ORIGINAL ARTICLE

Effect of Temperature on Chicken Egg Quality Available at Grocery Stores

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ABSTRACT

Introduction: Ever expanding nutritional needs of world population has been compensated by poultry products especially good quality eggs. Chicken egg is a complete, easily accessible, and cheaper source of proteins obtained from animal source. **Objectives:** The objective of this work was to assess the effect of temperature on different quality parameters of poultry eggs. **Methodology**: A total of 50 unfertilized eggs were purchased from poultry farm and grocery stores in winter and 50 in summer from the same farm and stores. Geometric parameters such as weight (W), length (L), and breadth (B) of individual eggs was measured. Formulae derived from geometry of ovoid were applied to measure volume (V), surface area (SA), shape index (SI) and geometric mean diameter (GMD) of eggs. The eggs were broken on flat surface. Albumen height (AH), albumen length (AL), albumen width (AW), yolk height (YH), diameter (YD) and pH of egg pulp were measured. Eggshells were dried and their weight was measured. Different formulae were applied to calculate pulp ratio, albumen index (AI), yolk index (YI) and haugh units (HU). **Results**: Independent sample *t* test was used to compare the geometric and internal egg quality parameters of summers and winter. There was insignificant difference in size, weight, and length of eggs, albumen height, yolk height, breadth, pulp ratio as well as pH of egg pulp ($p>0.05$). The calculated parameters during summer e.g. SI, SA, V, and GMD were 77.22± 1.93, 73.03± 7.22, 59.05±8.6 and 4.82±0.25 respectively. The SI, SA, V and GMD during winter were 73.66±1.74, 70.02 ± 2.86, 55.09 ±3.36 and 4.70 ±0.10 respectively. There was no significant effect of seasonal variation on geometric parameters. Internal egg quality parameters like AI, YI and HU during winter were 5.02%±1.8, 61.57%±14.9 and 68.51± 11.50 respectively whereas during summer AI, YI, HU were 4.08%±2.09, 57.87%±10.17 and 67.91±17.35 respectively. The pH of egg pulp was 7.54 in winter and 7.73 in summer. **Conclusion**: Geometric parameters showed insignificant differences in weight, and size but internal egg qualities were within normal range.

Keywords: Temperature, egg albumen, geometric parameters, egg quality, haugh score

INTRODUCTION

Ever expanding nutritional needs of world population has been compensated by poultry products especially good quality eggs. Chicken egg is a complete, easily accessible and cheaper source of proteins obtained from animal source. Pakistani population consumes meat 12.4 kg, milk 87.2 litter, eggs 2 dozen, and fish 3.5 kg/capita/year respectively to fulfill protein requirement. (1) Consumption of egg by pregnant women gives healthy birth outcomes. Eggs added in children diet promotes physical and brain development. (2) In addition to use in fresh meals prepared in our kitchens and restaurants eggs are consumed by food companies in their commercial recipes. Nature has enclosed all the essential ingredients in an ellipsoidal vessel of 50-60 cm. (3) An egg comprises of eggshell 9.5%, egg white 61.5% and egg yolk 29%. (4) In biological terms hen egg is a living cell which can produce a new life identical to its parents if ambient environment is provided.

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[(5)]The internal egg quality is dependent upon multiple factors: health of animals, natural factors, tainting, egg age, storage, temperature, humidity and handling during collection and transportation. [(4)] Chicken eggs have thousands of pores which can cause excessive water loss resulting in dehydration and loss of albumen quality. [(6)] There are extremes of temperature (4ºC-45 ºC) during summer and winter as well as poor control of environmental conditions for chicken eggs. The eggs are transported by open vans from poultry farms to the grocery shops and bakeries. The chain of supply has neither temperature regulation systems nor the procedure of 3-digit egg marking is being practiced by most of the poultry farmers. Grading of chicken eggs is carried out on the basis of size for consumer's convenience. It may be more economical and appropriate if eggs are graded based on mass. [(7)] In the light of poor temperature control and grading on the basis of size, it was imperative to evaluate the external as well as internal egg quality for determination of egg freshness. The aims of the present study were: [(1)] To examine the quality of eggs consumed as kitchen commodity. [(2)] To determine the effect of temperature on external and internal egg quality.

