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Original Article

# Analysis of the Quality of Eggs Marketed in Santarém, Brazil

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## ABSTRACT

Eggs are foods with almost perfect proteins, while also containing nutrients with high biological value. The purpose of this study was to evaluate the morphological structure, physical-chemical, nutritional, and microbiological parameters of commercial and free-range eggs sold in the municipality of Santarém, State of Pará, Brazil. The two types of eggs were also compared to check for any differences in quality. The evaluations were conducted on variables such as Haugh unit, weight loss, egg width and length, specific gravity, yolk and albumen dimensions, pH, among others. A total of 240 eggs were purchased in the Brazilian municipality of Santarém (2°45'06" S and 54°70'09" W). A statistical study was also performed using the BIOESTAT 5.0 statistical program. A comparison of the industrial and free-range eggs and their various parameters is given in the tables along with the coefficient of variation. The results obtained were satisfactory and showed that the quality of eggs is affected more by environmental factors than by the source from which they are obtained. The results were also compared to previously published literature, and it was determined that this study offers a better foundation for the nutritional examination of egg quality.

## INTRODUCTION

The Brazilian chicken egg production chain counts with advanced technology, utilizing genetics knowledge, strains with high productivity, nutrition, health, environment, and management. The system is unique in that it combines alternative production methods like the extensive or semi-extensive model with automated farms in battery cage systems (Vieira *et al.*, 2014). The 2022 Annual Report of the Brazilian Association of Animal Protein (ABPA) states that in 2021 roughly 55 billion eggs were produced, with an average annual consumption of 257 units per person. Only around 0.5% of this total is exported, with the Middle East accounting for the majority of buyers (71.1%) (ABPA, 2022).

The two distinct goals associated to egg production are incubation, designed for the reproduction of broilers and laying birds, and consumption, often known as table eggs, meant for direct or indirect human consumption. Eggs are a food with a high nutritional value and represent a cheap source of high-quality, biologically active proteins (e.g. ovoalbumin, conalbumin, ovomucoid, lysozyme, ovomucin, phosvitin, lipovitellin, livetin) (Moula *et al.*, 2013). They consist mainly of water, proteins and lipids, while also containing carbohydrates, minerals, and vitamins (Mazzuco, 2008; Fernandes *et al.*, 2015).

Markets and other outlets are predominantly supplied by egg production in traditional cages, guaranteeing that the general public has easy access to this meal as a source of animal protein (Banaszewska

*et al.*, 2020). In order to prevent coccidiosis, worms, and the hens' own ingestion of eggs, the cages used in this method do not require the usage of poultry litter, making it difficult for laying hens and eggs to come in contact with the excrement. Despite being an established process that ensures productivity, the management techniques frequently used in the production chain for commercial eggs can be viewed as controversial in the eyes of consumers more concerned with the issue of animal welfare, which has led to discussions and considerations about alternative forms of production (Hisasaga *et al.*, 2020).

Small and medium-sized farmers raise young birds and produce free-range eggs for their personal consumption or for sale at markets and other small businesses. They have high value in popular circuits are surrounded by preconceptions, such as the association of the "countryside" origin to a "natural" product, with advantages or qualities that are even more alluring than those found in farm eggs. Although eggs produced by alternative methods are commonly accessible on the market, little is known regarding their internal and external quality standards (Jalal *et al.*, 2006; Schwartz & Gameiro, 2017). Several factors can influence egg size and quality such as bird physiology, oviposition time, cage structure, number of birds per cage, egg collection frequency, bird age, nutrition, handling conditions, health status, temperature and humidity, genetics, and bird management (Vits *et al.*, 2005; Pettersson *et al.*, 2016). The factors that influence the quality of albumen and yolk are the time and temperature of egg storage, strain, and age of the laying hen, as well as nutritional management and health status. When the quality of eggs is unsatisfactory, it can cause economic losses to industries and damages to consumer health (Hisasaga *et al.*, 2020).

The quality of eggs for consumption includes a set of characteristics that influence the acceptability of the product by consumers, being determined by several external and internal aspects. External aspects related to egg quality involve the structure and hygiene of the shell, while internal aspects are related to the albumen, yolk, air chamber, color, odor, taste, and blood stains (Mendes, 2010; Henriques *et al.*, 2018). Like all animal products, eggs are perishable and start losing quality soon after oviposition, especially in the absence of adequate storage methods (Wardy *et al.*, 2010).

Eggs with poor-quality shells are undesirable mainly because they are considered the first protective barrier against the entry of microorganisms into the eggs (Mertens *et al.*, 2006; Janczak & Riber, 2015; Carvalho *et al.*, 2022). The egg is exposed to a series of factors that compromise the microbiological quality, ranging from employees, equipment, facilities, and management to the bird itself (Sharma *et al.*, 2022).

