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The effect of storage temperature and time on the quality traits and evaluation of fatty acid profile of backyard produced eggs

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Abstract
This study aimed to assess the impact of temperature and period of storage on the quality traits of backyard-produced eggs commonly found in villages of Kerala as well as examining their fatty acid profile. Eggs were divided into two groups and stored at room temperature and in a refrigerator. Evaluation of quality traits—Albumin Index (AI), Yolk Index (YI), Eggshell thickness, Yolk colour and Haugh unit (HU)—was conducted at 0, 7, 14, and 21 days. Fatty acid profile of an egg sample estimated and compared to that of the commercially produced egg.
Results demonstrated a rapid decline in internal quality within the first week for room-temperature-stored eggs. In contrast, refrigerated eggs-maintained freshness and quality traits consistently until day 21. Albumin Index, Yolk Index, and Haugh unit show significant reduction in room temperature kept eggs compared to refrigerated eggs (<0.001). Backyard Eggs demonstrate higher levels of Monounsaturated Fats (MUFA), and a lower Omega-6 to Omega-3 (ω-6/ω-3) ratio, suggesting a more balanced intake of essential fatty acid. These findings emphasize the significance of refrigeration in preserving the quality of backyard eggs over an extended storage period compared to room temperature and highlight potential health benefits associated with eggs from backyard sources.

Keywords: Backyard-produced eggs, temperature, storage period, quality traits, refrigeration, fatty acid profile, health benefits

Introduction
Eggs stand as a vital nutritional source abundant in high-quality protein, vitamins, and minerals. Moreover, they can provide essential fatty acids, fats to replace carbohydrates in diets, and induce satiety (Brand-Miller & Petocz., 1995; Vander Wal *et al*., 2005) [7, 30]. In India, egg production spans across commercial farms and backyard rearing systems in villages. Preserving the internal and external qualities of eggs until consumption is crucial in terms of public health and effective marketing. While numerous studies have focused on the quality changes of commercially produced eggs under various storage conditions, research on eggs sourced from backyard systems remains limited.
With increasing temperature, biochemical processes accelerate, resulting in carbon dioxide loss, a rise in internal pH (Alsobayel & Albadry, 2011) [5], and the release of water in albumin affecting its viscosity, while the diffusion of water into yolk diminishes its firmness (Akyurek *et al*., 2009) [4]. These alterations render eggs more susceptible to microbial contamination, whereas refrigeration delays this process. This study evaluates the speed of quality changes in two common village storage conditions: room temperature and refrigeration. The findings can be used for public awareness and marketing.
Additionally, eggs are a source of fatty acids. The monounsaturated fatty acids and Omega 6 to Omega 3 ratio in the diet are important in terms of cardiovascular diseases and inflammatory conditions. This study also evaluates the fatty acid profile of backyard eggs and compares it with commercially produced eggs.

Materials and Methods
Sample Collection: Fresh eggs were sourced from four different households in the Vandazhi Grama Panchayath, Palakkad District, Kerala.

The feeding practices slightly vary among these households. The birds were allowed to free-range during the daytime and were supplemented with a diet consisting primarily of rice bran, rice, wheat, coconut and groundnut oil cakes, kitchen waste, and leftover food.

Experimental Design: A total of 84 visually selected fresh eggs (not more than 4 days old) were obtained and divided into two treatment groups. Each group, comprising 36 eggs, was subjected to distinct storage conditions: one group was stored at room temperature, while the other was refrigerated at 5°C. Egg quality parameters were assessed at day 0 (pre-treatment), day 7, day 14, and day 21. Twelve samples from each treatment group were randomly selected for evaluation at each time interval.

Measurement Techniques: Egg weight was measured using a precise electronic balance with 0.01g accuracy. Eggs were broken onto a glass plate, and the height of the yolk and albumin were measured using Ame's tripod stand micrometer. Digital callipers were used to measure parameters such as length and width of the albumin and yolk, as well as the diameter of the yolk. Eggshell thickness was determined by averaging measurements from three regions: broad, narrow, and middle parts of individual eggshells using Screw gauge. Yolk colour was evaluated using a DSM Yolk Colour Fan, with values ranging from 1 (pale yellow) to 16 (dark orange).

