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Quality of free-range and commercial eggs subjected to different storage periods and temperatures

Qualidade de ovos caipiras e comerciais submetidos a diferentes períodos e temperaturas de armazenamento

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Abstract

The objective was to evaluate the internal quality of eggs from commercial laying hens and free-range hens subjected to different storage periods and temperatures. For the experiment, 280 eggs were randomly distributed into different treatments, adopting a completely randomized design, in a 2 x 7 factorial arrangement of two temperatures, seven storage periods, totaling 14 treatments with 10 replications. The treatments consisted of two storage conditions: under refrigeration ($6 \pm 1.0^{\circ}$ C) and at room temperature ($26.6 \pm 1.0^{\circ}$ C). Eggs were analyzed for 30 days, with evaluations in different storage periods (0, 5, 10, 15, 20, 25 and 30 days). For each storage condition, 140 eggs were separated, 70 commercial eggs and 70 free-range eggs. There was a linear increase in egg weight loss, yolk weight, albumen pH, yolk pH, length and width of albumen and yolk of commercial and free-range eggs, as the storage period increased. There was a linear reduction in weight, height and albumen index and in the yolk index of commercial and free-range eggs as the storage period increased, with more pronounced responses for eggs stored at room temperature (P<0.05). Albumen percentage was linearly reduced only for commercial eggs (P<0.05). Eggs kept at room temperature reduce their quality after 15 days of storage, and the storage under refrigeration for 30 days is recommended to preserve the shelf life of the egg for consumption.

Key words: birds; commercial eggs; animal products; shelf time

Resumo

Objetivou-se avaliar a qualidade interna de ovos provenientes de poedeiras comerciais e de galinhas caipiras submetidos a diferentes períodos e temperaturas de armazenamento. Para o experimento, foram utilizados 280 ovos. Os ovos foram distribuídos aleatoriamente nos diferentes tratamentos adotando-se o delineamento experimental inteiramente casualizado, em esquema fatorial 2 x 7, duas temperaturas, sete períodos de armazenamento, totalizando 14 tratamentos com 10 repetições. Os tratamentos consistiram em duas condições de armazenamento: sob refrigeração ($6 \pm 1,0^{\circ}$ C) e em temperatura ambiente ($26,6 \pm 1,0^{\circ}$ C). Os ovos foram analisados por um período de 30 dias, com avaliações realizadas em diferentes períodos de armazenamento (0, 5, 10, 15, 20, 25 e 30 dias). Para cada condição de armazenamento, foram separados 140 ovos, sendo 70 ovos comerciais e 70 ovos caipiras. Ocorreu aumento linear na perda de peso dos ovos, peso da gema, pH do albúmen, pH da gema, comprimento e largura do albúmen e da gema dos ovos comerciais e caipiras, à medida que se aumentava o período de armazenamento. Verificou-se redução linear no peso, altura e índice do albúmen e no índice da gema dos ovos comerciais e caipiras conforme se aumentava o período de armazenamento, com respostas mais acentuadas para ovos acondicionados em temperatura ambiente (P<0,05). A porcentagem de albúmen foi reduzida linearmente apenas para ovos comerciais (P<0,05). Ovos mantidos sob temperatura ambiente reduzem a sua qualidade a partir dos 15 dias de armazenamento, sendo o armazenamento sob refrigeração durante o período de 30 dias, or armazenamento, sendo o armazenamento sob refrigeração durante o período de 30 dias, o recomendado para preservar a vida de prateleira do ovo para consumo.

Palavras-chave: Aves; Ovos comerciais; Produtos de origem animal; Tempo de prateleira

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Introduction

Fresh egg in shell is produced by domestic poultry of any species⁽¹⁾, and considered an excellent resource with high biological value protein, widely consumed worldwide⁽²⁾ as it is a versatile and affordable food for all social classes.

It is rich in nutrients, such as essential amino acids, vitamins, minerals and fatty acids, in sufficient quantities and proportions to help the growth and maintenance of body tissues⁽³⁾, making the egg an excellent food to compose a healthy diet.

The breeding system adopted in Brazil, for the most part, comprises birds kept in commercial farms, in cages, with intensive production, due to the focus on genetic improvement, nutrition, reproductive management, health and ambience^(4,5). On the other hand, free-range eggs are produced by free-range birds under extensive system and exposed to fewer stressors than those in intensive rearing systems(6,7). In addition, the free-range chicken diet is based on products of plant origin and without the addition of dyes or synthetic pigments, according to the rules established by the Ministry of Agriculture, Livestock and Supply⁽⁸⁾.

In general, the quality of a fresh egg is related to characteristics affecting the acceptance of eggs by the consumer⁽⁹⁾. The quality of a fresh egg is related to its external and internal characteristics. External quality is determined by the shell quality, resistance to handling, egg weight, age, genetic origin and health of the hens, in addition to shelf life and storage conditions⁽¹⁰⁾. The internal egg quality is also an important aspect to consider, especially when addressing product marketing opportunities⁽⁹⁾. This is determined through the evaluation of physical-chemical and nutritional parameters⁽¹¹⁾.