Eggs that are not stored correctly are subject to internal contamination, with changes such as the reduction of emulsifying, viscosity, gelling, foaming and solubility properties occurring in the preparation of food systems, which impairs the final quality of the product (Moula *et al.*, 2013; Rao *et al.*, 2013). Among the pathogenic bacteria commonly associated with the deterioration of eggs *Salmonella spp*., *Staphylococcus aureus*., *Campylobacter jejuni*, *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Salmonella enteritidis* stand out (Stringhini *et al.*, 2009; Chen *et al.*, 2019; Hofer, 2021; Kulshreshtha *et al.*, 2021).

Environmental factors such as temperature and humidity have a significant impact on the quality of the shell. When temperatures are high and there is high relative humidity, the bird is out of its comfort zone and the quality suffers (Yimenu *et al.*, 2017). The heat loss system kicks in when birds are uncomfortable, causing them to breathe more quickly (hyperventilate), which causes their body heat to evaporate. As a result, the quality of the calcareous deposition that surrounds the egg is compromised, leading to eggs with thin shells (Pacheco *et al.*, 2022).

In this context, this study aimed to analyze the morphological structure, physical-chemical, nutritional, and microbiological parameters of commercial and free-range eggs sold in the municipality of Santarém, State of Pará, Brazil. Additionally, we compared these two varieties of eggs to see whether there were any quality variations.

## MATERIAL AND METHODS

### Study area

A total of 240 eggs were purchased in the Brazilian municipality of Santarém (2°45'06" S and 54°70'09" W), 120 of which were free-range eggs purchased at fairs, and 120 were industrialized eggs purchased in supermarkets (Figure 1). The eggs were acquired in July 2021, observing the expiration date for industrialized and free-range eggs (6 days since collection). Eggs of the large type were chosen (weighing between 55 and 59 g per unit). The collection was conducted with gloves and each egg was numbered and stored in clean paper boxes with dividers to avoid contact between them, and then they were stored in a refrigerator at a temperature around 4°C.

