


# VARIABILITY IN PROXIMATE AND MINERAL COMPOSITIONS OF YOLK AND ALBUMEN IN EGGS KEPT UNDER DIFFERENT STORAGE CONDITIONS

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 Supporting Information

**ABSTRACT:** This investigation evaluated the effects of storage length and temperature on the proximate and mineral compositions of yolk and albumen (white) of chicken eggs. A total of 720 eggs were used in a 4 X 2 factorial experiment consisting of four (0, 7, 14 and 21) storage days and two (room and cold) storage temperatures. Data obtained were subjected to ANOVA. The results showed no significant effect of storage length on crude protein and ash contents of the egg parts whereas carbohydrate increased significantly with increasing storage length. Also, protein and fat contents of the yolk were largely influenced ( $p < 0.05$ ) by storage temperature but nutrients in the albumen did not differ significantly between the two storage temperatures. On the other hand, mineral compositions of the egg components did not vary noticeably by storage temperature but storage length influenced some minerals considerably. It was noted that variations in proximate and mineral contents of egg yolk and albumen under different storage conditions exist; so, storage conditions (length and temperature) must be given much attention when keeping eggs prior to consumption in order to ensure nutrient quality.

**Keywords:** Egg quality, Egg protein, Nutrients, Storage length, Storage temperature.

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## INTRODUCTION

Chicken eggs are superior in carbohydrates, proteins, easily digestible fats, ash (minerals) and vitamins (Huopalahti et al., 2007; International Egg Foundation, 2014); thus, eggs are one of the utmost important sources of animal protein that provide good nutrition for human and animals at the least cost (Menezes et al., 2009). They are also treasured as raw materials for making curative and beautifying (cosmetic) products per their gelling, foaming, emulsifying (Matt et al., 2009) and thickening abilities – which however are largely determined by their exterior and interior properties (Koelkebeck, 2003). Egg quality is composed of traits such as: cleanliness, weight, shell quality, freshness, yolk and albumen indexes, Haugh unit and chemical composition that influence its acceptability to consumers (Song et al., 2002).

Generally, the biological/genetic composition (Rizzi and Marangon, 2012; Aygun and Yetişir, 2014; Jahedi et al., 2020), age (Rizzi and Marangon, 2012; Aygün and Nariç, 2017) and management (King'ori, 2012; Grochowska et al., 2019) of layers in addition to the conditions of egg storage (time, temperature, humidity, air movement and handling) are common factors associated with the level of quality loss of the egg (Stadelman et al., 1995; Oliveira and Oliveira, 2013). Nys et al. (2004) reported that, eggs display very consistent composition with respect to their nutritional composition but may change at different periods and temperatures of storing them.

For instance, in a trial, eggs collected from various retail shops (which may be at different stages of storage) had crude protein contents of 8.64, 7.98, 7.60 and 7.78 g/egg (Attia et al., 2014). Ogunwale et al. (2015) also noticed highly significant effect of storage time on crude protein content in eggs from Black Bovan nera chickens; eggs stored for 0 day (11.45%) was significantly lower in protein than those stored for 7, 14, 21 and 28 days which was found to be 11.54, 11.60, 11.55 and 11.59% respectively. Comparably, Dudusola (2009) found out that quail eggs stored for day 0 (35.3%) and 4 (33.0%) were similar but significantly higher in crude protein than those stored up to day 7 (30.5%) and 21 (27.4%) and subsequently proposed that, quail eggs should be stored for 4 days at room temperature to maintain desired internal quality. Usually, eggs stored in cold condition for several months above 85% humidity or at any temperature under highly sufficient humidity and long storage time would spoil when exposed to warm temperature because conditions may be favourable for the penetration of micro-organisms (Seidler and Hilmi, 2003). However, packing eggs under modified atmosphere could increase their internal quality up to the 28<sup>th</sup> day of storage (Giampietro-Ganeco et al., 2015).