Albumen has a great influence on the internal quality of the intact egg, controlling the position of the yolk⁽¹²⁾. Solomon⁽¹³⁾ emphasized that, when a fresh egg is broken, yolk is turgid and located in a central position, surrounded by thick and thin albumen. However, when an old egg is broken, yolk is flabby, off-center and surrounded by a large area of liquid. In addition to these factors, other parameters such as egg diameter, eggshell, yolk color and the weight and pH of the white and yolk allow a more complete characterization of egg quality⁽⁹⁾.

Eggshell consists mainly of calcium carbonate (CaCO₃) (about 94% weight), and represents approximately 10% of the egg. The porous structure is semipermeable, limiting the passage of air and water⁽¹⁴⁾. Shell color influences regional consumer demand, but does not affect quality or flavor. Variations in shell color are related to the genetics of the hen, eggs with white or brown shell color are more commonly found⁽¹⁵⁾. Eggs from free-range hens have tougher shells compared to commercial eggs, as a result of the greater exposure of birds to sunlight, which allows for greater synthesis of vitamin D, in addition to higher calcium intake due to extensive grazing^(15,16).

Yolk color varies depending on the diet of the laying hen, and eggs from free-range hens have a darker yolk compared to commercial eggs, due to the higher content of beta-carotene, alpha-tocopherol and polyphenols. However, yolk color is not related to the nutritional value of an egg^(17,18).

In order to use the full nutritional potential of the egg, it is necessary to preserve this food at an ideal temperature and for an appropriate storage period, since the egg is a perishable food that can quickly lose its quality during the period between storage and consumption⁽¹⁹⁾. During storage, egg loses moisture through evaporation from shell pores at a rate that is influenced by room temperature. In this period, there is also a loss of carbon dioxide, which, combined with a reduction in moisture, raises the pH of the albumen and yolk, with a reduction in the height, thickness and percentage of the albumen, as well as a flattening of the yolk and, as a result, a reduction in egg weight^(20, 21, 22, 23).

According to the Brazilian legislation(8), the recommended temperature for storing fresh eggs varies between 8 and 15 °C, with the relative humidity of the air between 70 and 90%, for 30 days. However, according to the quality management system, regulation on egg for human consumption of the Ministry of Agriculture, Livestock and Supply (Ordinance $138^{(24)}$; Normative Instruction DIVISA/SVS $4^{(25)}$ and RDC $35^{(1)}$), the shelf life of eggs sold fresh is 30 days, and only refrigeration is recommended during storage in the commercial establishment. In addition, Resolution RDC $216^{(26)}$ establishes that, when kept out of refrigeration, eggs must be used within a maximum of one week.

Storage time and temperature for free-range eggs are reasons for research⁽²⁷⁾, since the legislation does not have specific regulations for free-range eggs, using recommendations for commercial eggs. Thus, there is a need for studies on the effect of the interaction time versus room temperature on the quality of eggs produced by commercial hens and free-range hens in semi-arid regions, in order to elucidate the ideal storage period and temperature for the preservation of egg quality, providing a suitable product for the consumer market.

Considering the importance of conservation and maintenance of the nutritional characteristics of the egg, the objective was to evaluate the internal quality of eggs from commercial laying hens and eggs from free-range hens stored for 30 days under refrigeration and at room temperature.

Material and methods

Experimental location

The experiment was conducted at the Poultry Laboratory of the Campus of Agricultural Sciences, Federal University of São Francisco Valley (CCA/UNIVASF), Petrolina, state of Pernambuco, Brazil (9°19'28" South latitude, 40°33'34" West longitude, 393m altitude). The climate is hot semi-arid⁽²⁸⁾, with a rainy season (BSh), average annual rainfall of 376 mm, average annual temperature of 26 °C and average relative humidity of approximately 61%.

Egg collection

A total of 280 eggs were used, 140 commercial white eggs (G) and 140 red free-range eggs (C). White eggs came from a commercial farm, where the hens were about 85 weeks of age, kept in an intensive rearing system. Red eggs were purchased from a small local producer. Eggs were selected so that they did not show deformation and/or cracks. Subsequently, eggs were classified according to weight, in which commercial white eggs were glassified into category type 1 (67 g \pm 2.94 g average weight) and red free-range eggs into category 2 (60 g \pm 4.93 g average weight).

After collection, eggs were stored under refrigeration (16 °C and 70% relative humidity; RH) for two days. Subsequently, all eggs were weighed on a digital scale (Tecnal, SHI-BL-3200H, Piracicaba, state of São Paulo, Brazil), identified and then packed in cellulose trays with a capacity of 30 eggs.

Treatments

Eggs were distributed in a completely randomized design, 2 x 7 factorial arrangement, totaling 14 treatments, with 10 replications. The treatments consisted of two storage conditions: under refrigeration ($6 \pm 1.0^{\circ}$ C and 74% RH) and at room temperature ($26.6 \pm 1.0^{\circ}$ C and 56% RH). Eggs were analyzed for a period of 30 days, with evaluations in different storage periods (0, 5, 10, 15, 20, 25 and 30 days). Each storage condition was evaluated with 140 eggs, 70 commercial eggs and 70 free-range eggs. During the entire experimental period, the maximum and minimum temperatures of the storage sites were recorded daily by digital thermo-hygrometers distributed in the storage room at room temperature and inside the refrigerator.