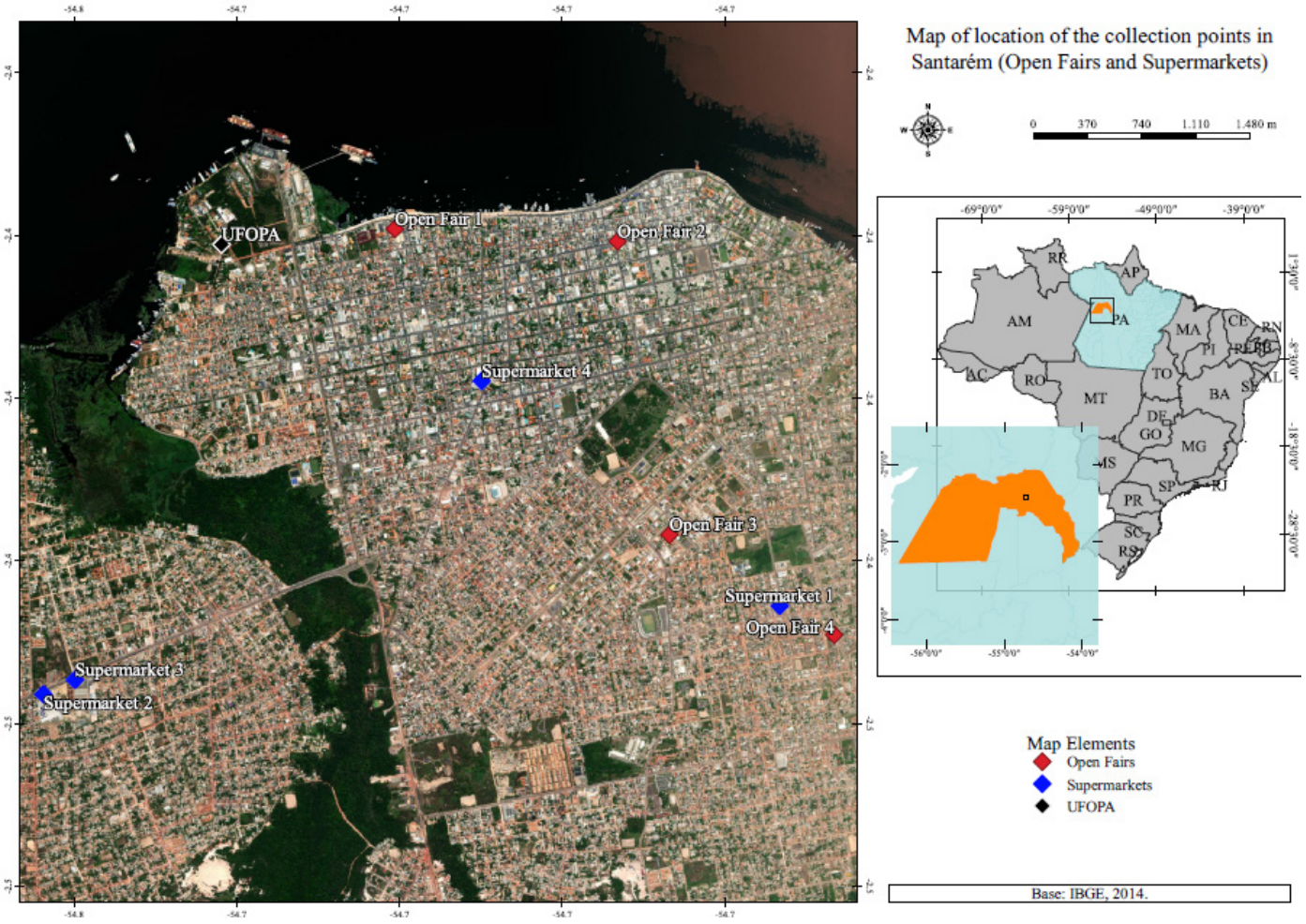


**Figure 1 –** Study area: map of the municipality of Santarém: urban area, collection sites.

Soon after collection, 15 eggs from each production system (industrial and free range) were separated for microbiological analysis, and 15 eggs others from each for bromatological analysis. The eggs for the bromatological analyzes were broken, separated into yolk and albumen, and kept in the freezer for total freezing of the samples. After 48 hours in the freezer, drying started through the lyophilization process. On the following day, 30 eggs from each production system were subjected to physical-chemical analysis, configuring day 0 of the experiment. Moreover, 30 eggs from each production system were analyzed after 7 and 21 days after acquisition. The following characteristics were analyzed: egg weight, height and diameter of albumen and yolk, weight of yolk and albumen, pH of yolk and albumen, yolk color, and shell thickness and weight, all of which are necessary for the calculation of parameters related to egg quality.

## Microbiological analysis

Fifteen eggs from each production system were aseptically separated from the total set of samples for microbiological analysis. Three eggs were used to form a sample unit from each collection site. Before starting the analyses, the three eggs were disinfected with 70% alcohol, broken, and the liquid was homogenized in a sterile beaker, and 25g of the sample was weighed and placed in sterilized containers. Then, 225 ml of 0.1% peptone water was added for a $10^{-1}$ dilution, and the mixture was homogenized with a sterile glass rod. From this initial dilution ($10^{-1}$), serial dilutions of $10^{-2}$ and $10^{-3}$ were made in test tubes containing 9 ml of sterile 0.1% peptone water. All microbiological procedures were performed according to already published protocol (Ehsani *et al.*, 2019).

## 2.3 Microbiological procedures

### *Standard count on Mesophilic Aerobic Heterotrophic Bacteria (MAHB) plates*

To proceed with the MAHB analysis, 200 µL of each dilution were inoculated by spread plate in Petri dishes containing standard counting agar (PCA). After plate incubation at 35 ± 2ºC for 24 - 48 hours, the

colonies present in them were counted. Counts were performed in a colony counter, on plates that had dilutions between 30 and 300 colony forming units (CFU).

### *Coagulase and catalase-positive staphylococci count*

Direct Count in Plates was conducted using the technique of seeding in a solid medium: "Spread Plate" (count on surface). Selected serial decimal dilutions were made ($10^{-1}$, $10^{-2}$ and $10^{-3}$) and 0.1mL of the inoculum was added to Petri dishes containing Baird Parker Agar (BP – KASVI®) supplemented with egg yolk solution and potassium tellurite (which causes the microorganism to reduce aerobically and anaerobically, producing black colonies). It was homogenized using a Drigalski loop, allowed to dry, and incubated upside down in an oven at 36°C for 30 to 48 hours. After this time, the reading of 30-300 colonies and the CFU/g count was obtained. Then, 3 typical colonies (bright black with an opaque ring and surrounded by a light halo) and 3 atypical (greyish or bright black, without halos) were selected and seeded using a bacteriological loop in TSA (Trypticase Soy Agar) medium. - KASVI®) and were incubated in an oven at 37 °C for 24 hours. Finally, morphotinctorial examination was performed by Gram stain and microscopy, Coagulase, and Catalase tests. To detect the production of catalase after adding a drop of 3% hydrogen peroxide on a microscope slide, an elevation of typical colonies for newly grown staphylococci was deposited, and the presence or absence of the decomposition reaction of staphylococcus was observed. Catalysis of hydrogen peroxide into water and oxygen by the bacteria was observed through the formation of gas (bubbles). For coagulase, the microscopy slide technique was used where clot formation was observed after the addition of rabbit plasma.

