Effect of Storage Time at Low Temperature on Physico-Chemical Characteristics of Eggs from Hens Kept in Backyard System in the City of Iasi in Romania

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Abstract
Egg production is an excellent solution to the acute shortage of animal protein. Despite its ability to be stored for several days at room temperature, eggs are an extremely perishable foodstuff whose optimal conservation standards must be mastered. The present study was carried out with the aim of evaluating the effect of storage time at low temperature on the physico-chemical characteristics of eggs from hens kept in backyard systems in the city of Iasi in Romania. For this purpose, 180 hen eggs were purchased on the local market. Once in the laboratory, the eggs were cleaned, marked and then stored in the refrigerator at a temperature of approximately 5ºC. The next day and every 7 days after, external characteristics (weight, measurements, shape indexes, volume and density) of all the eggs were evaluated and 60 eggs were then used for the analysis of internal characteristics (yolk and albumen indexes, Haugh unit, proportion of white, yolk, shell, edible parts) and chemical characteristics (pH, dry matter content, ash, proteins and lipids) of the eggs. The main results show that, except for density, which decreased with storage time, other external characteristics of eggs remained similar. The same was true for the major internal parameter characteristics. The pH of the yolk and albumen increased with the storage time, which also led to a variation in the contents of dry matter, ash, lipids and proteins of the egg components. It was concluded that although refrigeration allows the external characteristics of eggs from the backyard system to be kept relatively constant, storage time does, however, affect the chemical characteristics of the eggs. Refrigeration of eggs slows down but does not prevent the deterioration of the eggs and consequently they cannot be stored indefinitely.

Keywords: hen egg, conservation time, chemical characteristics, courtyard.

1. Introduction

Eggs are one of the easiest and most widely consumed foods for human feeding. Because of their high concentration of nutrients, eggs are a well-known source of high quality protein that should be considered part of a healthy diet and almost complete with vitamins and trace elements [1-4].

Egg quality can be affected by ambient conditions such as storage temperature and moisture [5]. These alterations consist of a loss of water, carbon dioxide which leads to an increase in egg pH [6, 7] and liquefaction of the egg white resulting from a reduction in the binding capacity of the ovomucine and lysozyme contained in the albumen with storage time [8]. This has the effect of reducing the freshness of the egg, which is commonly evaluated by the Haugh's index whose formula takes into account the albumen height and the egg weight [9]. Physico-chemical qualities are

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also influenced by the rearing system [10-12]. Eggs from aged hens are generally larger and have a thinner eggshell [5, 13, 14].

Hens nutrition, in particular their supply of calcium, phosphorus and vitamin D, influences eggshell thickness and resistance to breakage. Using greenery helps to improve the yolk color, while a low pH in drinking water densifies the albumen and improves its height. However, organic acids in feed do not affect this parameter [6].

Typical of backyard or backyard flocks is that hens can express their natural behaviour by scavenging, dust bathing and also feeding on grasses and other elements not always available in other farming systems [15-17]. Egg quality is nearest to that of eggs obtained from organic farming and the yolk color is natural [18].

How does refrigerating affect the quality of backyard eggs? Our study is a comparative analysis of the external, internal and chemical characteristics of eggs from hens kept in backyard systems as a function of storage time at low temperatures.

2. Materials and methods

Study area

This study was carried out at the Animal Production Management Department, Faculty of Zootechny of the University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania. The city of Iasi is located in the North of Romania, it covers an area of 94km2 and counts 320,000 inhabitants. Climate is continental. Over the year, average temperature is 9.8°C and rainfall averages 430.5 mm.

Animal material

180 eggs from hens raised in backyard system were bought from 6 breeders selling their products at the Alexandru Cell bun market in Iasi, which has the particularity of receiving small farmers from all over the region once a week.

At the laboratory, eggs were cleaned, marked and then stored in the refrigerator at 5ºC. The next day and then every 7 days, the external characteristics of all eggs were evaluated and 60 eggs were then used for the analysis of internal and chemical characteristics of eggs.

External characteristic

Weighing and measuring the egg

Eggs were individually weighed on an electronic scale branded Shimadzu UX4200H, with a capacity of 320 g and 0.01 g accuracy.

Large diameter and height of the eggs were measured using a digital caliper with 150mm range and 0.01mm accuracy.

