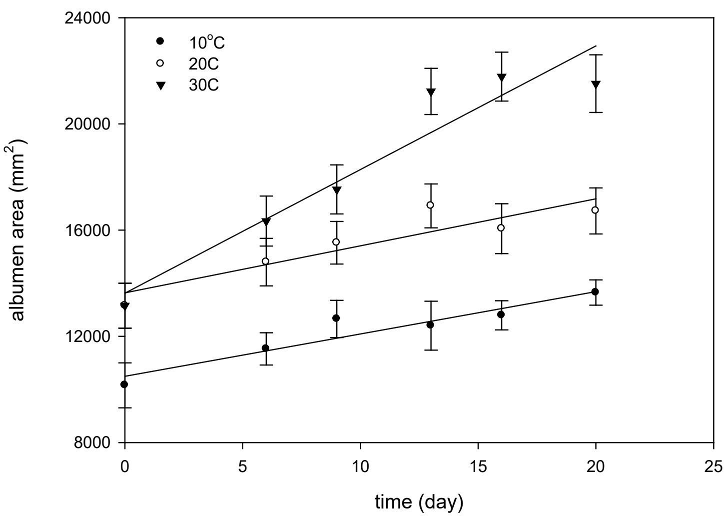

Figure 4. Changes in albumen area of egg samples stored at 10, 20 and 30°C

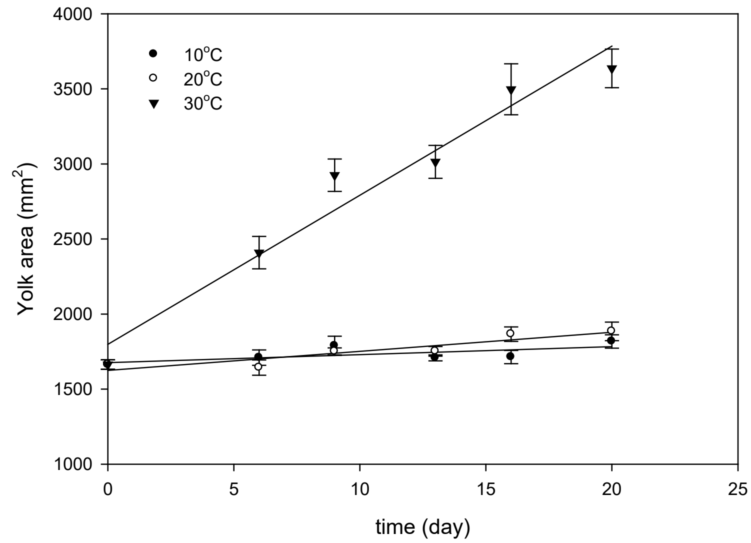

Figure 5. Changes in the yolk area of egg samples stored at 10, 20 and 30°C

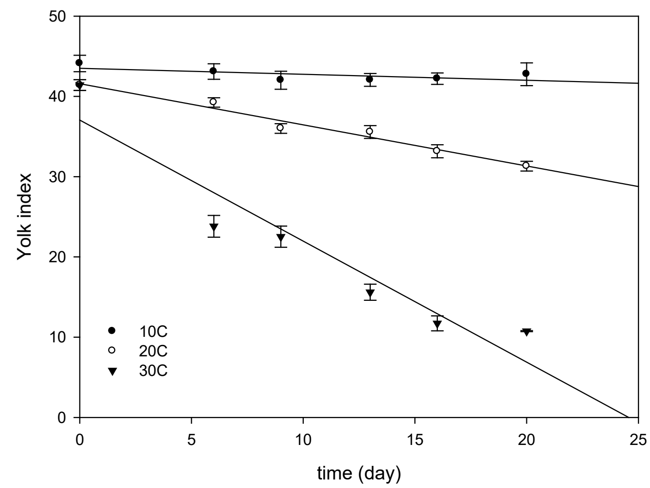

Figure 6. The yolk index of egg samples at 10, 20 and 30°C

Temperature Effect on Egg Quality

First, the natural logarithms of the rate constants were plotted against the inverse of absolute temperature, and then the straight-line relationship gave an Arrhenius-type behaviour. Table 3 shows egg samples' calculated activation energies (EA) for different quality parameters (weight change, Haugh Unit, pH, albumen and yolk area, albumen and yolk index). In the literature, activation energy is defined as the evaluation of the sensitivity of foodstuffs to temperature (Labuza, 1982). It was determined that the sensitivity of egg quality parameters to temperature decreased in the order of Haugh Unit, yolk index and area, white index and area, pH and weight. Haugh Unit is the most sensitive among all these because Haugh Unit is a relationship between the weight of the eggshell and flux height; also, both parameters are affected by storage temperature. Egg yolk is more affected by temperature changes than white. This may be due to the higher solids concentration in the egg yolk. Therefore, as the egg ages, water enters the white, causing the yolk to increase in size and become less viscous.