### *Presence/absence of Salmonella spp.*

For the analysis of Salmonella spp., using the surface plating technique (spread plate), an aliquot of 200 µL was removed from each dilution ($10^{-1}$, $10^{-2}$ and $10^{-3}$) and then inoculated in a Salmonella-Shigella Agar (SS – Difco®) medium. The inoculum was then spread evenly until absorption on the agar surface, and the plates were subsequently incubated in an oven at 35 ± 2 °C for 24-48 hours to read and observe typical colonies for *Salmonella* spp. (Ehsani *et al.*, 2019).

## RESULTS AND DISCUSSION

### Microbiological quality and sanitary status of industrial and free-range eggs

All samples analyzed had none of the bacteria used as a quality indicator by the current legislation used in this research. Both samples of free-range and commercial eggs analyzed showed the absence of *E. coli*, coagulase-positive *Staphylococcus aureus,* and *Salmonella* (Table 1).

**Table 1 –** Result in CFU $g^{-1}$, or presence (+) and absence (-) of the microbiological quality indicators analyzed in the free-range and commercial egg liquid sold in Santarém, Pará.

| Microorganism | Free-range eggs | | | | Industrial eggs | | | |
|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | S1 | S2 | S3 | S4 |
| *E. coli* | - | - | - | - | - | - | - | - |
| *Staphylococcus aureus* | - | - | - | - | - | - | - | - |
| Salmonella Shigella | - | - | - | - | - | - | - | - |
| MAHB (UFC $g^{-1}$) | - | - | - | - | $49x10^3$ | 235 | $15,8x10^2$ | - |

F (fair); S (supermarket); UFC (Colony Forming Unit); - (absent); MAHB (Mesophilic Aerobic Heterotrophic Bacteria)

After microbiological analysis, it was found that free-range eggs showed an absence of all the bacteria that usually contaminate eggs, cause quality decline, and consequently affect consumer health. On the other hand, in conventional systems there is a certain standard for production processes, hence they generally present a better microbiological quality. In the study by Ferreira *et al.*, 2017, it was observed that egg contamination is more related to the way in which they are stored than to the way they are produced, considering that the wrong handling and lack of adequate hygiene in the environment where this egg will be stored can compromise the quality of the shell, allowing the penetration of bacteria such as *Salmonella* spp.

Silva Rumao *et al.* (2020) found in their study that among the egg samples analyzed from a public market in São Luís, Maranhão, 12.5% showed the presence of Salmonella spp. inside the egg and 17.5% of the samples contained the same bacteria in the shell. This finding reveals the sanitary and hygienic deficit of some egg producers and the inadequate management attitudes employed at the place of sale of the product. These include the cleaning or washing of the eggs so that they are more presentable to the consumer's eyes, which culminates in the removal of their protective film, the first protective barrier against the invasion of microorganisms. In this context, there is a need to update, harmonize, and consolidate technical rules

and regulations related to food. According to studies showing that the main cause of diarrhea outbreaks in Brazil is Salmonellosis, and also for other reasons, the National Health Surveillance Agency approved a resolution in 2009 that obliges producers to put a warning to consumers on packaging and consumption of eggs.

As for the industrial eggs, it was also verified that no bacteria described in the product's regulations (*E. coli*, *Staphylococcus aureus,* and *Salmonella*) were found in the analyzed samples (Table 1). This is in line with (Degenhardt & Pereira, 2020), who tested 48 commercial eggs for the presence of Salmonella and found pathogens in none of the samples among the three analyzed parts, which according to the authors is suggests a satisfactory and effective handling and conservation method of eggs.

The standard plate count of Mesophilic Aerobic Heterotrophic Bacteria indicates the hygienic-sanitary quality of the food. Despite not being on the list of microbiological quality indicators in products of animal origin, mesophilic aerobic heterotrophic bacteria are considered an important parameter that reflects the sanitary conditions of the places of storage and transport of food.

In this research, the presence of mesophilic aerobic heterotrophic bacteria in (industrial) eggs in three of the four analyzed supermarkets was observed. The highest count was found in the S-1 samples, followed by S3 and S2. These data suggest that transport and storage conditions favor the contamination of eggs by bacteria from the MAHB group, which are typically environmental. Considering that among these there may be others that are potential pathogens or opportunists and are part of the MAHB population, this poses a risk to the consumer.

These microorganisms are a significant group that grows well in the range of 20°C to 45°C, related to the hygienic-sanitary condition, where most of the pathogens of interest in food are present (Jay, 2005). They live in all environments, in the air with dust particles, in the soil, in the water, and even in the human body. Despite not being the focus of inspection by health surveillance agencies and not having parameters that stipulate the amount that is tolerated in food, these bacteria can compromise the quality of eggs and even cause damage to human health.

Silva *et al.* (2007) offers useful information in assessing food quality, as high populations of bacteria may be related to deficiencies and/or failures in sanitation, process control, or ingredient quality. Despite there not being legislation regarding the microbiological standard for MAHB, Silva *et al.* (2007) and Morton (2001) described that the maximum number of these species (in raw ground beef, for example) should not exceed $1.0x10^5$ CFU $g^{-1}$.