Egg Shape Index was obtained by dividing large diameter by height and multiplying by 100 [19].

*Shape index =[Diameter (mm)/Height (mm)]*100*

The specific gravity assessment of eggs was based on Archimedes' principle. Eggs were weighed in the air. Water weight (at 22 °C) displaced by the eggs was determined by immersing the eggs in the water of a beaker on the same scale [20]. The specific gravity of the eggs was then determined using the equation:

Specific gravity = [Weight of egg in air (g)/Weight of water displaced (g)]

Egg volume was obtained by placing the egg in a graduated cylinder containing a known volume of water. Egg volume (Vo) was determined by the difference between the volume after introduction of the egg (Vf) and the initial volume of water (Vi).

Vo=Vf-Vi

Internal characteristics of the egg

Throughout the trial, the following data were collected after the eggs were broken: diameter and height of the dense albumen and yolk, yolk color, weight of the white, yolk and shell, and shell thickness.

Measuring and weighing of albumen, yolk and shell

Eggs were broken individually and their contents carefully placed on a 40cm x 40cm glass slab placed on a flat and stable surface. The diameter and then the height of the dense albumen and yolk were then measured using the digital caliper. For height measurement, the caliper was attached to tripods to be perpendicular to the glass plate.

Yolk color was assessed according to the method of [21] which uses a range of yolk colors (Yolk Color Fan® scale, Roche).

After separating albumen from yolk using a 100ml syringe, yolk was weighed using an electronic balance (Denver Instruments 214) with a capacity of 210 g and an accuracy of 0.0001 g.

The shells were washed with water to remove the remaining albumen and then dried before being weighed. Albumen weight was obtained by calculating the difference between whole egg weight and the weight of the yolk and shell.

Aw=Ew-(Yw+Sw)

With: Aw: Albumen weight, Yw: Yolk weight, Ew: Whole egg weight, Sw: Shell weight

Using a caliper attached to a tripod, the thickness of the shell was taken from shell fragments taken from the large side, the large diameter and the small side of the egg.

The different weights and measurements on the internal and external egg characteristics were used to calculate:

- *Proportion of egg components (%) = [Shell weight;yolk;white weight (g) /Egg weight (g)]*100*
- *Percentage of edible matter (%) = proportion of yellow + proportion of white*
- *Egg constituent index (%) = [Yolk; white height (mm)/Yolk; white diameter (mm)]*100*
- *Haugh unit (HU) = 100 log (H+7.57 - 1.7P0.37)*

Where H = Albumen height (mm); P = Egg weight (g); 7.57 = Albumen height correction factor and 1.7= Egg weight correction factor [22].

Albumen and yolk were homogenized separately in large glass petri dishes. pH values were obtained by inserting the tip of a pre-calibrated electronic pH meter into each of them. pH values were noted once the number had stabilized on the display of the pH meter. The procedure was repeated 5 times for each sample and the average was taken.

Analysis of chemical characteristics

AOAC Method No. 925.30 [23] was used to evaluate the dry matter in pre-dried eggs obtained by dehydration in a Memmert SEU 700 forced-air oven at 70ºC.

Crude ash content was evaluated by incineration at 550ºC in a Super Therm C311 incinerator after pre-combustion with a Bunsen tray until cessation of smoking in accordance with AOAC Specification 900.02 [23].

Crude protein (CP) was obtained from the evaluation of total nitrogen content by the Kjeldahl method, applied on a Velp Scientifica DK 6 digestion system and UDK 7 distillation system, according to AOAC Method No. 925.31 [23]. Finally, the total nitrogen content was multiplied by 6.25, which generated the crude protein content. Total lipid content in the form of crude fat was determined by AOAC method No. 925.32 [23], using a Velp Scientifica Soxhlet SER 148 extractor.

Statistical analysis

Data were expressed as a mean ± standard deviation over the mean. One-factor analysis of variance (ANOVA) was used following the general linear model to compare means of the different parameters. When differences between means were significant, Duncan's test was used to separate them at the 5% significance level. IBM Statistic SPSS 25.0 and Excel 2016 were also used for the data processing and illustration.

3. Results and discussion

Results

Eggs external characteristics

The changes in external characteristics of eggs from the backyard system as a function of storage time are shown in Figure 1. Although similar ($p > 0.05$) with storage time, egg weight decreased from 64.66±9.78g to 63.71±9.45g between the 7th and 21st day of storage in refrigerator. Egg diameter, height, shape index and volume were also not affected by egg storage time.