METHODOLOGY

The experiment was conducted at Multidisciplinary Laboratory run by Allied Health Sciences and Azra Naheed Medical College, Superior University, Lahore. A total of 100 unfertilized eggs were collected and examined in two phases. The protocol was perused from the Head of Food Sciences and Nutrition Department in December 2021. Different rural and urban grocery stores were marked randomly, and chicken eggs were collected. Fresh eggs were collected from a poultry farm and treated as control group. The same poultry farm and grocery shops were approached to collect second sample in June 2022. Deformed, blood stained or cracked eggs were excluded during selection. All the samples were examined on the same day. Weight of every egg was measured to 0.01 accuracy by digital balance electronic balance BS-600H). Moreover, vernier caliper of 0.05mm precision was used to measure length and maximum breadth of individual egg. The formulae derived from the geometry of ovoid were applied to V, SA, SI and GMD of eggs.

i- $GMD = (LB^2)^{1/3}$. [(7)]
ii- $V = (0.6057-0.0018B) L*B^2$. [(8)].
iii- $SA= (3.155 - 0.0136L+0.0115B) L*B$. [(8)]
iv- $SI = (B/L) x100$.

Where: B= breadth of egg; L= length of egg.

The eggs were broken on a flat surface and allowed to settle for 3 minutes. The AL, AB and AH of egg white were noted. Similarly, the YB and YH were measured. The freshness of eggs was determined by applying the methods reported by different workers to calculate haugh units HU), albumen index (AI) and yolk index (YI).

AI = (albumen height/ long diameter+ short diameter of albumen/2) x100). [(9)]

YI = (yolk height/yolk diameter/2) x100. [(9)]

$HU= 100 \times (\text{Log}_{10}\, h - 1.7\, w^{0.37} + 7.6)$ [(10)]

HU ranges from 0-130. The higher the value of HU, the better-quality freshness of sample. Category AA $\geq$72, A 71-60, B 59-31 C $\leq$ 30. Category C is unfit for human consumption. [10] The eggshells were dried and weighed to calculate the egg pulp (EP) and shell/pulp ratio.

EP= Egg W- Egg Shell weight g

Ratio= EP/W x 100 %. The higher the EP ratio better the egg quality.

Egg yolk and albumen were beaten thoroughly with eggbeater and pH was measured with "pH Tester PH-107". [11] Mean and standard deviation of all groups were calculated. The formulae derived from the geometry of ovoid were applied to measure volume and surface area of eggs. Measurements of geometric mass models was accomplished by independent sample t test. Microsoft Excel Worksheet of Window 2013 was used to calculate Haugh units, yolk, and albumen index. Statistical Package for the Social Sciences (SPSS) version 21 was used to evaluate the data. P-value was calculated with 5% significance level.

RESULTS

The geometric parameters and internal egg quality parameter of eggs collected in summer and winter were compared by using independent *t* test. The geometric parameters, W, L, B, eggshell weight and pulp ratio in summer were 57.0g, 5.71cm, 4.4cm, 6.02g and 89.681% respectively as compared to 56.5g, 5.74cm, 4.22cm, 5.83g and 89.412% in winter, Table 1. The difference between W, L and eggshell weight of summer and winter groups was insignificant (p-value >0.05), however, the B of eggs in winter was significantly higher than summer group (p-value=0.00).

Table-1 Geometric Parameters of Chicken Eggs Collected in Different Seasons:

SEASONS	**GROUPS**	W g)	L cm)	B cm)	Shell g)	Pulp Ratio %
Winter	Control n=10)	54.17	5.67	4.16	6.14	87.485
	Urban n=20)	57.42	5.66	4.33	5.6	89.523
		60.3	5.87	4.22	5.9	89.666
	Rural n=20)	53.62	5.58	4.10	6.22	90.221
		57.10	5.96	4.34	5.3	90.191
	Mean	56.518	5.74	4.22	5.832	89.412
	SD	2.69	0.14	0.10	0.383	1.135
Summer	Control n=10)	53.90	5.64	4.33	6.76	88.665
	Urban n=20)	60.80	5.50	4.39	6.37	90.224
		59.90	5.94	4.48	6.19	90.216
	Rural n=20)	63.40	6.04	4.68	6.20	88.399
		46.90	5.41	4.12	4.60	90.718
	Mean	56.98	5.71	4.40	6.02	89.681
	SD	5.92	0.25	0.18	0.74	1.042

Where: W=egg weight, L=egg length, B=egg breadth, n=100 intact eggs.