Information on the effect of storage temperature on nutritional composition of eggs is non-completed, limited or not readily accessible, whereas Dudusola (2009) suggested that refrigeration at low temperatures is the best storage condition for quail eggs. Freezing subjects microbial activities to dormancy and slows the movement of molecules to keep food safe at a constant temperature of -17.78°C even though food quality may suffer with prolonged freezer storage –

but the freezing process per se does not destroy nutrients (USDA, 2013; Taylor, 2019). Eggs are best stored at a temperature of about  $-1^{\circ}\text{C}$  and 80-85% relative humidity (Seidler and Hilmi, 2003) while cold storage at  $-1.5^{\circ}\text{C}$  could keep eggs for 6-9 months (Belitz et al., 2009).

A study had shown that, seafoods frozen or kept in frozen condition certainly lose quality (Mackie, 1993). The loss in quality may be due to changes in muscle integrity, proteins and lipids (Solanki et al., 2011); as cellular disintegration during cold storage can cause acid hydrolysis of lipids to free fatty acids (Mazrouh, 2015).

In tropical countries such as Ghana, eggs collected from layer chickens are kept for several days under temperatures varying from  $19-22^{\circ}\text{C}$  until they are sold out; the eggs are sold to retailers who may resell them in open markets at temperatures ranging from  $28-34^{\circ}\text{C}$  or to consumers who either store them under room temperature or refrigeration at  $5^{\circ}\text{C}$  prior to consumption (Hagan and Eichie, 2019). Despite the facts that the quality of eggs changes under different storage conditions, enough work had not been done to investigate the effects of the various storage conditions on the nutritional composition of eggs – specifically those from the domestic chicken (Bashir et al., 2015) compared to their physical characteristics. Therefore, this research work investigated the variability in nutrient composition of yolk and albumen of chicken eggs stored under different conditions.

## MATERIALS AND METHODS

### Study area

Eggs used for the study were obtained from the Teaching and Research Farm of the School of Agriculture, University of Cape Coast, Ghana and the experiment was conducted at the Nutrition Laboratory of the same University. The study area has average minimum and maximum temperatures of  $21^{\circ}\text{C}$  and  $32^{\circ}\text{C}$  correspondingly and an annual rainfall of 1300 mm (Kruenti et al., 2022).

### Experimental design and data collection

Eggs were collected from a flock of 42 weeks old brown layer chickens fed a standard layer mash made of 18% crude protein and 3200 kcal/kg ME. All essential vaccinations and medications were duly followed. Eggs were randomly collected on the same day between 8:00 – 8:30 am from the farm, examined, cleaned with a dry cloth, packed onto paper crates in a carton and transported to the laboratory within 30 minutes. A  $4 \times 2$  factorial experimental design involving four storage days (0 [D1], 7 [D2], 14 [D3] and 21 [D4]) and two storage temperatures (room temperature [T1]:  $21-32^{\circ}\text{C}$  and cold temperature [T2]:  $5^{\circ}\text{C}$ ) was used. Seven hundred and twenty eggs were randomly selected and divided into eight treatment groups, with 90 eggs in each treatment; each treatment had three replicates (R1, R2 and R3) of 30 eggs. The treatment structure is as follows: treatment 1 and 2 (D1T1R1, D1T1R2, D1T1R3 and D1T2R1, D1T2R2, D1T2R3), 3 and 4 (D2T1R1, D2T1R2, D2T1R3 and D2T2R1, D2T2R2, D2T2R3), 5 and 6 (D3T1R1, D3T1R2, D3T1R3 and D3T2R1, D3T2R2, D3T2R3), 7 and 8 (D4T1R1, D4T1R2, D4T1R3 and D4T2R1, D4T2R2, D4T2R3) respectively and identified with permanent marker accordingly. Treatments 1, 3, 5 and 7 were stored under room temperature ( $21-32^{\circ}\text{C}$ ) for the respective days whereas 2, 4, 6 and 8 were stored in a LOGIK CB BC-90 Fridge at  $5^{\circ}\text{C}$  (Hagan and Eichie, 2019). Analysis was done on storage days 0, 7, 14 and 21. Before analysis was carried out, the eggs were broken onto a petri dish using a scalpel and the yolk entirely separated from the white (albumen) using a plastic yolk separator. Extra eggs stored for each treatment, replaced eggs that did not have yolk well separated from the albumen. All yolks as well as albumen per storage condition were poured into a clean and sterilized beaker each, labelled and centrifuged to homogenize. Proximate analysis was done by the methods of the Association of Official Analytical Chemists (AOAC, 2012). Mineral elements (phosphorous, calcium, potassium, sodium, iron, copper and zinc) were also determined by methods of the AOAC (2012). All equipment were sterilized before each experiment; but were also regularly and thoroughly cleaned with distilled water and tissue paper after each experimental group.