Weight loss

At the end of each storage period, the weights of eggs, albumen, yolk and shell were measured. Weight loss of whole eggs during storage was calculated using the equation proposed by Akter et al.⁽²⁹⁾:

Total weight loss (g)= initial weight – final weight

Weighing

After weighing, each egg was broken into a flat and smooth polyethylene surface and the yolk was separated from the albumen. Chalaza was carefully removed using tweezers. Before weighing, yolk was wrapped in paper towel to remove the adhered albumen. Shell was washed and dried at room temperature ($26 \,^{\circ}$ C) for 48 h, and then weighed. The percentages of yolk, shell and albumen were obtained by calculating the ratio: specific component weight/egg weight multiplied by 100, as presented by Lana et al.⁽³⁰⁾.

Albumen and yolk measurements and indices

Measurements of height, length, width and diameter of the albumen and yolk were taken with a digital caliper. A distance of 1 cm from the yolk was respected to measure the thick albumen. Data obtained in the measurements were used to calculate the indices⁽¹⁹⁾:

Albumen index (%)= albumen height / albumen diameter * 100

Yolk index (%) = yolk height/ yolk diameter * 100

Yolk color

Yolk color was evaluated visually and individually using the Yolk Color Fan⁽³¹⁾, which has a range of color intensity values ranging from 1 (pale yellow) to 16 (dark orange). The test was always performed by the same individual and under the same lighting conditions⁽³²⁾.

pH determination

Values of pH were measured individually in the albumen and in the yolk with a benchtop pH meter, dipping the probe into the sample solution⁽³³⁾.

Statistical analysis

Data were analyzed using the PROC GLM of the Statistical Analysis System University Software (SAS University) by means of analysis of variance and regression of parameters as a function of storage time at room temperature and under refrigeration. The significance of the parameters estimated by the models and the values of the coefficients of determination were adopted as criteria for selecting the regression models. To estimate the regression equation between pressure and volume data, the PROC REG procedure was used. The following statistical model was used:

$Y = \mu + Tj + eij$

where: μ = overall mean; Tj = days of storage as a function of temperature; eij = residual error.

Results and discussion

The increase in the storage period resulted in a linear increase in the weight loss of commercial (Table 1) and free-range (Table 2) eggs, with greater losses observed for eggs stored at room temperature (P<0.05), with an observed loss of 2.81 g for commercial eggs and 2.05 g for free-range eggs under this storage condition (Tables 1 and 2, respectively). This weight loss is related to moisture loss and the outflow of gases to the environment, as a consequence of the increase in pore size of the shell as the egg ages.

For Eke et al.⁽³⁴⁾, the greater weight loss in eggs stored at room temperature compared to those stored under refrigeration occurs because the cuticle covering the air pores of the shell of eggs stored in ambient conditions dries faster and begins to shrink, therefore, increasing shell porosity at a faster rate, facilitating the escape of carbon dioxide (CO_2) and water produced by biochemical reactions in the albumen of eggs. This escape resulted in a linear reduction in the albumen weight of commercial and free-range eggs (Tables 1 and 2, respectively;

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P<0.05), which became watery, which contributed to the egg weight loss.

The reduction in albumen weight was more pronounced in eggs stored at room temperature (commercial eggs and free-range eggs, Tables 1 and 2, respectively; P<0.05) compared to the average weight of eggs kept under refrigeration. Comparing the albumen weight of commercial and free-range eggs on day 0 with the day 30 of eggs stored at room temperature, a reduction of 10.48 g was found for commercial eggs (Table 1) and 4.57 g for free-range eggs (Table 2). For eggs subjected to refrigeration, there was a reduction of 5.57 g for commercial eggs (Table 1) and 3.66 g for free-range eggs (Table 2).

The reduction in albumen weight (commercial eggs and free-range eggs, Tables 1 and 2, respectively; P<0.05) and albumen % (commercial eggs; Table 1; P<0.001) and a

linear increase in commercial and free-range egg yolk weight with increasing storage period were probably the result of the biochemical reactions occurring in the egg, in which albumen enzymes hydrolyze the amino acid chains and release the water associated with proteins, which causes albumen thinning, loss of its viscosity, leaving it more spread out⁽³⁵⁾. Free water migrates from the albumen to the yolk, increasing its weight. This process tends to occur more slowly in eggs kept at refrigerated temperature, while at room temperature, eggs are more susceptible to these chemical reactions⁽³⁾. Thus, it can be inferred that during the storage period, water contained in the yolk can also migrate to the albumen. This was also reported by Luo et al.⁽³⁶⁾, who analyzed the effect of storage temperature on the quality of eggs stored for 84 days. Nevertheless, the authors suggest further studies to elucidate the reason why this water migration occurs.