The internal egg quality parameters, AL, AW, AH, YD and YH and pH were 7.76cm, 7.13cm, 0.80cm, 4.86cm, 1.71cm and 6.96 respectively in summer control group and 9.48cm, 7.55cm, 0.68cm, 4.07cm, 1.52cm and 6.53 in winter control group. In the eggs collected from grocery stores, these were 11.6cm, 9.5cm, 0.5cm, 4.6cm, 1.3cm and 7.81 in winter and 14.3cm, 12.0cm, 0.56cm, 4.6cm, 1.34cm and 7.66 in summer respectively. All parameters were significantly better in control group as compared to grocery stores group in both summer and winter. AL, AW and pH were significantly higher in summer than winter but there was no significant difference in AH, YW and YH of summer and winter groups, Table 2.

Table-2 Internal egg quality parameters of chicken eggs in taken in summer and winter:

SEASONS	GROUPS	AL (cm)	AB (cm)	AH (cm)	YB (cm)	YH (cm)	pH
Winter	Control n=10)	9.582	7.655	0.672	4.070	1.520	6.533
	Urban n=20)	13.536	12.164	0.318	5.045	1.040	7.850
		12.433	10.258	0.475	4.382	1.425	8.225
	Rural n=20)	9.98	6.99	0.6	3.986	1.65	7.15
		10.625	8.775	0.613	5.025	1.438	8.031
	Mean	11.643	9.546	0.501	4.609	1.388	7.814
	SD	1.415	1.9031	0.118	0.447	0.220	0.405
Summer	Control n=10)	7.760	7.130	0.80	4.860	1.71	6.96
	Urban n=20)	13.08	10.11	0.69	4.71	1.71	7.94
		12.88	9.8	0.66	4.9	1.29	7.81
	Rural n=20)	16.03	15.09	0.32	4.73	1.18	7.45
		15.44	13.01	0.357	4.19	1.18	7.465
	Mean	14.357	12.002	0.506	4.632	1.34	7.666
	SD	1.610	2.515	0.19	0.307	0.252	0.246

Where: AL= Albumen length, AB=Albumen breadth, AH= Albumen height, YB= Yolk breadth, YH= Yolk height, pH= pH of egg pulp.

The SI, SA, V and GMD were 73.34%, 67.44cm^2, 52.0cm^3 and 4.61cm respectively in winter control group whereas 76.77%, 70.48cm^2, 55.81cm^3 and 4.73cm were in summer control group; 73.66%, 70.02cm^2, 55.09cm^3 and 4.70cm in winter grocery stores group and 77.22%, 73.03cm^2, 59.05cm^3 and 4.82cm in summer grocery stores group respectively. The AI, YI and HU during winter control group were 7.81%, 74.69% and 83.53; in winter grocery stores group were 5.02%, 61.57% and 68.51; in summer control group were 10.75%, 70.37% and 91.21; and in summer grocery stores group were 4.08%, 57.87% and 67.91 respectively. Table 3. The difference in albumen index between control groups and mean of rural and urban group was 35% in winter and 62% in summer. For yolk index, this difference was 17.56% in winter and 17.76% in winter. For Haugh Unit, this difference was 15.02% in winter and 25.55% in summer.

Table-3 Geometric and internal quality parameters derived by formulae:

SEASONS	GROUPS	SI (%)	SA (cm^2)	V (cm^3)	GMD (cm)	AI (%)	YI (%)	HU
Winter	**Control n=10)**	73.34	67.44	52.00	4.61	7.81	74.69	83.53
	Urban n=20)	76.51	70.75	56.11	4.74	2.48	41.23	50.16
		71.88	70.34	55.31	4.71	4.19	65.04	67.41
	Rural n=20)	73.45	65.53	49.81	4.54	7.07	82.79	79.11
		72.81	73.47	59.12	4.82	6.33	57.23	77.37
	Mean	73.66	70.02	55.09	4.70	5.02	61.57	68.51
	SD	1.559	2.756	3.252	0.098	1.969	14.416	11.927
Summer	**Control n=10)**	76.77	70.48	55.81	4.73	10.75	70.37	91.21
	Urban n=20)	79.82	70.31	55.83	4.73	5.95	72.61	82.79
		75.42	76.17	62.60	4.92	5.82	52.65	81.10
	Rural n-20)	77.48	81.18	68.98	5.10	2.06	49.89	46.35
		76.16	64.48	48.81	4.51	2.51	56.32	61.39
	Mean	77.22	73.03	59.05	4.82	4.08	57.87	67.91
	SD	1.509	5.690	6.854	0.197	3.130	9.332	16.392

Where SI (%) = Shape Index, SA= Surface Area, V= Volume of egg, GMD= Geometric Mean Diameter, AI=Albumin Index, YI=Yolk Index, HU= Haugh Unit

DISCUSSION

Pakistan has a diverse climate and temperature range, from an average of 9°C in December to 39°C in June. This study was carried out in the suburbs of Lahore. The grid reference of Lahore city is 31° 34' 55.3620'' N and 74° 19' 45.7536'' E. [12] Geometric parameters of eggs provide precise quantification of egg profile. This information is used as powerful tool for ecology, morphology and biological characteristics in different animal species. Many parameters are significantly affected by seasonal variations. The control group of summer and winter did not show any significant effect of temperature. A number of other factors related to chicken e.g., age, diet, atmosphere etc. may affect the quality of eggs. However, among the eggs collected from grocery stores, there was a significant decrease ($p<0.05$) in all internal quality parameters as compared to controls, and this difference was more marked in summer samples. Among the geometric parameters, the difference in W was insignificant. Most of the eggs were of 57g, large size per dozen weight greater than 680g). Some other studies had similar egg weights of 62 g [10] and 59g, [13} while many workers had reported smaller egg weight 41 g [14] and 38 g. [15]

Egg SI reflects the eggshell shape such as sharp (<72%), standard (72% to 76%) and round (>76%). (16) The SI of winter eggs was standard (73%) but round in summer (77%). Kumar et al. (2022) also mentioned the shape index of 74% in Aseel eggs. (14) Aseel breed is mostly found in subcontinent. The SA measured by composite equations ranges from 70-74 cm^2(17). The mean SA in winter and summer were 70.02 cm^2 and 73.03 cm^2 respectively. Seasonal changes had insignificant effect on geometric parameters. Similarly, V of eggs ranged between 52-59 cm^3. There was no effect of seasonal variation on egg V. There was no significant difference in the GMD of eggs during summer and winter. It was 4.70cm in winter and 4.82cm in summer. This is in accordance with findings of Cunningham *et al.,* (1960), and Sauveur and Picard, (1987), which states that in the absence of other factors, the seasonal changes have no impact on fresh egg quality. (18, 19) The geometrical parameters, such as egg V, SA, etc., are nowadays being used in numerical simulation of the eggshell behavior under mechanical loading (20) or under thermal treatments (21), (22). We can also predict egg qualities, breeding egg hatchability, and hatchling size using these parameters. (23) Normal egg W has 9-12% eggshell and 88-91% pulp. We observed 89.4% pulp in winter and 89.6% in summer. Hussain et al. (2018) has reported 87.4% to 88.83% pulp in different breeds. (24) There was marked decrease in internal parameters, and changes were more in summer than winter.

In past, YI and AI were used as a parameter of egg quality. In our study, YI decreased significantly in grocery stores samples (61% and 57%) as compared to control groups (74% and 70%) in winter and summer respectively. However, the changes were equivalent in both seasons (17.56% and 17.76%) and the seasonal change in YI was insignificant. Samli et al. (2005) have reported reduction in YI at 29^{o}C from 44% to 39% and 32% after 5 and 10 days respectively. (10) Other studies have shown the YI of 36%-38% (14), 41% (24) and 45%. (25) We have observed that quality of albumen is the most sensitive parameter to seasonal variation. Albumen length, width and index were affected significantly in summer as compared to winter (p-value=0.000 in all groups). The AI of control groups were 7.81 & 10.75. Kumar, et al. (2021) reported AI 8.5 to 9.2, (14) while Hussain et al. (2018) calculated 7.9 AI in his study. (24) Egg consumer industries mostly rely on HU which was introduced by Raymond Haugh in 1937. (26) HU has replaced YI and AI. HU ranges from 0-130. The higher value indicates better egg quality. Haugh Unit <30 are grade C eggs and considered unfit for human consumption.