### Data analysis

Data collected were subjected to ANOVA using the GLM procedure of Minitab (version 18). Differences in means were separated using the Tukey Pairwise Comparisons Method at 5% level of significance. The model used was:

$$Y_{ij} = \mu + SD_i + ST_j + \varepsilon_{ij}$$

Where:  $Y_{ij}$  = the dependent variable;  $\mu$  = the general mean;  $SD_i$  =  $i$ th observation of storage day;  $ST_j$  =  $j$ th observation of storage temperature;  $\varepsilon_{ij}$  = the random error associated with the dependent variable.

## RESULTS AND DISCUSSION

### Effects of storage day on proximate composition of egg yolk and albumen

Table 1 shows effects of storage day on proximate composition of egg yolk and white (albumen). There was no significant ( $p>0.05$ ) effect of storage length on crude protein and ash content of the egg parts. According to Réhault-Godbert et al. (2019), storage duration and conditions are associated with protein degradation, which corroborates the insignificant incessant declination in yolk and albumen protein with advancement in storage day as observed in the current work. The marginal changes detected in the protein content of the egg components are in agreement with

Dudusola (2009) who had found significant differences in the proximate composition at various days of keeping quail eggs except for protein. The significant ( $p < 0.05$ ) reduction in yolk fat with increasing storage time and that of the albumen fat at day 14 may be due to lipid oxidation and degradation. This reduction in the fat content of yolk and albumen confirms the results reported by Ogunwole et al. (2015), Ebegbulem and Asukwo (2018) and Luo et al. (2020) who found lipid content of eggs to decrease though insignificantly as storage length increased. These findings support the idea that egg if needed, must be kept, for a short time to maintain its protein and fat at optimum level (Ogunwole et al., 2015; Ebegbulem and Asukwo, 2018). However, the continual reduction detected in yolk fat in the current work is an indication that prolonged storage could be used to reduce the high fat content of egg yolk for people who need low fat. On the other hand, ash content would not change between fresh and stored eggs which diverges from the non-substantial increases in ash content of eggs kept at the respective storage days as informed (Ebegbulem and Asukwo, 2018). The outcome of the present research indicates that carbohydrate composition of the chicken egg parts would increase with increasing storage length up to the 21<sup>st</sup> day, which could be due to the loosening/permeability of the vitelline membrane to glucose as storage days increase, thereby facilitating both in vivo and in vitro fixation of free glucose by the yolk and albumen proteins (glycoproteins) and thus increasing carbohydrate content (Réhault-Godbert et al., 2019). This makes stored eggs a better option over fresh eggs in making 'energy remedies' for people during emergencies. In addition, the variations seen in the proximate composition of the egg components during the storage periods confirm various works (Stadelman et al., 1995; Rizzi and Marangon, 2012) that as eggs age, their nutritional composition is affected. Nevertheless, the results of the current work are comparable to global standard reference values of egg proximate analysis as found in the USA (USDA, 2016) and the UK (Roe et al., 2013; McCance and Widdowson, 2021).