The presence of BHT (butylated hydroxytoluene) is associated with contamination of foods such as meat in countries that are part of the European Union, where meat inspection is very strict, and meats that present maximum values for this type of microorganism of $5×10^6$ CFU $g^{-1}$ are considered fit for consumption. In Europe, this type of pathogen is responsible for foodborne outbreaks and infections (Fenelon *et al.*, 2019).

## Chemical composition of eggs

For the albumen chemical composition of free-range and commercial eggs evaluated at time zero, there was a significant effect ($p$<0.05) on the values of dry matter, crude protein, and lipids, with no significant difference ($p$>0.05) for mineral matter (Table 2). The results obtained corroborate the study by Saccomani (2015), where the values of lipids and crude protein also showed a significant difference for the different groups studied. However, no statistical difference was observed for dry matter.

**Table 2 –** Dry Matter (DM), Mineral Matter (MM), Crude Protein (CP) and Lipids (L) of the albumen expressed on the basis of dry matter.

| Eggs | DM (%) | MM (%) | CP (%) | L (%) |
|---|---|---|---|---|
| Free-range eggs | $36.17^a$±7.34 | $5.25^a$±0.32 | $33.06^a$±6.32 | $12.98^a$±1.58 |
| Industrial eggs | $17.97^b$±2.73 | $5.51^a$±0.19 | $16.89^b$±2.61 | $9.82^b$±1.17 |
| CV% | 17.75 | 4.77 | 17.29 | 12.05 |

Means followed by the same letter in the same column do not differ statistically (Tukey 5%); Normality: Shapiro Wilk; CV: Coefficient of variation.

As for the proximate analysis of the yolk of free-range and commercial eggs, there was a significant effect ($p$<0.05) on the values of dry matter, mineral matter, and crude protein (Table 3). In view of this, it is important to consider that the difference in mineral matter is related to the rearing system and the amount of foods rich in carbohydrates, fibers, carotenoids and minerals ingested by laying hens in the free-range system, since they live cage-free and have a more diverse diet when compared to laying hens that live in confinement and have a nutritional intake tailored to the purpose of production (Santos *et al.*, 2019).

For the ether extract variable, there was no statistical difference between the eggs of the two analyzed systems. However, it was found that the

values of lyophilized egg yolk with an amount of dry matter, protein, ether extract, and mineral matter were equal to 95%, 30%, 55%, and 4%, respectively (Cruz *et al.*, 2022). The results found in this study also corroborate the findings by Saccomani (2015), who was able to observe values for dry matter, crude protein, ether stratum, and mineral matter for eggs produced in a cage battery system equal to 98.66 %, 35.56%, 51.10%, and 3.64%, respectively, and values equal to 98.30%, 33.89%, 50.68%, 3.57%, for the same parameters for eggs produced in a free-range production system (with access to the paddock area for grazing).

**Table 3 –** Dry Matter (DM), Mineral Matter (MM), Crude Protein (CP) and Lipids (L) of the yolk expressed on the basis of dry matter.

| Eggs | DM (%) | MM (%) | CP (%) | L (%) |
|---|---|---|---|---|
| Free-range eggs | 83.03a±4.07 | 3.58±a0.05 | 30.50±a2.44 | 59.94±a1.39 |
| Industrial eggs | 45.84b±2.47 | 3.39±b0.11 | 15.03±b2.25 | 58.55±a3.03 |
| CV% | 5.15 | 2.23 | 11.50 | 3.75 |

Means followed by the same letter in the same column do not differ statistically (Tukey 5%); Normality: Shapiro Wilk; CV: Coefficient of variation.

## Internal and external quality of eggs

There was an interaction ($p$<0.05) for egg weight in the conventional production system (Table 4) with storage time, that is, there was a reduction in the average egg weight at 21 days of storage. The data of this study corroborate the findings of Medeiros *et al.* (2022) who observed a gradual loss in egg weight during 21 days of storage, around 0.32g per day.

Regarding free-range eggs, although there was a loss in weight in the course of storage, the difference in grams between day 0 and day 21 was 2.94g, thus not showing statistical significance for the weight of free-range eggs (Table 4). The results found in this research are similar to those described by Sabino *et al.* (2022) who verified in their study that the weight of free-range eggs tends to show gradual losses depending on the storage time and the environment in which they are stored.

The variation in egg weight during prolonged storage time, according to Yi *et al.* (2014), is due to its loss of water to the environment through its membranes and also the shell. Furthermore, according to the author, this loss is related to temperature, air circulation, and relative humidity of the storage place. In storage conditions with high temperatures and relative humidity, losses tend to increase more than in eggs that are kept at lower temperatures or submitted to refrigeration, for example (Pissinati *et al.*, 2014).