Specific gravity decreased with storage time. From 1.075±0.01 on the 7th day of storage to 1.06±0.003 on the 21st day. However, the densities recorded between 14 and 21 days of storage were similar.

Eggs internal characteristics

Measurements and Haugh and yellow indexes as a function of storage time

As shown in figure 2, internal characteristics of eggs from backyard hens vary with storage time. The yolk index decreased from 37.64±2.53 (Day 7) to 36.23±7.47 (Day 21). Albumen index decreased from 12.54±8.10 on Day 7 to 8.34±5.07 on Day 21. Nevertheless, the values of these different parameters remained similar ($p > 0.05$) regardless of the storage time at low temperatures.

Haugh index dropped from 67.30±22.5 to 62.26±12.61 between the 7th and 21st day of storage without any significant difference ($p > 0.05$).

Proportion of Yolk, white and edible matter percentage.

It can be seen from Table 1 showing the variation in the proportions of edible matter with storage time that the proportions of albumen, yolk and total edible matter were not affected by storage time. However, there was a slight increase in the percentage of total edible matter throughout storage. The highest proportion (90.25±0.94%) was observed in eggs with 21 days of storage and the lowest (90.06±1.25%) at 7 days of egg storage.

The effect of storage time on shell characteristics (Table 2) shows that shell represents less than 10% of the egg weight. The proportion of the shell and large side thickness were not affected by egg storage time. In contrast, the thickness of the middle and small end of the shell increased significantly between 7 days of storage and other periods that were otherwise similar. The average shell thickness followed the same trends as the thickness of the large side.

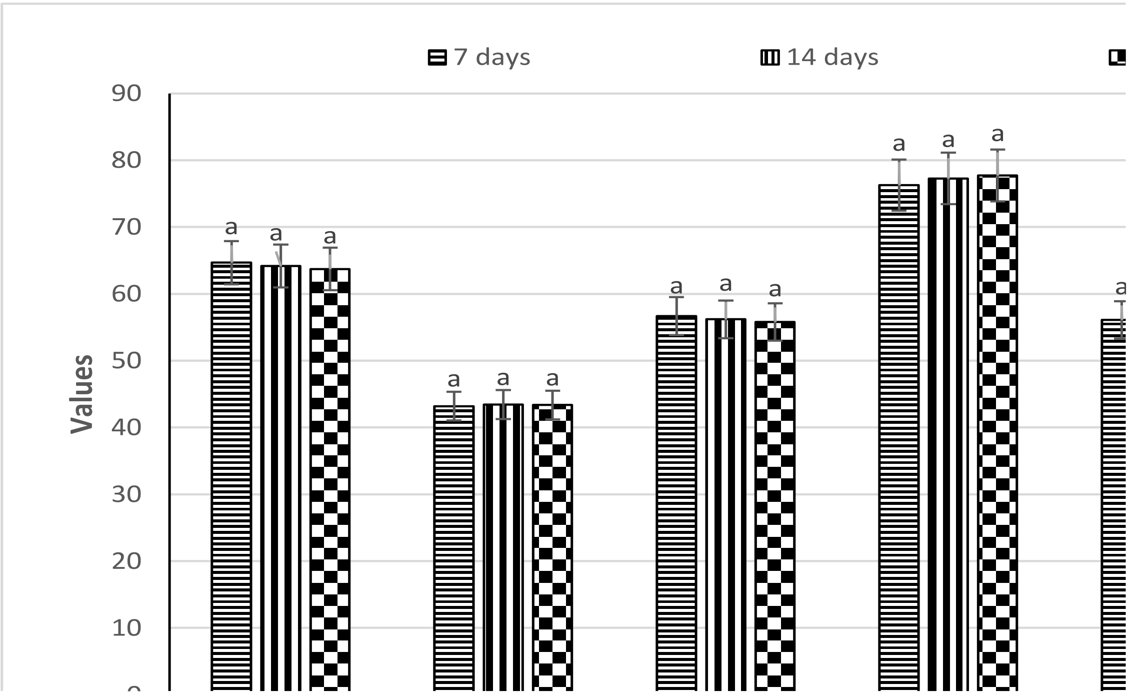

Figure 1. External characteristics of eggs from the backyard system as a function of storage time