Table 3. Calculated activation energies of the egg samples at each test parameter

Quality Parameter	Activation Energy (kcal/mole)	R^2
Weight Change	3190.630	0.921
Haugh Unit	48467.396	0.933
pH	7472.829	0.996
The Area of Albumen	9136.013	0.994
The Area of Yolk	24776.768	0.938
Albumen Index	13216.958	0.841
Yolk Index	25722.170	0.979

(R^2: coefficient of determination)

Shelf-life Determination Due to Different Quality Parameters

Two important parameters were determined according to the temperature dependence on the activation energy. Haugh unit is the most sensitive parameter to temperature (Kemps et al., 2006; Kul & Şeker, 2004). Yolk index, yolk area, albumen index, and albumen area are the next sensitive parameters after the Haugh unit. However, when the indices and Haugh unit parameters were checked, the same parameter, height, was observed. Therefore, if the Haugh unit is chosen as a shelf-life determination parameter, the area, height, and weight values should be considered simultaneously. Since pH is an important indicator of egg deterioration (Kemps et al., 2006), the changes in pH values were chosen as the second important parameter for measuring the egg's shelf life.

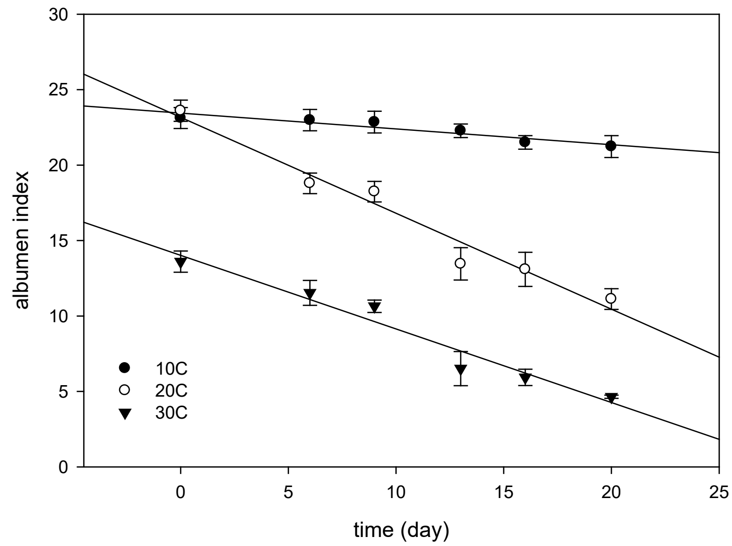

Figure 7. Albumen index of egg samples at 10, 20 and 30°C

If the end of the experiment is considered the end of the consumer's acceptance of the egg, the remaining quality parameters appear to be different in percentage. In order to decide the shelf life, an endpoint value must be assigned. Since there was no criterion for any egg quality parameter or egg shelf-life determination, it was assumed that 14% of the Haugh unit values were lost and 86% remained. For the case of pH value, a similar approximation was used also. Hence, the final quality parameter "A" in Equation 4 for the Haugh unit can be considered as "0.86Ao", and the final quality parameter "A" for the pH parameter can be considered as "(1/0.86) A_o". Because the samples' pH increased during storage, the highest pH value for our sample was calculated as 8.3 (≈ 7.14*1/0.86) and used in the calculation of values in Table 4. The shelf life of the egg samples at different temperatures was determined by substituting the new values obtained into the expression of the zeroth order ratio (Table 4-A). It was found that according to the Haugh Unit, at 10°C, the shelf life of the egg studied was found to be 238 days (Table 4-A). It was questionable to accept. Hence, it is clear that the most important step in shelf-life estimation for any food is assigning the most important parameter that must be followed and then assigning an endpoint at which the food is no longer acceptable. Then, instead of 0.86, 0.90 was inserted in the calculations, and the obtained shelf-life values were also listed in Table 4-B. It was found that a change from 0.86 to 0.9 caused such a huge change in shelf life.

Table 4. Calculated shelf-life values of the eggs using changes in Haugh Unit and pH values at 10, 20 and 30°C

	Shelf life (d)					
	A Temperature (°C)			B Temperature (°C)		
	10	20	30	10	20	30
Haugh Unit	238.9	28.9	6.9	167.2	24.3	5.9
pH	58.3	38.7	24.2	38.2	25.3	15.8

Conclusion

In this study, the following results were obtained by investigating egg quality parameters, their temperature sensitivity and egg shelf life: It was understood from the results that as shell eggs age, significant physical changes occur in the yolk, white, egg weight and shell colour. During the ageing process, the flocked area and pH of the whole egg increased, while the weight and flock height of the shell egg decreased. Some of the egg quality parameters, such as egg weight, pH, Haugh Unit, colour, white area and white height, showed zero order of degradation kinetics. Lightness (L) and redness (a) can indicate colour quality. While HU was found to be the most sensitive parameter to temperature, weight change was the least affected parameter. It has been understood that pH is an important parameter in determining shelf life. Egg shelf life decreased as the storage temperature increased. In order to estimate shelf life, especially for perishable food products, the most critical step is defining the value of the deterioration mechanism causing food not to be consumable. Any misleading in the calculation will cause either the loss of food itself, though it still has value, or the loss of food safety.

Compliance with Ethical Standards

Conflict of interest: The author(s) declares that they have no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: The authors declare that this study does not include experiments with human or animal subjects, so ethics committee approval is not required.

Data availability: Data will be made available on request.

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Disclosure: -

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