Calculation of Indices: The Albumen Index was derived from the ratio of albumen height to albumen diameter. Similarly, the Yolk Index was calculated using the ratio of yolk height to yolk diameter. The Haugh Unit (HU) was computed using the formula: $HU = 100 * \log (h + 7.57) - (1.7 * w^{0.37})$, where 'h' represents albumen height in millimetres and 'w' signifies egg weight in grams.

Statistical Analysis: The obtained results underwent analysis of variance (ANOVA), and differences were assessed using 't' test. Statistical analysis was conducted using SPSS version 24.0.

Fatty Acid Profile of the Egg Yolk Analysis: A representative sample from the backyard produced eggs as well as a sample of commercially produced egg were analysed for its fatty acid profile at Animal Feed Analytical and Quality Assurance Laboratory, Namakkal using Gas Chromatography as per (O'Fallon *et al*., 2007) [22]. The values were compared and contrasted.

Results and Discussion

The effect of storage period and temperature on the selected egg quality traits are summarised in table No. 1

Table No. 2 provides the relative values of fatty acid profile analysis for backyard and commercial eggs.

Table 1: The effect of storage period and temperature on the selected egg quality traits are summarised

Variation in Egg Quality Parameters according to Time and Temperature															
	Albumin Index			**Yolk Index**			**Yolk Colour**			**Shell Thickness**			**Haugh Unit**		
Time	**Room**	**5 °C**	**p value**	**Room**	**5 °C**	**p value**	**Room**	**5 °C**	**p value**	**Room**	**5 °C**	**p value**	**Room**	**5 °C**	**p value**
0 Day	0.08ᵃ±0.0	0.08±0.0		0.37ᵃ±0.01	0.37±0.01		4.70ᵃ±0.21	4.70ᶜ±0.21		0.34±0.01	0.34±0.01		78.65ᵃ±3.36	78.65±3.36	
7 Day	0.05ᵇ±0.01	0.06±0.01	0.095	0.24ᵇ±0.01	0.35±0.02	0.000	5.67ᵃ±0.24	7.50ᵇ±0.73	0.024	0.35±0.01	0.34±0.02	0.591	64.23ᵇ±4.63	69.43±4.73	0.445
14 Day	0.02ᶜ±0.00	0.07±0.00	0.000	0.24ᵇ±0.02	0.37±0.02	0.000	7.64ᵇ±0.34	8.00ᵇ±0.33	0.462	0.32±0.01	0.35±0.01	0.002	56.20ᵇ±2.68	77.49±2.21	0.000
21 Day	0.03ᶜ±0.01	0.07±0.01	0.000	0.11ᶜ±0.03	0.37±0.01	0.000	7.40ᵇ±0.50	9.64ᵃ±0.36	0.001	0.34±0.01	0.35±0.01	0.822	53.99ᵇ±5.61	78.12±2.70	0.000
p value	0.000	0.478		0.000	0.860		0.000	0.000		0.093	0.562		0.001	0.220	

When comparing the effect of temperature on the quality traits at specific intervals, The Yolk Index (YI) significantly reduced (<0.01) in 7-day old eggs kept at room temperature (YI-0.24) compared to the refrigerated eggs (YI-0.35). Albumin Index (AI) and Haugh Unit (HU) did not show significant variation between these two groups at this point. Whereas, through day 14 and finally towards day 21 there was a highly significant (<0.01) progressive reduction found in the values of Albumin Index, Yolk Index and Haugh Unit in room temperature (AI - 0.03, YI- 0.11. HU-53.99) stored eggs than the eggs stored in the fridge (AI - 0.07, YI- 0.37. HU - 73.12)

With respect to interaction of time alone on individual treatment groups, there was a significant reduction (<0.01) in Albumin index and Yolk index with respect to (0 to 21) day ageing in eggs kept at room temperature (Day 0; AI - 0.08, YI - 0.37. Day 21; AI - 0.03, YI- 0.11). Whereas in refrigerated eggs these indices were almost steady though out the 21-day period without significant variation