**Table 4 –** Average weight of commercial and free-range eggs.

| Eggs weight | Evaluation days | | | CV% |
|---|---|---|---|---|
| | 0 | 7 | 21 | |
| Industrial egg | 59.97$^{a}$±7.68 | 59.03$_{a}$±6.62 | 54.90$^{b}$±8.33 | 13.17 |
| Free-range egg | 51.11$^{a}$±6.25 | 50.87$^{a}$±6.82 | 48.17$^{a}$±5.39 | 11.06 |

Means followed by the same letter on the same line do not differ statistically (Tukey 5%); Normality: Shapiro Wilk; CV: Coefficient of variation.

Albumen heights for both industrial and free-range eggs did not show significant differences during the 21 days of storage, which can be explained by the storage at low temperatures (approximately 4ºC) (Table 5). The variations in the height of the albumen of the eggs is also associated with the non-homogenization thereof. The data found in this research diverged from what was found by (Henriques *et al.*, 2018), who observed a reduction in the size of the albumen height of the eggs over the days of treatment. It is important to understand that albumen size is important for egg quality assurance. From the moment it reduces its size and loses consistency, the integrity of the egg is compromised. This decrease is usually associated with changes in storage, notably caused by fluctuations in temperature; therefore, the longer the storage, the lower the height of the albumen (Medeiros *et al.*, 2022).

Lana *et al.* (2017) observed that the storage period and the egg chemistry itself can influence albumen decrease. This decrease in albumen height is associated with the prolonged period of egg storage and high temperatures, resulting in albumen liquefaction. This is mainly due to the dissolution of the chalazae, in addition to the degradation of albumin protein by carbonic anhydrase, resulting in water and carbon dioxide ($CO_2$). In this process, the water passes from the albumen to the yolk by osmosis, causing the weakening of the vitelline membrane (Rutz *et al.*, 2005).

The Haugh unit is used as a parameter to assess egg quality because it correlates egg weight with albumen size, yielding an internal quality coefficient. The lower this value, the worse the quality of the egg. In this context, values greater than 72 represent high-quality eggs (AA), between 60-71 eggs of intermediate quality (A), and below 60 eggs of low quality (B and C) (Saccomani, 2015). That being said, there was a statistically significant difference in this study, $p$<0.05, attributed to the 21st day of evaluation, which is the highest value for the variable analyzed. It can also be observed that commercial eggs evaluated on days 0 and 7 presented low quality, with Haugh units

between 54.54 and 59.44, respectively (Table 5). This result may have been influenced by the heterogeneity of the samples, indicating a possible difference in the age of the eggs sold, considering that they were all acquired on the same day, which indicates the same expiration date.

Regarding the quality of eggs, using the Haugh unit as a parameter, Narushin *et al.* (2021) attributed this loss to different types of storage and temperature, considering that eggs stored under refrigeration showed better quality than those stored at room temperature.

The HU values found in the free-range eggs were 68.14 on day 0, 64.37 on day 7, and 68.38 on day 21. That being said, it was observed that the HU variation of free-range eggs showed no statistical difference and can be classified as being of intermediate quality (A), being therefore good for consumption. This differs from the findings of Sabino *et al.* (2022), which showed a progressive linear loss of HU over the course of 36 days when evaluating free-range eggs stored in fresh environments, mainly between days 0 and 24. In the study by Lana *et al.* (2017), a decrease in egg quality was also observed from the moment of laying, resulting in an initial standard of excellence with HU equal to 94.62 and decreasing to 30.82 in 30 days.

**Table 5 –** Quality of eggs from the industrial and free-range system, based on the physical analysis of the variables.