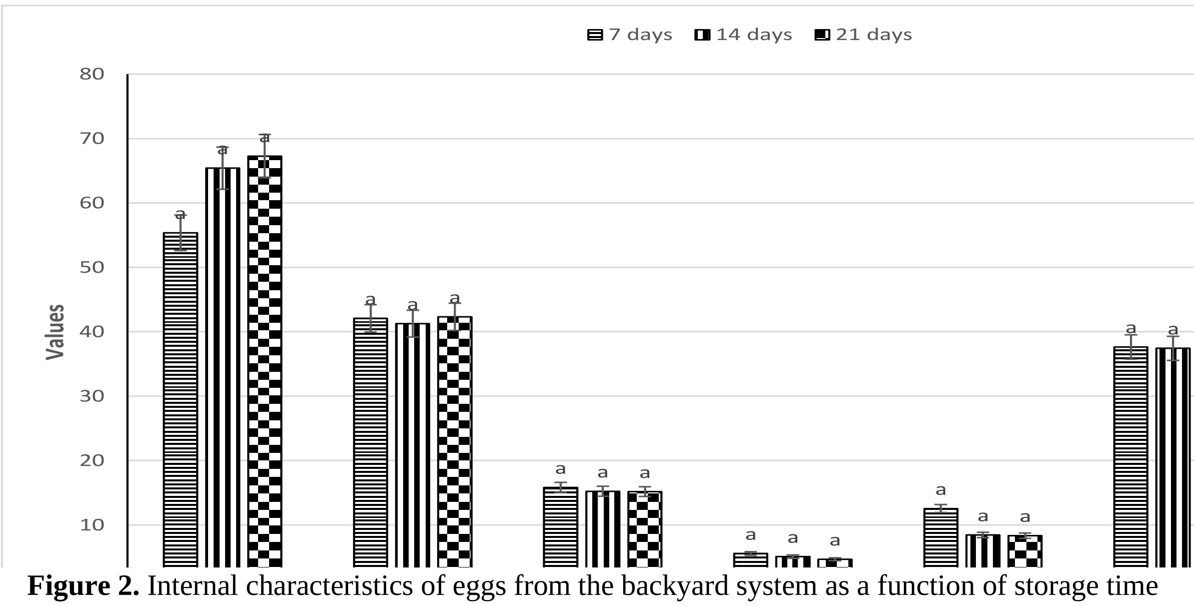

Figure 2. Internal characteristics of eggs from the backyard system as a function of storage time

Table 1. Variation of edible matter with storage time

Storage time	Albumen proportion (%)	Yolk proportion (%)	Edible matter (%)
7 days	64.11±2.58 [a]	25.95±2.00 [a]	90.06±1.25[a]
14 days	64.52±2.17 [a]	25.62±1.69 [a]	90.14±0.63[a]
21days	63.83±2.69 [a]	26.42±2.32 [a]	90.25±0.94[a]
Mean	64.16±2.42	25.99±1.98	90.15±0.94

a: on the same column, values assigned to the same letter are not different (P >0.05)

Table 2. Eggshell characteristics variation as a function of storage time

Storage time	Proportion (%)	Large side thickness	Medium side thicnesss	Small side thichness	Thickness me
7 days	9.75±0.94 [a]	0.40±0.04 [a]	0.39±0.03 [a]	0.37±0.03 [a]	0.38±0.03 [a]
14 days	9.86±0.63 [a]	0.41±0.03 [a]	0.42±0.03 [b]	0.42±0.02 [b]	0.42±0.02 [b]
21days	9.40±1.25 [a]	0.40±0.03 [a]	0.42±0.02 [b]	0.41±0.03 [b]	0.41±0.02 [b]
Mean	9.85±0.94	0.40±0.03	0.41±0.03	0.40±0.03	0.40±0.03

a,b: on the same column, values assigned to the same letter are not different (p >0.05)

Table 3. Variations of yolk color and pH with storage time

Storage time	Yolk color	Albumen pH	Yolk pH
7 days	9.80±1.93 [a]	8.63±0.24 [a]	6.12±0.08 [a]
14 days	10.00±3.16 [a]	8.66±0.08 [ab]	6.26±0.05 [b]
21days	10.30±2.54 [a]	8.79±0.03 [b]	6.28±0.03 [b]
Mean	10.03±2.51	8.70±0.16	6.23±0.09

a,b: on the same column, values assigned to the same letter are not different (p >0,05)