The deterioration of Yolk Index, Albumin Index, and Haugh unit in room temperature observed in this study is in agreement with previous reports (Tona *et al*., 2004; Jones & Musgrove., 2005; Samli *et al*., 2005; Akyurek *et al*., 2009; Yimenu *et al*., 2017) [29, 12, 24, 4]. As an egg ages, biochemical processes, mainly carbonic acid breakdown occurs that deteriorate the quality of proteins, leading to the release of carbon dioxide and water. Consequently, albumin becomes more fluid, causing water to diffuse into the yolk, stretching the vitelline membrane (Karoui *et al*., 2005) [13] and flattening the yolk (Scott & Silversides, 2000; Silversides & Budgell, 2004) [26] (Huang *et al*., 2012) [33]. As per the egg grading system in the USA, eggs are categorized into AA (≥72), A (71–60), B (59–30), and C (<29) based on their HU (Haugh Unit) values. Eggs with HU values below 60 are deemed unsuitable for consumption (Nematinia & Abdanan Mehdizadeh, 2018) [21]. Similarly, the Yolk Index, indicating freshness, deems values above 0.38 as exceptionally fresh, 0.28 to 0.38 as fresh, and below 0.28 as regular quality (Aikpitanyi & Imasuen, 2023) [3].

In the present study, neither time nor temperature interaction caused consistent changes in the quality of eggshell thickness in either group throughout the considered period. This is in agreement with Adamski *et al*. (2017) [17] who reported unchanging physical characteristics and consistent shell thickness during storage, implying that the mineral compounds within the eggshell impart long-term stability and mechanical resilience to deformation. On the contrary, Maria *et al*. (2020) [16] showed, as the age advances eggshell thickness reduces in layers and storage in room temperature also causes further reduction in shell thickness in eggs from aged birds. Moreover, factors such as breed, bird age, and diet influence shell thickness with the most significant thinning occurring later in the laying period (Fathi *et al*., 2019) [10]. Eggshell thickness is also related to water loss according to environmental temperature and humidity (Veldsman *et al*., 2020) [31].

Additionally, in the present study, yolk color increased due to aging by an average of 2-3 points in both treatment groups from day 0 to 21. This is in agreement with previous studies (Lee *et al*., 2016) [15]. In contrast, others observed a significant reduction in yolk color due to aging (Jin *et al*., 2011; Martínez *et al*., 2021; Tjatur Nugroho Krisnaningsih *et al*., 2022) [11, 17, 28]. On the other hand, at lower temperatures, the current study found no significant changes in the Albumin Index, Yolk Index, and Haugh unit values until day 21. A similar result in Haugh unit was obtained by Jin *et al*. (2011) [11], while this differs from other studies where a noticeable but lesser reduction in these parameters was observed (Akyurek *et al*., 2009; Martínez *et al*., 2021) [4, 17], albeit not as extensive as in room temperature conditions. This discrepancy could be attributed to certain limitations in the current study compared to planned studies that specifically focus on particular breeds and ages. Such targeted studies ensure uniformity in egg qualities among selected samples, resulting in more consistent treatment outcomes. While the eggs collected for the present study displayed initial variations in breed, bird age, alterations in feeding practices, and differences in health status among households. These factors potentially influence individual egg characteristics such as size, albumin and yolk height, and yolk color (May & Stadelman., 1960; Bell *et al*., 2001; Martinez *et al*., 2012) [20, 18]. Consequently, inconsistencies in quality indices within groups and during treatment were anticipated. This obstacle might impede the precise measurement of trends in the considered indices, although an overall trend could still be identified.

Table 2: Fatty Acid Composition - Comparison between Backyard and Commercial Eggs

Fatty Acid %	Backyard Egg	Commercial Egg
Myristic Acid	0.91	0.05
Palmitic Acid	31.26	29.40
Stearic Acid	14.13	11.99
Behenic Acid	3.99	5.38
Oleic Acid	36.21	33.68
Palmitoleic Acid	4.24	4.18
Arachidic Acid	0.49	0.13
Linoleic Acid	6.24	10.35
Eicosapentaenoic Acid	0.37	0.79
Docosahexaenoic Acid	1.91	1.56
Linolenic Acid	0.21	0.11
Total SFA	50.29	46.82
Total MUFA	40.45	37.86
ω-6 FA	6.73	10.48
ω-3 FA	2.49	2.46
ω-6/ω-3	2.295	4.26
Total PUFA	9.22	12.94
Total UFA	49.67	50.8
PUFA/SFA	0.183	0.276
SFA/UFA	1.01	0.922