| Parameters | Evaluation days | | | CV% |
|---|---|---|---|---|
| | 0 | 7 | 21 | |
| Albumen height (mm) | | | | |
| Industrial egg | 3.84$^{a}$±1.27 | 4.16$^{a}$±1.08 | 4.61$^{a}$±1.32 | 31.10 |
| Free-range egg | 4.54$^{a}$±0.91 | 4.25$^{a}$±1.35 | 4.45$^{a}$±0.75 | 24.25 |
| Haugh Unit | | | | |
| Industrial egg | 54.54$^{b}$±14.16 | 59.44$^{b}$±11.42 | 66.03$^{a}$±10.84 | 20.65 |
| Free-range egg | 68.14$^{a}$±6.89 | 64.37$^{a}$±11.34 | 68.38$^{a}$±7.53 | 14.55 |
| Yolk index | | | | |
| Industrial egg | 0.19$^{a}$±0.06 | 0.18$^{a}$±0.05 | 0.21$^{a}$±0.05 | 29.30 |
| Free-range egg | 0.24$^{a}$±0.05 | 0.26$^{a}$±0.07 | 0.23$^{a}$±0.05 | 24.73 |
| Specific gravity | | | | |
| Industrial egg | 1.007$^{a}$±0.005 | 1.005$^{a}$±0.001 | 1.005$^{a}$±0.002 | 0.28 |
| Free-range egg | 1.009$^{a}$±0.013 | 1.006$^{a}$±0.008 | 1.004$^{b}$±0.013 | 3.66 |
| Bark weight | | | | |
| Industrial egg | 5.68$^{a}$± 0.86 | 5.86$^{a}$±0.65 | 5.16$^{b}$±1.05 | 14.41 |
| Free-range egg | 4.72$^{a}$± 0.88 | 4.51$^{a}$±0.72 | 4.79$^{a}$±0.58 | 15.22 |
| % of bark | | | | |
| Industrial egg | 9.48$^{a}$±0.89 | 9.96$^{a}$±0.85 | 9.38$^{a}$±1.29 | 10.07 |
| Free-range egg | 9.20$^{b}$±1.08 | 8.89$^{b}$±1.00 | 9.97$^{a}$±0.95 | 11.34 |
| bark thickness | | | | |
| Industrial egg | 0.43$^{a}$± 0.05 | 0.43$^{a}$±0.05 | 0.39$^{b}$±0.09 | 14.80 |
| Free-range egg | 0.35$^{a}$± 0.08 | 0.31$^{a}$±0.06 | 0.33$^{a}$±0.10 | 23.71 |

Means followed by the same letter on the same line do not differ statistically (Tukey 5%); Normality: Shapiro Wilk; CV: Coefficient of variation.

Another important factor to assess egg quality is the yolk index, since it uses height and width dimensions to verify the consistency of the egg yolk (Santos Neto, 2019). Considering this, no statistical difference was observed during the 21 days in this study of industrial and free-range eggs. However, the values ranged between 0.18 and 0.26 (Table 5), below the values of 0.40 and 0.42 considered ideal (Li *et al.*, 2017). This low index can be attributed to genetic factors and even the age of hens, considering that older birds tend to have more liquid albumen, reduced laying frequency, and reduced yolk production from the hepatic synthesis in the follicles (Oliveira *et al.*, 2020). It is valid to consider that with mounting storage time, some phenomena such as evaporation by temperature and osmolarization of the albumen into the yolk occur, causing the decrease of the yolk index (Paiva *et al.*, 2019).

The ideal specific gravity (SG) value, according to theorists in the area, is 1.08 g $mL^{-1}$, with values ≤1.07 g $mL^{-1}$ considered of low quality (Henriques *et al.*, 2018). The values referring to the GE of eggs from the industrial and free-range production system were lower than what was considered ideal, with values between 1.004 and 1.009 g $mL^{-1}$ (Table 5).

Corroborating this study, Oliveira *et al.* (2020) also observed lower SG values in all groups of eggs that were evaluated and submitted to treatment, including the values of the control group whose SG observed was 1.033 g $mL^{-1}$.

Therefore, when evaluating SG, it is important to consider factors such as the age of the hen, the nutritional intake to which the animal is submitted, and how the egg is stored, given that very dry environments can cause the albumen to evaporate and consequently cause a decrease in density (Henriques *et al.*, 2018; Oliveira *et al.*, 2020). Saccomani (2015) also found similar SG values below the standard considered ideal in different types of rearing systems and temperatures, attributing such decrease to biochemical factors that occur in the egg during the storage period.

Eggshell weight is an important characteristic to study because the shell is essential for the maintenance of egg content, being associated with its resistance, impacts in the moment of laying, collection, selection, and during distribution logistics (Tomaszewski *et al.*, 2022). The percentage of shells ranged from 8.9 to 10.0% (Table 5). These levels differ from those found by Medeiros *et al.* (2022), who found values equal to 12% of the egg size. However, (Carvalho *et al.*, 2022) also found that the average percentage of bark was

also below 10%. The authors did not observe statistical variation influencing egg quality during the 30 days of storage.

The difference in the percentage of the shell may be related to the storage method and the nutritional management of laying hens. It was also observed that free-range eggs had lower percentages when compared to commercial eggs. This is associated with the way laying hens are raised, given that free-ranging birds tend to ingest more organic matter and some insects, while confined layers have a balanced nutritional supplementation with calcium and other minerals (Cunha *et al.*, 2017). This shows the decrease in egg quality during the storage interval, data also found by (Holanda *et al.*, 2020) when analyzing the main aspects related to the processing of eggs intended for consumption in Brazil.

In relation to shell thickness, it was observed in this work that commercial eggs showed a decrease on the 21st day, with a statistical significance of $p \leq 0.05$. This finding corroborates a study carried out by Pires *et al.*, 2020 that found egg thickness of commercial laying hens equal to 0.36 mm.