Chemical characteristics of eggs

Yolk coloration and pH

It can be seen from Table 3 presenting the variations in yolk color and pH with egg storage time that, although not significant, the intensity of yolk color increased with storage time. The pH of the albumen was higher than yolk pH. It ranged from 8.63±0.24 (Day 7) to 8.79±0.03 (Day 21) for yolk and from 6.12±0.08 (Day 7) to 6.28±0.03 (Day 21) for albumen.

Dry matter, ash, protein and fat content

In figure 3, the chemical characteristics of eggs from the backyard rearing system as a function of albumen storage time are shown. It can be seen that the dry matter of the albumen (Fig 3 A) decreased from day 7 to day 21 of storage. The highest dry matter was found in the 7-day eggs (12.84±0.03%) greater than the 14-day eggs (12.76±0.02%), which in turn were higher than the 21-day eggs (11.51±0.01%).

Yolk dry matter accounts for about half of the fresh sample. There was a significant reduction in dry matter content (48.35±0.03%) at 14 days (50.66±0.00%) of storage compared to the values recorded at 7 (50.65±0.05%) and 21 days, which were otherwise similar. Estimated ash content (Fig 3 B) was affected by storage time. Albumen ash content decreased significantly throughout the storage period. Eggs on day 7 had a higher value (0.89±0.13) than those on day 14 (0.80±0.05) which were also higher than day 7 (0.72±0.002).

Yolk ash content, in contrast to albumen, increased with storage time. Highest ash content was observed at 21 days (3.67±0.10%) and the lowest at 7 days (3.13±0.27%) of storage.

The results of the albumen and yolk protein assay (Fig 3 C) show that the yolk contains almost three times less protein than albumen. Variation in protein values with storage time is significant and follows exactly the same trend with albumen and yolk. As a result, it decreases between 7 (30.67±1.62 for yolk) and 14 days (78.06±0.53 for albumen) before rising again at 21 days to 81.38±0.30 and 32.57% for albumen and yolk respectively.