Haugh units in our control group of summer and winter were 91.21 and 83.53 respectively. This disparity in values may be dependent upon multiple factors like age, diet and housing capacity of sheds. The sample collected from grocery stores showed decreased HU. It was 67.91 (25.55% decrease) in summer and 68.51 (15.02% decrease) in winter as compared to controls. The quality of eggs decreased from AA grade of control group to A grade. Thus, it is the earliest sign of deterioration of egg quality and is strongly affected by seasonal change. Haugh unit has been most widely used for quality determination (25). Samli et al. (2005) has observed the decrease in HU from 91 to 40 with increasing time and temperature (10). Hussain et al. (2018) has reported 80-86 HU in his study. (24) Khan et al. (2013) observed the decrease in HU 83.1 to 75.9 within 8 days at 16 °C and 78% humidity. (15)

pH of egg contents is very sensitive sign of freshness. Prolonged storage will evaporate CO_2 through the pores in egg shell and increase pH. We have observed 6.53 and 6.96 pH in control groups. The pH of samples from grocery stores in winter and summer were 7.81 and 7.66 respectively. Pires et al. (2019) demonstrated storage at 20 °C for 8 weeks increase pH 9.18.[27] Adamski et al. (2017) also showed that change in pH is more pronounced with increased temperature whereas refrigeration delays the change in pH. [28]

No doubt storage at 5-7 °C can maintain the quality of eggs for longer time but consumption of electricity in hot weather will not keep it cost-effective for the consumers. It is hoped this exercise will give awareness about the freshness and quality of eggs.

This work gives an idea about the geometric values and freshness of the eggs but this information is deficient about caloric value and micronutrients like vitamins, minerals, proteins and probiotics. The eggs were not marked with laying dates so duration of storage and its correlation with freshness couldn’t be determined.

CONCLUSION

Seasonal variation had insignificant difference on geometric parameters. Internal egg quality was A grade in both groups. The pH was most sensitive parameters to analyze the freshness of eggs and change in Haugh Unit was most significant parameter to detect seasonal variation.

REFERENCES

1. Miranda JM, Anton X, Redondo-Valbuena C, Roca-Saavedra P. Rodrigue z JÁ, Lamas A, Franco CM, Cepeda A. Egg, and egg-derived foods: Effects on human health and use as functional foods. Nutrients. 2015;71):706-29.
2. Lutter CK, Iannotti LL, Stewart CP. The potential of a simple egg to improve maternal and child nutrition. Maternal & child nutrition. 2018 Apr;14: e12678.
3. Abeyrathne EN, Lee HY, Ahn DU. Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—A review. Poultry science. 2013 Dec 1;9212):3292-9.
4. Bovera F, Iannaccone F, Piccolo G, Meo CD, Russo F, Piscitelli D, Attia YA, Hassan SS, Nizza A. Effect of group size on performance and egg quality of laying hens during 20 to 36 weeks of age. Italian Journal of Animal Science. 2014 Jan 1;131):3148.
5. Fink DJ, Caplan AI, Heuer AH. Eggshell mineralization: a case study of a bioprocessing strategy. MRS Bulletin. 1992 Oct;1710):27-31.
6. Carvalho DC, Silva AW, Gois GC, Moraes EA, Antunes KV, Queiroz MA, Souza RD, Pinheiro SR, Taran FP. Quality of free-range and commercial eggs subjected to different storage periods and temperatures. Ciência Animal Brasileira. 2022 Mar 30;23.
7. Rashidi M, Gholami M. Prediction of egg mass based on geometrical attributes. Agriculture and Biology Journal of North America. 2011;24):638-44.
8. Narushin VG. Egg geometry calculation using the measurements of length and breadth. Poultry science. 2005 Mar 1;843):482-4.
9. Yannakopoulos AL, Tserveni-Gousi AS. Quality characteristics of quail eggs. British Poultry Science. 1986 Jun 1;272):171-6.