When considering factors that influence the decrease in eggshell thickness, Medeiros *et al.* (2022) cites temperature, considering that regions where the temperature is higher sometimes require laying hens to change their metabolism to stabilize body temperature. This causes hens to produce eggs with lower quality as compared to Brazilian regions with lower temperatures, such as the South and Southeast.

As for the shell thickness of free-range eggs, there was no statistical difference caused by storage time, considering $p \leq 0.05$. This is in disagreement with the study by Almeida *et al.* (2020) which found values equal to or greater than 0.33 mm in eggs from three different non-commercial laying hens. The evaluation of eggshell thickness is important to verify the resistance to physical damage and possible penetration of pathogenic microorganisms through the shell pores (Almeida *et al.*, 2020).

In this study, the percentage of albumen observed in the two production systems decreased in terms of storage time. The most expressive value was observed for industrial eggs (Table 6), where values were 61.22 and 56.87 on days 0 and 21, respectively, with a difference of 4.35.

Eggs from the free-range system had lower amounts of albumen but had less variation according to storage time, and also differed statistically with values of $p<0.05$ on days 7 and 21, with 55.84 and 52.17, respectively. Mazzuco (2008) states that the egg has approximately 63% of albumen, so the amount of shell and yolk is inversely proportional to the amount of albumen.

According to Rodrigues & Sala (2001), the decomposition of carbonic acid into carbon dioxide and water contributes to the loss of internal egg quality. This occurs due to the flow of carbon dioxide, which is taken to the exterior during its decomposition due to the porosity of the egg. The water that remains in the shell promotes the liquefaction of the albumen, making it less dense, causing a reduction in height, and causing an increase in pH, which leads to a process of chemical dissociation of the protein complex. One of the facts that may have contributed to the low albumen values in this study is the prolonged storage time and high temperatures to which eggs sold in the municipality of Santarém are subject.

As for yolk pH, there was a significant interaction with the storage time of eggs in the two production systems, as shown in (Table 6). This fact was not observed by Leandro *et al.* (2005) and Pascoal (2008) when studying eggs sold at fairs, grocery stores, and supermarkets. Yolk pH values found in the present study were higher than those found by these researchers, who obtained a range between 6.06 and 6.26.

Thus, they are outside the range considered ideal for fresh eggs, which should have a yolk pH equal to 6.0. After some time, this pH changes, increasing considerably due to the $CO_2$ content found inside the egg.

The albumen pH values of industrial eggs were not significant by Tukey's test at 5% significance. Similar results were also found by Pereira *et al.* (2014) in a study of eggs purchased in supermarkets. For the free-range eggs, there was a statistical difference, with a value on day 21 equal to 8.86.

According to Rodrigues & Sala (2001), the prolonged storage of eggs causes changes in some physical-chemical and sensorial characteristics of the albumen, such as loss of viscosity and increase in pH value. In fresh eggs, the albumen pH normally ranges from 7.6 to 7.9, while when they get older, carbon dioxide is released and pH values reach 9.5.

## CONCLUSION

The research was conducted to assess the quality of white eggs from various sources (supermarkets, free markets, and poultry farm eggs) in Brazil. The eggs have good microbiological quality, since no pathogens were detected in both free-range and

**Table 6 –** Quality of eggs from the conventional and free-range systems, based on the physicochemical analysis of the variables: albumen percentage, and yolk and albumen pH.

| Parameter | Evaluation days | | | |
|---|---|---|---|---|
| | 0 | 7 | 21 | CV% |
| % albumen Industrial eggs | 61.22$^{a}$±3.36 | 59.81$^{a}$±3.61 | 56.87$^{b}$±5.19 | 7.2 |
| Free-range eggs | 53.36$^{a}$±8.87 | 55.84$^{ab}$±4.68 | 52.17$^{ac}$±4.42 | 11.58 |
| yolk pH Industrial eggs | 6.34$^{b}$±0.23 | 6.51$^{b}$±0.37 | 6.62$^{a}$±0.32 | 4.83 |
| Free-range eggs | 6.48$^{a}$±0.32 | 6.52$^{a}$±0.27 | 6.40$^{b}$±0.23 | 4.98 |
| Albumen pH Industrial eggs | 9.09$^{a}$±0.22 | 9.02$^{a}$±0.10 | 9.07$^{a}$±0.09 | 1.68 |
| Free-range eggs | 9.05$^{a}$±0.22 | 9.02$^{a}$±0.11 | 8.86$^{b}$±0.24 | 2.48 |

Means followed by the same letter on the same line do not differ statistically (Tukey 5%); CV: Coefficient of variation.

industrial eggs. In general, free-range eggs presented a better nutritional quality than industrial eggs. The results show that, in general, eggs purchased both at fairs and supermarkets in Santarém have a low quality, which is probably associated with high temperatures in commercialization places. These results indicate that refrigerated storage of eggs is necessary in order to increase their shelf life and keep their internal qualities preserved for longe time.

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