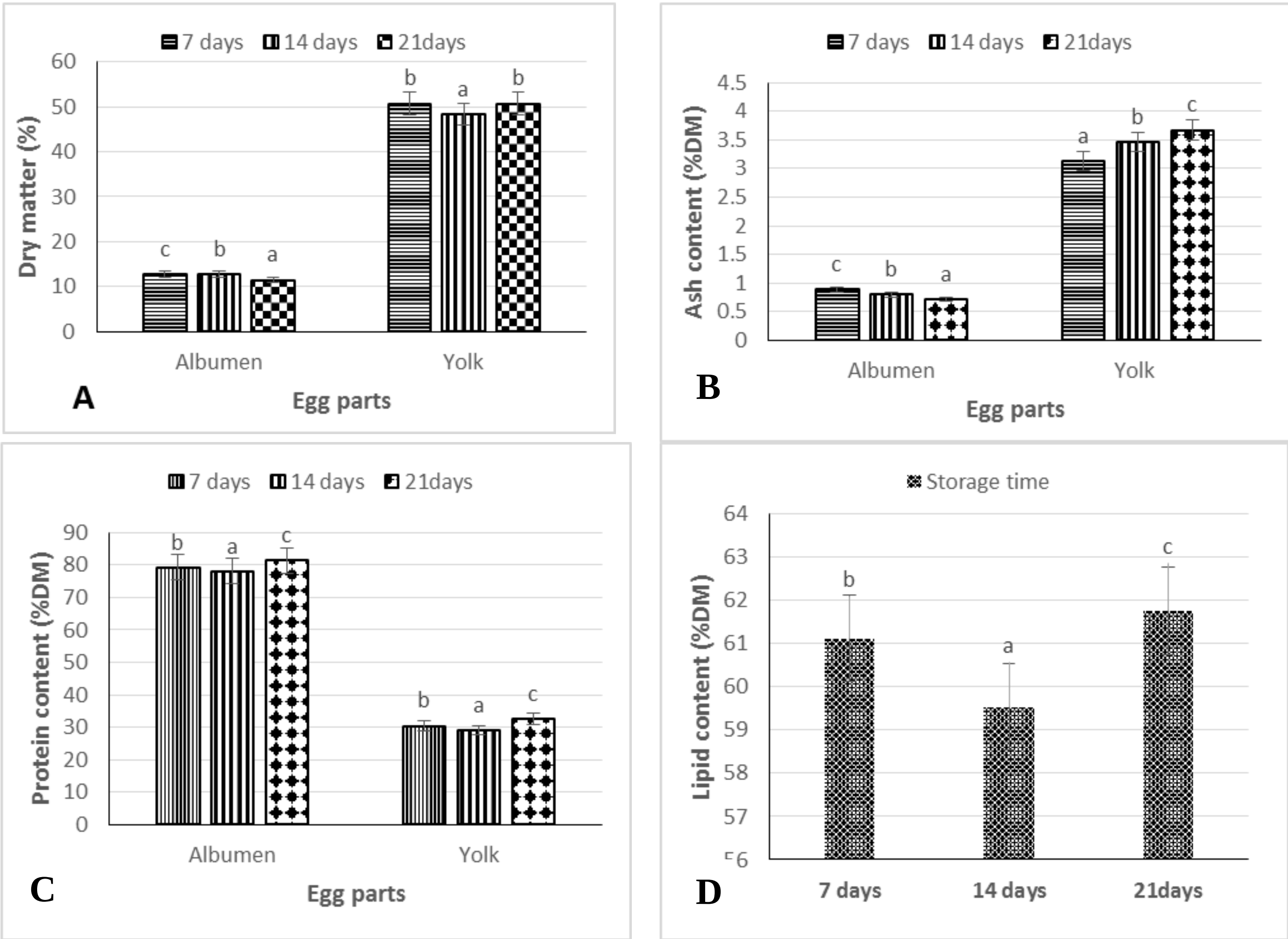

Figure 3. Chemical characteristics of eggs from the backyard system as a function of storage time A : Dry Matter; B : Ash; C : Protein; D : Lipid

Yolk lipid content (Fig 3 D) was also affected during storage. 21-day-old eggs had the highest lipid level (61.76±0.75%) and the lowest was obtained with 14-day-old eggs (59.53±0.34%).

Discussion

Measurement results show that mean egg diameters and heights were 44.46±0.11 and 58.47±0.43mm respectively. Our results are above values presented by Fayeye et al [24] in Morocco; Keambou et al [25] in West Cameroon and by Samandoulougou et al [26] in Burkina-Faso who noted values of 35.24, 48.58 and 54.26 mm egg heights respectively. They are also higher than the values of Zaaboube and Benrahou [27] on local hens bought in Algeria's markets. These observations can be explained by factors such as genetic type [28], feed and geographical areas [29], [29] that affect the weight, size and even color of eggs [4].

Regarding shape indexes, the average was 76.16±0.74%, which is higher than the values obtained by Markos [31], who found values ranging from 66.5 to 71.3; by Keambou et al [25], from whom values were close to 73, and by Samandoulougou et al [26], whose values ranged from 72 to 75%. According to King'ori [32], the size, age, state of health and internal structure of the hen are among the factors that can strongly influence the shape index of the egg. Overall, shape indexes found in this study are higher than the required standard of 75 for eggs to be packed in standardized packs [33]. This would be the result of a selection made over time on farm farms in Romania which also incorporates the introduction of heavy stock hens.

Although weight reduction is not significant with storage time, there has been a significant decrease in density, which could be justified by the loss of water contained in the albumen through the pores of the egg shell, whose calcium carbonate, the main constituent responsible for its rigidity, justifies the non-variation of egg volume with storage time [4].

By storing at room temperature, Youssef et al [34] saw a considerable reduction in weight, which was not the case with our eggs that were stored at low temperature. Refrigeration at an appropriate temperature slows down the aging of the egg [4] [32].

Yolk index analysis showed no significant difference between eggs of different weeks. This would be due to the storage conditions used. Our results reinforce those of Ouattara [35], Altan et al [36] and Jones and Musgrove [36] who found that the refrigerator delayed or even prevented the yolk index from falling.

Although we noted a relative reduction in albumen and Haugh indexes with storage time, they showed the same trends as the yolk index.