**Table 1 - Effects of storage day on proximate composition of egg yolk and white (albumen)**

Nutrient Composition (g/100g)	Storage day				p-value	
	Day 0	Day 7	Day 14	Day 21		
Egg Yolk	Protein	15.5	15.3	15.3	15.2	0.67
	Fat	29.0 <sup>a</sup>	28.1 <sup>b</sup>	27.6 <sup>c</sup>	27.4 <sup>c</sup>	0.01
	Ash	1.3	1.3	1.3	1.3	0.58
	Carbohydrate	1.6 <sup>b</sup>	2.2 <sup>b</sup>	3.9 <sup>a</sup>	4.3 <sup>a</sup>	0.01
Egg White (Albumen)	Protein	10.7	10.6	10.4	10.3	0.20
	Fat	0.2 <sup>a</sup>	0.2 <sup>a</sup>	0.1 <sup>b</sup>	0.2 <sup>a</sup>	0.03
	Ash	0.8	0.8	0.7	0.8	0.50
	Carbohydrate	0.7 <sup>c</sup>	1.6 <sup>b</sup>	2.4 <sup>ab</sup>	3.3 <sup>a</sup>	0.01

Means in rows with different superscripts are significantly different; p-value: probability value ( $p < 0.05$ ); g/100g: grams per 100 grams (%)

#### Effects of storage temperature on proximate composition of egg yolk and albumen

Results presented in Table 2 show that storage temperature substantially influenced crude protein and fat contents but not the ash and carbohydrate contents of the egg yolk. On the other hand, content of all studied nutrients did not significantly ( $P > 0.05$ ) differ in the egg white (albumen) between the two storage temperatures. Egg had been proclaimed a perishable food that requires cold storage to keep longer and maintain nutrient quality (Menusano, 2018). Again, earlier reports indicate that alterations of freshness criteria are accelerated at room temperature when compared to refrigerated conditions (Réhault-Godbert et al., 2019). Thus, the significantly lower values of protein and fat under the room temperature compared to the cold temperature confirm the fact that protein and lipid degradation is enhanced by room temperature rather than cold temperature. This agrees to the notion that, freezing as a common practice in the meat, fish and other animal protein-based industry, preserves quality for an extended time with minor alterations in the products' dimension (Obuz and Dikeman, 2003). The results also show that generally, there is more protein in egg yolk than the albumen as found under all storage conditions which validates the idea that many people anticipate more protein in egg yolk than the albumen (Kaewmanee et al., 2009). Therefore, consumers are advised to eat the egg yolk rather than the egg white if protein is required. The current data also suggest that people who have health challenges relating to cholesterol should not eat frozen eggs due to the high amount of lipid found in yolk from such eggs.

**Table 2 - Effects of storage temperature on proximate composition of egg yolk and white (albumen)**

Nutrient comp. (g/100g)	Egg Yolk				Egg White (Albumen)			
	Protein	Fat	Ash	CHO	Protein	Fat	Ash	CHO
Room Temp.	15.1 <sup>a</sup>	27.7 <sup>b</sup>	1.3	3.4	10.5	0.2	0.8	2.5
Cold Temp.	15.7 <sup>b</sup>	28.4 <sup>a</sup>	1.3	3.5	10.6	0.2	0.8	2.4
p-value	0.03	0.01	0.94	0.85	0.68	0.11	0.68	0.62

Means in a column with different superscripts are significantly different; Comp.: composition; Temp.: temperature; CHO: carbohydrate; p-value: probability ( $p < 0.05$ ); g/100g: grams per 100 grams (%)