 Table 1. Weights, percentages, pH values of egg components and yolk color of commercial eggs stored at different temperatures for 30 days of storage

Tommonotuno				Storage		SEM	P value					
Temperature	0	5	10	15	20	25	30	- Average	SEM	L	Q	
Weight eggs (g)												
A (26.6°C)	68.54	68.14	65.48	64.63	66.27	65.98	64.41	66.21	2.88	0.061	0.340	
R (6°C)	66.33	64.09	66.36	64.05	63.99	62.94	66.46	64.89	2.84	0.205	0.187	
Egg weight loss (g)												
A (26.6°C)	0.00	1.07	1.86	3.04	3.82	4.51	5.41	2.81	2.10	<0.001ª	0.067	
R (6°C)	0.00	0.34	0.79	1.16	1.60	2.52	2.68	1.29	1.09	<0.001 ^b	0.059	
Shell weight (g)												
A (26.6°C)	5.94	5.88	5.90	6.17	6.33	6.30	5.90	6.06	0.47	0.143	0.118	
R (6°C)	5.73	5.84	6.00	6.10	5.88	6.13	6.18	5.98	0.46	0.202	0.746	
Shell percentagem (%)												
A (26.6°C)	8.67	8.99	8.90	9.59	9.8 + 7	9.84	9.38	9.32	0.69	0.269	0.386	
R (6°C)	8 4 2	9.03	9.09	9 1 9	8 86	9.58	9 30	9.07	0.67	0.093	0 294	
R(0 C)	0.12	2.05	2.02	,,	Albumen w	veight (g)	2.20	2.07	0.07	0.095	0.291	
A (26.6°C)	43.66	42.16	40.21	38.80	38.31	34.73	33.18	38.72	3.15	<0.001°	0.852	
R (6°C)	43.72	41.39	41.18	40.84	40.17	39.26	38.16	40.67	2.86	0.001 ^d	0.664	
	10172	11107		10101	Albumen perc	centage (%)	20110	10107	2.00	01001	0.001	
A (26.6°C)	63.67	64.16	64.36	62.40	60.69	60.87	60.22	62.34	12.88	<.0001°	0.137	
R (6°C)	62.04	59.78	63.06	52.71	59.76	54.20	58.89	58.63	2.73	<.0001 ^f	0.763	
					Yolk wei	ght (g)						
A (26.6°C)	17.44	18.94	19.43	19.45	20.17	22.03	24.85	20.33	2.68	<0.001 ^g	0.051	
R (6°C)	18.39	18.70	19.10	19.76	18.63	19.59	21.13	19.33	1.70	0.018^{h}	0.227	
					Yolk percen	ntage (%)						
A (26.6°C)	27.66	27.43	26.63	28.55	30.41	30.04	30.17	28.70	7.70	0.397	0.155	
R (6°C)	28.76	30.34	28.08	37.45	30.64	36.39	31.79	31.92	2.56	0.901	0.370	
, ,					pH albi	umen						
A (26.6°C)	9.30	9.31	9.44	9.45	9.47	9.48	9.55	9.43	0.08	< 0.001 ⁱ	0.100	
R (6°C)	9.11	9.17	9.19	9.24	9.24	9.28	9.34	9.22	0.11	0.009^{j}	0.376	
, ,					pH y	olk						
A (26.6°C)	6.09	6.24	6.26	6.48	6.82	6.88	6.89	6.52	0.16	<0.001 ^k	0.485	
R (6°C)	6.08	6.09	6.18	6.25	6.29	6.31	6.37	6.22	0.11	< 0.0011	0.172	
. ,					Yolk c	olor						
A (26.6°C)	9.10	8.80	8.60	8.60	8.20	9.00	8.60	8.70	28.03	0.110	0.092	
<u>R (6°C)</u>	8.50	8.10	8.90	8.60	9.00	9.20	9.20	8.78	0.61	0.083	0.224	

A= room temperature; R= under refrigeration; SEM= standard error of the mean; L= significant for linear effect; Q= significant for quadratic effect. Significant at 5% probability level. Equations: $\hat{Y}^a=0.123+0.09x$, R²=0.88; $\hat{Y}^b=0.19-0.17x$, R²=0.94; $\hat{Y}^c=36.99-0.17x$, R²=0.72; $\hat{Y}^d=36.79-0.08x$, R²=0.21; $\hat{Y}^c=17.20+0.044x$, R²=0.80; $\hat{Y}^i=16.56+0.02x$, R²=0.83; $\hat{Y}^a=9.41+0.002x$, R²=0.60; $\hat{Y}^i=9.29+0.005x$, R²=0.53; $\hat{Y}^i=6.20+0.01x$, R²=0.66; $\hat{Y}^j=6.14+0.01x$, R²=0.56