10. Samli HE, Agma A, Senkoylu N. Effects of storage time and temperature on egg quality in old laying hens. Journal of Applied Poultry Research. 2005 Oct 1;143):548-53.
11. Lapao C, Gama LT, Soares MC. Effects of broiler breeder age and length of egg storage on albumen characteristics and hatchability. Poultry science. 1999 May 1;785):640-5.
12. Sajjad SH, Batool R, Qadri ST, Shirazi SA, Shakrullah K. The long-term variability in minimum and maximum temperature trends and heat island of Lahore city, Pakistan. Science International. 2015 Mar 1;272):1321-5
13. Eke MO, Olaitan NI, Ochefu JH. Effect of storage conditions on the quality attributes of shell table) eggs. Nigerian Food Journal. 2013 Jan 1;312):18-24.
14. Kumar M, Dahiya SP, Ratwan P, Sheoran N, Kumar S, Kumar N. Assessment of egg quality and biochemical parameters of Aseel and Kadaknath indigenous chicken breeds of India under backyard poultry farming. Poultry Science. 2022 Feb 1;1012):101589.
15. Khan MJ, Khan SH, Bukhsh A, Abbass MI, Javed M. Effect of different storage period on egg weight, internal egg quality and hatchability characteristics of Fayumi eggs. Italian Journal of Animal Science. 2013 Jan 1;122): e51.
16. Sarica M, Onder H, Yamak US. Determining the most effective variables for egg quality traits of five hen genotypes. International journal of agriculture and biology. 2012 Apr 1;142).
17. Hamilton RM. A comparison of the various equations published for the estimation of characteristics of hen’s eggs, the importance of reporting the compression rate for shell strength measurements, and distinction between egg specific gravity and density. World's Poultry Science Journal. 2022 May 11:1-20.
18. Cunningham FE, Cotterill OJ, Funk EM. The Effect of Season and Age of Bird: 1. On Egg Size, Quality and Yield. Poultry Science. 1960 Mar 1;392):289-99.
19. Sauveur B, Picard M. Environmental effects on egg quality. In ‘Egg Quality—Current Problems and Recent Advances’.Eds RG Wells and CG Belyavin.) pp. 219–34.
20. Perianu C, De Ketelaere B, Pluymers B, Desmet W, DeBaerdemaeker J, Decuypere E. Finite element approach for simulating the dynamic mechanical behaviour of a chicken egg. Biosystems engineering. 2010 May 1;1061):79-85.
21. Sabliov CM, Farkas BE, Keener KM, Curtis PA. Cooling of shell eggs with cryogenic carbon dioxide: a finite element analysis of heat transfer. LWT-Food Science and Technology. 2002 Nov 1;357):568-74.
22. Denys S, Pieters JG, Dewettinck K. Computational fluid dynamics analysis of combined conductive and convective heat transfer in model eggs. Journal of Food Engineering. 2004 Aug 1;633):281-90.
23. Severa L, Nedomová Š, Buchar J, Cupera J. Novel approaches in mathematical description of hen egg geometry. International Journal of Food Properties. 2013 Oct 3;167):1472-82.
24. Hussain J, Javed K, Hussnain F, Musarrat S, Mahmud A, Mehmood S. Quality, and sensory attributes of eggs from different chicken genotypes in Pakistan. JAPS: Journal of Animal & Plant Sciences. 2018 Dec 1;286)

25. da Silva Pires PG, Bavaresco C, Moraes PD. Hundred years of knowledge on table egg refrigeration. World's Poultry Science Journal. 2022 Aug 21:1-3.
26. Haugh RR 1937), 'The Haugh unit for measuring egg quality', US Egg Poultry Magazine, 43, 522–555, 572–573.
27. Pires PG, Pires PD, Cardinal KM, Leuven AF, Kindlein L, Andretta I. Effects of rice protein coatings combined or not with propolis on shelf life of eggs. Poultry Science. 2019 Sep 1;989):4196-203.
28. Adamski M, Kuźniacka J, Kowalska E, Kucharska-Gaca J, Banaszak M, Biegniewska M. Effect of storage time on the quality of Japanese quail eggs coturnix coturnix japonica). Polish Journal of Natural Sciences. 2017;321).