### Effects of storage day on mineral composition of egg yolk and albumen

Table 3 indicates effects of storage day on mineral composition of egg yolk and albumen. The results show no significant ( $P>0.05$ ) effect of storage day on potassium in egg yolk and egg white as well as on calcium, sodium, copper and zinc contents of egg white. This demonstrates stability of these mineral elements during storage even though there are marginal intermittent changes in their values as storage day increases. Contrariwise, there were magnificent ( $P<0.05$ ) variations in the phosphorous, calcium, sodium, iron, copper and zinc contents of the yolk as well as the phosphorus and iron composition of the egg white. Eggs are made up of significant amounts of several mineral elements (Gutierrez et al., 1996) that play different roles in the development of the human body. But the amount of the mineral elements in an egg's edible parts can vary at different egg ages (storage periods). High concentration of phosphorous and iron could be contained in the yolk from fresher eggs than stored eggs and this is an indication that, people who are deficient in these minerals should make fresh eggs their choice. On the other hand, storing eggs up to 14 days will yield higher concentrations of phosphorus and iron in egg white (albumen) and the highest concentration of calcium in the egg yolk. Consequently, people especially children who need more calcium for bone and teeth formation should be given food supplements made of yolk obtained from eggs stored up to the 14<sup>th</sup> day. The high levels of calcium and phosphorus in the egg parts at the various storage days means that, eggs whether fresh or stored up to day 14, are necessary for optimal bone development particularly in children to prevent rickets and osteomalacia (Erkan and Ozden, 2007). The current results support the substantial and irregular levels of elemental calcium in egg yolk reported by Stadelman et al. (1995). Though the amount of potassium in the chicken egg components might not be affected by storage time, eggs stored up to day 7 are most recommended for consumption if potassium is needed. On the other hand, egg storage could go up to day 21 for consumers who need high amount of sodium and copper from the yolk. Concentration of zinc in egg yolk was higher than that of the albumen; indicating that people who need more zinc should prioritize yolk from stored eggs rather than fresh ones. The opinion that potassium and sodium are the major minerals in albumen whiles calcium, potassium and phosphorus are the major elements in yolk (Stadelman et al., 1995; Roe et al., 2013; USDA, 2016; McCance and Widdowson, 2021) is completely supported by the current data. The results of this study thus confirm the fact that, the relative content of egg minerals and other nutrients may vary from one national reference to another but remains globally comparable (Roe et al., 2013).

**Table 3 - Effects of storage day on mineral composition of egg yolk and egg white (albumen)**

Mineral composition ( $\mu\text{g/g}$ )		Storage day				p-value
		Day 0	Day 7	Day 14	Day 21	
Egg Yolk	Phosphorous	1513.8 <sup>a</sup>	1444.3 <sup>b</sup>	1363.9 <sup>c</sup>	1376.4 <sup>c</sup>	0.01
	Calcium	1420.2 <sup>b</sup>	1984.9 <sup>ab</sup>	2424.3 <sup>a</sup>	2330.5 <sup>a</sup>	0.01
	Potassium	1655.3	2449.9	1604.0	1553.8	0.13
	Sodium	439.6 <sup>ab</sup>	455.8 <sup>b</sup>	511.0 <sup>ab</sup>	725.5 <sup>a</sup>	0.03
	Iron	45.1 <sup>b</sup>	43.6 <sup>b</sup>	41.7 <sup>a</sup>	43.9 <sup>b</sup>	0.02
	Copper	1.2 <sup>a</sup>	1.2 <sup>a</sup>	1.7 <sup>a</sup>	2.4 <sup>b</sup>	0.01
	Zinc	29.3 <sup>a</sup>	29.5 <sup>a</sup>	31.1 <sup>b</sup>	34.2 <sup>b</sup>	0.02
Egg White (Albumen)	Phosphorous	113.3 <sup>a</sup>	143.2 <sup>b</sup>	174.6 <sup>b</sup>	135.9 <sup>ab</sup>	0.03
	Calcium	86.3	86.2	85.9	83.5	0.16
	Potassium	1946.8	2188.4	2036.3	2121.4	0.36
	Sodium	1082.3	1209.0	1243.2	1121.7	0.52
	Iron	0.9 <sup>a</sup>	0.7 <sup>a</sup>	1.6 <sup>b</sup>	0.6 <sup>a</sup>	0.01
	Copper	0.4	0.6	0.7	0.5	0.22
	Zinc	0.3	0.2	0.2	0.5	0.11