Tomporature				Storage p	- Auguago	SEM	P value					
Temperature	0	5	10	15	20	25	30	- Average	SEN	L	Q	
Weight eggs (g)												
A (26.6°C)	59.43	60.24	59.85	58.44	56.78	58.38	60.65	59.11	3.99	0.402	0.156	
R (6°C)	55.06	59.08	58.04	55.64	59.10	55.45	56.12	56.93	4.05	0.084	0.279	
Egg weight loss (g)												
A (26.6°C)	0.00	1.03	1.99	2.87	3.44	4.56	5.22	2.05	2.14	0.033ª	0.051	
R (6°C)	0.00	0.34	0.90	1.40	1.75	2.36	2.46	0.99	2.14	$< 0.001^{b}$	0.066	
Shell weight (g)												
A (26.6°C)	5.61	5.65	5.96	5.68	5.63	5.87	6.43	5.83	0.71	0.835	0.077	
R (6°C)	5.69	6.01	5.66	5.56	5.76	5.68	5.63	5.71	0.62	0.924	0.628	
					Shell perc	centage (%)	1					
A (26.6°C)	9.44	9.38	9.93	9.72	9.93	10.09	10.57	9.86	4.22	0.177	0.233	
R (6°C)	10.29	10.16	9.74	9.99	9.74	10.25	10.01	10.03	0.79	0.071	0.111	
					Albumen	weight (g)						
A (26.6°C)	37.05	36.55	33.72	35.67	34.38	31.08	32.48	34.42	3.39	<0.001°	0.940	
R (6°C)	38.13	37.30	36.04	35.86	35.15	35.09	34.47	35.58	3.38	0.036 ^d	0.163	
				1	4lbumen pe	ercentage (%	%)					
A (26.6°C)	62.34	63.28	61.15	62.06	59.18	59.95	58.75	60.96	7.25	0.064	0.154	
R (6°C)	59.42	58.30	62.31	55.77	60.94	58.57	59.66	59.28	2.45	0.054	0.155	
					Yolk w	eight (g)						
A (26.6°C)	16.76	17.33	17.45	18.55	18.68	19.01	19.29	17.86	2.23	0.024°	0.052	
R (6°C)	16.23	16.46	16.46	16.61	17.02	17.37	17.47	16.80	1.54	< 0.001 ^f	0.979	
					Yolk perc	entage (%)						
A (26.6°C)	28.23	27.34	28.92	28.22	30.88	29.96	30.68	29.18	3.64	0.052	0.191	
R (6°C)	30.28	31.55	27.95	34.24	29.32	31.18	30.32	30.69	2.19	0.132	0.311	
					pH al	lbumen						
A (26.6°C)	9.26	9.36	9.36	9.37	9.43	9.44	9.45	9.38	0.07	<.0001 ^g	<.0001	
R (6°C)	9.07	9.15	9.22	9.23	9.24	9.28	9.28	9.21	0.08	<.0001 ^h	0.058	
					рН	' yolk						
A (26.6°C)	6.19	6.21	6.31	6.33	6.39	6.53	6.59	6.36	0.17	0.003 ⁱ	0.052	
R (6°C)	6.12	6.16	6.20	6.26	6.27	6.29	6.38	6.24	0.10	0.019 ^j	0.182	
					Yolk	color						
A (26.6°C)	9.60	8.90	8.50	8.80	9.30	10.20	10.20	9.36	1.55	0.707	0.828	
<u>R (6°C)</u>	9.80	8.70	8.40	9.00	9.40	9.20	9.70	9.17	1.30	0.398	0.588	

 Table 2. Weights, percentages, pH values of egg components and yolk color of free-range eggs stored at different temperatures for 30 days of storage

A= room temperature; R= under refrigeration; SEM= standard error of the mean; L= significant for linear effect; Q= significant for quadratic effect. Significant at 5% probability level. Equations: $\hat{Y}^a=0.123+0.09x$, $R^2=0.88$; $\hat{Y}^b=0.19-0.17x$, $R^2=0.94$; $\hat{Y}^c=36.99-0.17x$, $R^2=0.72$; $\hat{Y}^d=36.79-0.08x$, $R^2=0.21$; $\hat{Y}^c=17.20+0.044x$, $R^2=0.80$; $\hat{Y}^i=16.56+0.02x$, $R^2=0.83$; $\hat{Y}^a=9.41+0.002x$, $R^2=0.60$; $\hat{Y}^b=9.29+0.005x$, $R^2=0.53$; $\hat{Y}^i=6.20+0.01x$, $R^2=0.66$; $\hat{Y}^i=6.14+0.01x$, $R^2=0.56$

Albumen pH of commercial and free-range eggs increased linearly with increasing days of storage, according to room and refrigerated temperatures (commercial eggs and free-range eggs, Tables 1 and 2, respectively; P<0.05). The pH values found are within the pH range considered adequate for eggs subjected to different storage periods $(9.0-9.7)^{(37)}$. For the yolk pH, a linear increase in yolk pH of commercial and free-range eggs was also found with increasing days of storage and as a function of room and refrigerated temperatures (commercial eggs and free-range eggs, Tables 1 and 2, respectively; P<0.05), between 6.0 and 6.9, established by

Dutra et al. $^{(38)}$ for eggs subjected to different storage periods.

There was no effect of storage period as a function of temperature on egg weight, shell weight and percentage, and yolk percentage of commercial eggs (Table 1; P>0.05). There was no effect of storage period as a function of temperature on egg weight, shell weight and percentage, albumen percentage and yolk percentage of free-range eggs (Table 2; P>0.05). Many consumers believe that the main differences between commercial and free-range eggs are: shell color, egg size and yolk color. When these consumers arrive at the supermarket and

come across white-shelled eggs and brown-shelled eggs, they prefer the latter because they believe that these have greater nutritional value than white eggs. However, it is worth mentioning that the eggshell color does not interfere with its quality and there is no nutritional difference between white and brown eggs⁽³⁹⁾. In fact, eggshell color is a genetic characteristic of the bird and its intensity is one of the basic selection criteria, especially considering ornamental breeds⁽⁴⁰⁾. Yolk color varies according to the amount of pigment ingested⁽⁴¹⁾.