Backyard eggs show a higher total saturated fats (SFA) percentage of 50.29% compared to 46.82% in commercial eggs. Conversely, the total monounsaturated fats (MUFA) in backyard eggs amount to 40.45%, slightly exceeding the 37.86% found in commercial eggs. Regarding polyunsaturated fatty acids (PUFA), backyard eggs exhibit 9.22%, while commercial eggs show a higher PUFA content at 12.94%. Backyard eggs have notably lower Omega-6 fatty acids (ω-6 FA) at 6.73% versus 10.48% in commercial eggs. Meanwhile, the Omega-3 fatty acids (ω-3 FA) present marginal differences, with backyard eggs at 2.49% and commercial eggs at 2.46%. Additionally, the backyard eggs reveal a lower Omega-6 to Omega-3 ratio (ω-6/ω-3) of 2.295, while commercial eggs exhibit a higher ratio of 4.26.

According to Sarma *et al*. (2017) [25], the fatty acid composition of eggs gathered from local breeds in various regions of Tamil Nadu indicated SFA levels between 49.21 to 46.42, PUFA levels spanning from 19.19 to 5.7, and an omega-6 to omega-3 ratio ranging from 6.5 to 1.55. These findings align with the present study. However, in eggs from free-range and indigenous sources, Cherian *et al*. (2002), Polat *et al*. (2013) [23], and Kian & Gharooni (2016) [14] identified lower levels of saturated fatty acids (SFA) compared to the current study (34.6, 29.66, and 36.6, respectively). Additionally, Cherian *et al*. (2002) [8] and Polat *et al*. (2013) [23] noted higher levels of polyunsaturated fatty acids (PUFA) (18.1, 31.28), while Cherian *et al*. (2002) [8] and Kian & Gharooni (2016) [14] reported higher omega-6 to omega-3 ratios (28.9, 8.1) compared to the findings of the present study.

According to Massiera *et al*. (2010) [19] and others, excessive consumption of omega-6 polyunsaturated fatty acids (PUFA) and an imbalanced (ω-6/ω-3) ratio, typically between 10:1 and 20:1 in Western diets, contributes to the development of various health issues, including cardiovascular problems, cancer, inflammation, autoimmune diseases, disrupted brain growth, insulin resistance, depression, and premature aging (Bourre, 2005; Adkins & Kelley, 2010) [6, 2]. Similarly, Simopoulos (2010) suggested that aiming for a balanced ratio of 1:1 to 2:1 for (ω-6/ω-3) fatty acids in the diet promotes optimal health.

In the present study, backyard eggs demonstrate higher levels of monounsaturated fats (MUFA), potentially offering cardiovascular benefits by improving lipid profiles and reducing heart disease risk. Conversely, commercial eggs exhibit elevated polyunsaturated fatty acids (PUFA), particularly Omega-6 fatty acids, raising concerns due to the potential for excessive Omega-6 intake, which may contribute to inflammation and related health issues. Additionally, backyard eggs present a lower (ω-6/ω-3) ratio, suggesting a more balanced intake of essential fatty acids that could potentially reduce the risk of inflammatory conditions. In contrast, the higher (ω-6/ω-3) ratio in commercial eggs may lead to an imbalance, contributing to inflammation, cardiovascular diseases, and other health complications associated with exaggerated Omega-6 intake.

Although backyard eggs show a better Omega-6 to Omega-3 ratio, potentially aiding cardiovascular health, their storage quality might differ due to varied handling and diets. Conversely, commercial eggs may maintain consistent nutrient profiles and storage quality owing to controlled production methods.

Conclusion

This research illuminates the significant impact of storage temperature on the quality traits of backyard-produced eggs in Kerala. While room temperature storage led to a rapid decline in quality within the initial week, refrigeration-maintained freshness and traits consistently for 21 days ($p<0.001$ for Albumin Index, Yolk Index, and Haugh Unit). Moreover, the higher Monounsaturated Fats (MUFA) and balanced (ω-6/ω-3) ratio in backyard eggs compared to commercially produced ones suggest a healthier dietary fatty acid source. These findings underscore refrigeration's crucial role in preserving superior egg quality over extended periods and highlight potential health benefits associated with consuming eggs from backyard sources.

Conflict of Interests

There is no conflict of interest.

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