We also observed a non-significant decrease in the proportions of albumen and total edible matter and an increase in the proportion of yolk; results that corroborate those reported by Youssef et al [32]. This may be due to the aging of the eggs, which begins with a loss of water by the albumen, which leads to a loss of weight in the albumen, thus justifying the decrease in the proportions of albumen and the increase in the proportion of yolk, given that it loses almost no water during this time [4].

Our results contradict those of Monira et al [28] who observed a decrease in shell thickness as a function of the egg conservation period in summer at ambient temperature in Bangladesh.

The intensification of yolk color in our work would be the cause of the enzymatic reactions taking place in the egg during its conservation. Indeed, according to Tremolieres [37] it can occur during the conservation of the egg, color anomalies due to the release of volatile gases following reactions of hydrolysis of lipids by lipases and phosphatases. Our values were higher than those reported by Ledvinka et al [38] with eggs from laying hens of different strains reared in cage and litter systems. This difference could be attributed not only to the genetic type, but also and especially to the husbandry system. It is indeed known that hens reared in backyard systems have the possibility to supplement their feed with greenery, which acts by increasing the intensity of yolk coloration [39, 4]. Our results show a significant increase in the pH of the albumen and egg yolk with storage time. This increase would result from physico-chemical modifications with notably a loss of carbon dioxide taking place in the different compartments, which lead to an increase in this parameter. Although the trend is similar, our results were weaker than those reported by [40] when studying the effect of storage mode and storage time on the quality of farm eggs in Senegal. This could be due to the genetic type of the hens and the farming system as well.

Findings from our work show that egg yolk contains 4 times more dry matter than albumen. Indeed, the egg white is mostly made up of water which, during the plumping process, passes through egg membranes to dilute the dense albumen, thus increasing the volume of the egg before it forms a shell in the uterus [4]. Moreover, a significant reduction in the dry matter content of the egg albumen can be observed with the storage duration, while the dry matter content of the yolk remains almost identical. It could be explained by the loss of water contained in the egg white by evaporation through the eggshell pores. Our results are similar to those reported by [4] for hens. They are also in opposition to those found by Moran [41] who observed an increase in the dry matter content of the yolk throughout the storage of turkey eggs. This could be explained by the anatomical and physiological differences of each species that would lead to products with different characteristics [42].

Albumen ash accounts for less than 1% of the dry matter and decreases with storage time. Yellow ash is between 3 and 4% of dry matter and increases with storage time. Our results are higher than those obtained by Samandoulougou et al [26] who found 2.62 for the yolk ash content of local hens in Burkina Faso. These differences could be due to the storage method used, the genetic type, feeding and environmental conditions.

Due to its very low content (0.2-0.4%) in albumen [4, 44], the effect of storage time on the lipid content of the white has not been studied. The yolk results are slightly lower than those (52.9%) of AEB [44] in the United States, but higher than results from Samandoulougou et al [26] who obtained 32.76 for eggs from local hens in Burkina Faso. These results would be due to genetic type, environment and bird feeding.

Protein levels varied throughout storage. However, these results are lower than those obtained by [4] who obtained a rate of 33%. Divergence in the results may be due to the way in which our eggs are stored.

Protein contents of the albumen and yolk varied irregularly although they increased between 7 and 21 days of storage. Our results are similar to those of [4] but slightly lower than the 84.6% AEB value [44]. Apart from feeding, this difference could be due to the ageing of the egg resulting in water loss by evaporation [45], osmotic exchange between the yolk and the white of the egg and enzymatic reactions that show anaphylactic reactions due to the presence of decarboxylation amines [2, 37, 45].

4. Conclusions

At the end of our study on the effect of storage time at low temperature on the physico-chemical characteristics of eggs from hens reared in backyard systems in the city of Iasi in Romania, it was concluded that:

- external characteristics of the eggs remained constant except for the density, which decreased with the storage time;
- With the exception of the eggshell thickness which increased, all other internal egg characteristics have remained unchanged over the storage time;
- pH of the yolk and albumen increased with storage time, which also affected the dry matter, ash, lipid and protein contents of the egg components.

Although refrigeration keeps the external characteristics of eggs from the backyard system relatively constant, the storage time nevertheless affects their chemical characteristics. Refrigeration of eggs slows down but does not prevent the aging of eggs and therefore they cannot be stored indefinitely.

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