There was no effect of the storage period as a function of temperatures on yolk color, with a variation from 8.45 to 8.90 for the yolk of commercial eggs (Table 1; P>0.05) and from 8.45 to 9.95 for the yolk of free-range eggs (Table 2; P>0.05). It was observed that free-range eggs showed greater variation in yolk color compared to commercial eggs. This was expected, due to the greater amount of xanthophyll pigments (carotenoids) in the diet of free-range hens. These pigments directly influence the color of the yolk and, because free-range hens are raised in extensive systems, their diet is quite varied, eating mainly vegetables, which are rich in carotenes, and laying

eggs with more intensely colored yolks than birds raised in commercial systems that often receive low-pigment feeds^(41,42).

It is possible to determine the quality of the egg through the height of the thick albumen, because as the egg gets older, the proportion and height of the thick albumen are reduced. As a result, the amount of thin albumen is increased, it has greater fluidity and, as a result, it becomes less consistent, causing a loss in height. This process is accelerated when eggs are stored at high temperatures⁽⁴³⁾. This was verified in the present study, in which commercial and free-range eggs stored at room and refrigerated temperatures linearly reduced albumen height with increasing storage period, with lower albumen heights observed for eggs stored at room temperature when compared to those stored in a refrigerated environment (commercial eggs and free-range eggs, Tables 3 and 4, respectively; P<0.001), since the high temperature accelerates the albumen thinning process, because it increases the loss of CO₂ to the external environment through a concentration gradient.

Table 3. Height, length and width of albumen and yolk of commercial eggs stored at different temperatures during 30 days of storage

Temperature -	Storage periods								SEM	P value	
	0	5	10	15	20	25	30	Average	SEM	L	Q
Albumen height (mm)											
A (26.6°C)	5.69	4.33	3.56	2.54	2.08	2.04	1.94	3.26	2.52	<0.001ª	0.060
R (6°C)	5.71	5.40	5.24	5.10	4.90	3.99	3.42	4.82	0.82	$< 0.001^{b}$	0.214
Albumen length (mm)											
A (26.6°C)	107.45	122.19	133.28	140.20	141.95	143.05	143.19	133.05	26.79	<0.001°	0.919
R (6°C)	110.91	114.83	116.30	117.87	119.50	121.84	122.39	117.66	7.82	$< 0.001^{d}$	0.641
Albumen width (mm)											
A (26.6°C)	87.93	110.32	119.25	120.39	129.98	131.98	135.16	119.29	26.56	<0.001°	< 0.001
R (6°C)	88.17	90.64	93.35	94.44	96.34	99.80	100.05	94.69	6.96	$< 0.001^{\mathrm{f}}$	0.826
					Yolk heigh	ht (mm)					
A (26.6°C)	15.83	13.25	11.08	9.16	7.74	6.82	6.27	10.02	8.83	$< 0.001^{g}$	0.090
R (6°C)	16.38	15.93	15.64	15.19	15.12	14.10	14.00	15.19	1.21	$< 0.001^{h}$	0.733
Yolk length (mm)											
A (26.6°C)	46.67	50.88	54.08	56.38	58.33	62.67	65.16	56.31	12.32	$< 0.001^{i}$	0.632
R (6°C)	46.05	46.17	46.57	46.64	46.96	50.22	50.31	47.56	1.67	$< 0.001^{j}$	0.056
					Yolk widt	h (mm)					
A (26.6°C)	44.02	49.44	52.18	53.95	56.28	59.63	61.76	53.89	20.08	$< 0.001^{k}$	0.232
R (6°C)	44.18	44.42	44.54	44.95	45.337	47.32	47.75	45.50	3.79	0.0041	0.319

A= room temperature; R= under refrigeration; SEM= standard error of the mean; L= significant for linear effect; Q= significant for quadratic effect. Significant at 5% probability level. Equations: $\hat{Y}^{a}=5.30-0.14x$, R²=0.84; $\hat{Y}=5.90-0.07x$, R²=0.89; $\hat{Y}^{c}=116.16+1.13x$, R²=0.80; $\hat{Y}^{d}=112.13+0.37x$, R²=0.97; $\hat{Y}^{c}=98.32+1.40x$, R²=0.86; $\hat{Y}^{r}=88.59+0.41x$, R²=0.98; $\hat{Y}^{e}=14.83-0.32x$, R²=0.96; $\hat{Y}^{b}=16.41-0.08x$, R²=0.96; $\hat{Y}^{i}=47.39+0.59x$, R²=0.99; $\hat{Y}^{i}=45.28+0.15x$, R²=0.77; $\hat{Y}^{k}=45.57+0.55x$, R²=0.98; $\hat{Y}^{i}=43.65+0.12x$, R²=0.85

As a result of albumen thinning with increasing days of storage, a linear increase can be found for the length and width of the albumen of commercial and free-range eggs stored for 30 days as a function of room and refrigerated temperatures, with a more pronounced growth in the eggs kept in the room temperature (P<0.001; Tables 3 and 4).