Means in rows with different superscripts are significantly different;  $\mu\text{g/g}$ : microgram per gram; p-value: probability value ( $p < 0.05$ )

### Effects of storage temperature on mineral composition of egg yolk and albumen

The results presented in Table 4 show that, the mineral composition of the domestic chickens' egg would not vary ( $P>0.05$ ) under the different storage temperatures; implying that the mineral elements studied in the current research are more stable under room and cold temperatures. However, phosphorous, calcium, potassium, iron and copper contents may be numerically more in yolks from eggs stored under room temperature than those stored under cold temperature while the opposite should be expected for sodium and zinc contents of the same egg part. On the other part, phosphorus, calcium, potassium and sodium were numerically more in albumen (egg white) obtained from the refrigerated eggs than those from room temperature. In literature, information on the effects of storage temperature on mineral composition of chicken eggs are not readily available as notified by Bashir et al. (2015) and other works (Stadelman et al., 1995; Réhault-Godbert et al., 2019). Again, the results obtained in this study compares well with reference values in other parts of the world (Stadelman et al., 1995; Roe et al., 2013; USDA, 2016; McCance and Widdowson, 2021)

**Table 4 - Effects of storage temperature on the mineral composition of egg yolk and albumen**

Mineral Comp. (µg/g)	P	Ca	K	Na	Fe	Cu	Zn	P	Ca	K	Na	Fe	Cu	Zn
Storage Temp.	Egg Yolk							Egg White (Albumen)						
Room Temp.	1400.6	1357.9	1248.8	531.0	37.4	1.1	30.8	155.1	78.9	1569.4	1795.0	0.8	0.3	0.2
Cold Temp.	1392.1	1255.9	1186.8	599.7	33.8	0.9	32.0	161.2	81.2	1619.3	1827.5	0.6	0.3	0.3
p-value	0.52	0.34	0.10	0.45	0.21	0.10	0.22	0.29	0.32	0.13	0.23	0.15	1.00	0.42

Comp.: composition; Temp.: temperature; P: phosphorous; Ca: calcium; K: potassium; Na: sodium; Fe: iron; Cu: copper; Zn: zinc; µg/g: microgram per gram; p-value: probability (P < 0.05)

## CONCLUSION

Owing to the fact that storage conditions affect egg quality and the importance of yolk and albumen (white) in the egg processing industry, for products and people that need high protein, eggs should be refrigerated and not stored beyond day 14 but prolonged storage could be used to decrease fat content of eggs for products and people that need low fat. Also, the energy (carbohydrate) level of egg yolk and albumen can increase as storage length increases; therefore, food supplements used as 'energy remedies' should be made from eggs stored up to the 21<sup>st</sup> day. Potassium in egg yolk and egg white as well as calcium, sodium, copper and zinc contents of egg white are not affected by storage day whereas magnificent variations in the phosphorous, calcium, sodium, iron, copper and zinc contents of the yolk as well as the phosphorus and iron composition of the egg white are expected as storage day advances. Lastly, the aforementioned mineral elements may remain stable under both room and cold temperatures. Generally, the results of this study show that the nutritional composition of egg yolk and albumen may change when eggs are stored up to different storage days and under different temperatures. Subsequently, proper attention must be given to the period of time and temperature under which eggs are kept during distribution and sale by egg sellers or by consumers in order to ensure quality. It should however be noted that, the results of the present research are limited to 42 weeks old brown layer chickens, fed a layer mash of 18% crude protein and 3200 kcal/kg metabolizable energy and thus, the outcomes may not be generalised for other layers of diverse ages that are fed on different diet.

## DECLARATIONS

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### Availability of data and materials

Not applicable

### Authors' contributions

All authors contributed equally to this work.

### Competing interests

The authors declare that they have no competing interests.

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