With 30 days of storage, an increase of 35.74 mm and 47.23 mm was observed, respectively, for the length and width of the albumen of commercial eggs kept at room temperature and for commercial eggs kept in refrigeration, the length and width of the albumen increased by 11.48 mm and 11.88 mm, respectively, in relation to the initial measurement (Table 3). Regarding

the free-range eggs at room temperature, there was an increase of 35.20 mm and 36.10 mm for the length and width of the albumen, and for eggs under refrigeration, increases of 21.26 mm and 8.85 mm for the length and width of the albumen were obtained in relation to the initial measurement, at the end of the 30-day evaluation (Table 4).

Table 4. Height, length and width of albumen and yolk of free-range eggs stored at different temperatures for 30 days of storage

Tomponature		Storage periods								P va	alue
Temperature	0	5	10	15	20	25	30			L	Q
Albumen height (mm)											
A (26.6°C)	5.87	4.73	3.56	3.50	3.44	2.89	2.85	3.84	17.23	$<.0001^{a}$	0.489
R (6°C)	5.07	4.91	4.67	4.64	4.56	4.22	4.06	4.59	0.87	$<.0001^{b}$	0.806
Albumen length (mm)											
A (26.6°C)	98.85	113.36	128.21	129.89	131.17	133.22	134.05	124.11	12.41	<.0001°	0.173
R (6°C)	101.96	103.90	104.63	106.13	109.67	120.71	123.22	110.03	14.26	$<.0001^{d}$	0.131
Albumen width (mm)											
A (26.6°C)	81.20	100.38	102.17	108.77	115.86	117.19	117.30	106.12	20.34	<.0001°	0.413
R (6°C)	88.12	88.67	88.73	89.16	89.64	93.10	96.97	90.63	10.14	$< .0001^{f}$	0.421
			Ye	olk height (mm)						
A (26.6°C)	16.28	14.99	13.46	11.52	11.38	8.60	8.23	12.02	5.27	<.0001g	0.817
R (6°C)	16.10	15.85	15.57	15.52	14.14	13.96	13.62	14.97	1.01	0.042^{h}	0.807
			Ye	olk length (mm)						
A (26.6°C)	43.21	47.96	48.96	51.41	51.77	53.63	53.82	50.09	2.32	$<.0001^{i}$	<.0001
R (6°C)	44.53	44.99	45.58	45.70	46.40	46.80	47.47	45.92	1.23	$<.0001^{j}$	0.813
			Y	olk width (1	nm)						
A (26.6°C)	41.30	46.81	47.53	48.99	49.81	51.53	51.86	47.12	5.52	<.0001 ^k	0.255
R (6°C)	42.42	42.46	42.69	42.91	43.09	43.52	44.06	43.02	1.44	<.00011	0.622

A= room temperature; R= under refrigeration; SEM= standard error of the mean; L= significant for linear effect; Q= significant for quadratic effect. Significant at 5% probability level. Equations: $\hat{Y}^{a}=5.21-0.91x$, R²=0.82; $\hat{Y}^{b}=5.07-0.03x$, R²=0.96; $\hat{Y}^{c}=108.22+1.06x$, R²=0.76; $\hat{Y}^{d}=99.06+0.73x$, R²=0.86; $\hat{Y}^{c}=89.46+1.11x$, R²=0.85; $\hat{Y}^{f}=86.74+0.26x$, R²=0.75; $\hat{Y}^{e}=15.32-0.22x$, R²=0.59; $\hat{Y}^{b}=16.32-009x$, R²=0.91; $\hat{Y}^{i}=45.21+0.33x$, R²=0.88; $\hat{Y}^{i}=44.50+0.09x$, R²=0.99; $\hat{Y}^{k}=43.47+0.28x$, R²=0.70; $\hat{Y}^{i}=42.22+0.05x$, R²=0.93

Similar to the results observed in the present study, Altunatmaz et al.⁽⁴⁴⁾ evaluated the influence of storage temperature and time on the internal and external quality of eggs stored at room temperature (25 °C) and under refrigeration (5 °C) for 28 days, and reported that the albumen height is significantly reduced with the extension of the storage period as a function of storage temperature, which directly affects the dimensions of length and width of the albumen of the eggs studied. The authors emphasize that these changes are more pronounced for eggs subjected to 25 °C, when compared to 5 °C.

The increase in days of storage of commercial and freerange eggs as a function of room and refrigerated temperatures resulted in a decreasing linear effect for yolk height, with a more pronounced reduction observed for eggs kept at room temperature (9.56 mm commercial eggs

versus 8.05 mm free-range eggs), when compared to those kept under refrigeration (2.38 mm commercial eggs versus 2.48 mm free-range eggs) at the end of the 30 days of evaluation (commercial eggs and free-range eggs, Tables 3 and 4, respectively; P<0.05). For measurements of length and width of the yolk, there was a linear increase as the days of storage of commercial and free-range eggs increased, depending on the room and refrigerated temperatures (commercial eggs and free-range eggs, Tables 3 and 4, respectively; P<0.05). Larger length and width dimensions were verified for eggs stored at room temperature, with increases in yolk length and width of 18.49 mm and 17.74 mm, respectively, for commercial eggs (Table 3; P<0.05) and 10.61 mm and 10.56 mm, for free-range eggs (Table 4; P<0.05) at the end of the 30 days of evaluation. Eggs stored in a refrigerated environment

had their initial measurements of length and width of the yolk increased at the end of the 30 storage days, but with a lower intensity of increase in the measurements in relation to eggs subjected to room temperature.

Corroborating the results of the present study, Feddern et al.⁽³⁾ evaluated egg quality under different storage conditions, seasons and laying hen lines, observed that the length and width of the yolk of all eggs analyzed showed a significant increase during the storage period and a reduction in yolk height values.

The albumen index is directly correlated with height measurements and the diameter of the thick albumen. In this study, the storage period caused a linear reduction for the albumen index of commercial and free-range eggs (P<0.05; Figure 1), with lower albumen indices for eggs kept at room temperature, with a reduction from 0.073

(day 0) to 0.014 (day 30) at the end of the storage period for commercial eggs (Figure 1A) and from 0.073 (day 0) to 0.022 (day 30) at the end of the storage period for freerange eggs (Figure 1B). The reduction found in the albumen index evidences the loss of egg quality throughout the storage period, especially for eggs stored at room temperature.

The increase in storage days as a function of temperature also caused a reduction in the yolk index of commercial and free-range eggs (P<0.05; Figure 1), with lower yolk index for eggs stored at room temperature. Considering the yolk index presented by Dutra et al.⁽³⁸⁾ for the classification of fresh eggs (0.30-0.50), it can be inferred that the quality of yolks of commercial and free-range eggs stored under refrigeration presented higher quality than those stored at room temperature.



Figure 1. Albumen and yolk indices of commercial and free-range eggs stored at different temperatures during 30 days of storage. (Figure 1A - albumen index of commercial eggs; Figure 1B - yolk index of commercial eggs; Figure 1C - albumen index of free-range eggs; Figure 1D - yolk index of free-range eggs). Equations: albumen index of commercial eggs at room temperature: $\hat{Y}=0.06-0.0013x$, $R^2=0.91$; albumen index of commercial eggs under refrigeration: $\hat{Y}=0.06-0.003x$, $R^2=0.89$; yolk index of commercial eggs at room temperature: $\hat{Y}=0.34-0.05x$, $R^2=0.95$; yolk index of commercial eggs under refrigerator: $\hat{Y}=0.36-0.09x$, $R^2=0.99$; albumen index of free-range eggs at room temperature: $\hat{Y}=0.06-0.0013x$, $R^2=0.97$; albumen index of free-range eggs under refrigerator: $\hat{Y}=0.36-0.09x$, $R^2=0.99$; albumen index of ree-range eggs under refrigerator: $\hat{Y}=0.36-0.0013x$, $R^2=0.93$; yolk index of free-range eggs under refrigerator: $\hat{Y}=0.36-0.0013x$, $R^2=0.93$; yolk index of free-range eggs under refrigerator: $\hat{Y}=0.36-0.0013x$, $R^2=0.93$; yolk index of free-range eggs under refrigerator: $\hat{Y}=0.36-0.0013x$, $R^2=0.93$; yolk index of free-range eggs under refrigerator: $\hat{Y}=0.36-0.004x$, $R^2=0.79$

According to Qi et al.⁽³⁷⁾, when the yolk index is lower than 0.25, it means that the yolk is very fragile. Commercial eggs stored at room temperature had the lowest yolk index (0.18), which is related to the lower average height (10.02 mm) that the yolks of these eggs

presented compared to refrigerated eggs (15.19 mm), given that this index is based on the relationship between height and diameter, that is, the smaller the height and the larger the diameter, the lower the yolk index. According to Dutra et al.⁽³⁸⁾, high temperatures cause stretching and

increase in the permeability of the vitelline membrane, which accelerates the passage of water from the albumen to the yolk, which has higher osmotic pressure, causing it to lose its spherical shape and becomes more elongated and flattened, causing a reduction in the volk index.

According to the Brazilian legislation, refrigeration of eggs is not mandatory, and eggs must be stored at room temperature from the moment of laying to final distribution, being packaged under refrigeration only by consumers⁽²³⁾. Arruda et al.⁽⁴⁵⁾ mentioned that eggs stored for 28 days at room temperature, present changes in their quality, which reinforces the evidence of the importance of refrigeration in the preservation and storage of the egg. Normative instruction 1, of December 8, 2020, recommends a maximum period of 30 days for the storage of eggs using temperatures between 4 and 12 °C, with control of relative air humidity⁽⁴⁶⁾. Thus, it was observed that commercial and free-range eggs held better results for the characteristics evaluated when stored under refrigeration during storage for 30 days. However, the information established by the legislation is mainly related to commercial eggs, not providing accurate information regarding the shelf life of eggs produced by free-range hens^(47,48,49).

Conclusion

The quality of commercial and free-range eggs is influenced by temperature and storage periods. Commercial eggs stored at room temperature reduce their quality after 15 storage days. Free-range eggs stored at room temperature also reduce their quality after 15 storage days. It is recommended to store commercial eggs and free-range eggs under refrigeration for a period of 30 days, in order to preserve the shelf life of the egg intended for consumption.

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

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