



Physical-Chemical and Sensorial Quality of Eggs Coated With Copaiba Oil Biofilm and Stored At Room Temperature for Different Periods*

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ABSTRACT

The present study aimed at evaluating the effects of a coating based on copaiba oil on the quality of eggs stored for different periods. Eggs were coated with copaiba oil solutions (4, 8, 12, 16, or 20% copaiba oil) or not (control) and stored for 1, 7, 14, 21, 28 or 35 days at room temperature. The following parameters were evaluated: egg weight loss, egg components (albumen, yolk, and eggshell) relative weights, egg specific gravity, Haugh units, eggshell quality, and sensorial attributes. Eggs stored for 35 days presented the highest weight losses. Coating (20% copaiba oil solution) reduced egg weight loss up to 41.02%. Coated eggs presented better internal quality (yolk and albumen heights, specific gravity, and eggshell strength). Sensorial attributes were negatively affected by storage time (aroma and flavor), and copaiba oil solution (aroma, appearance, and flavor), with the natural odor of copaiba described by the tasters. Egg internal, external, and sensorial quality worsened with storage time. Solutions containing 16 and 20% copaiba oil can be used as a biofilm to coat eggs, preserving its internal and external quality, and may affect their sensory characteristics, and therefore, may be a viable alternative for maintaining the internal quality of eggs stored at room temperature for long periods.

INTRODUCTION

The egg is considered as one of the most complete foods in the human diet, especially due its composition, as it is rich in vitamins, minerals, fatty acids and proteins of excellent biological value (Rêgo *et al.*, 2012). According to the Brazilian Association of Animal Protein (ABPA, 2017), there was a considerable increase in the *per capita* consumption of eggs between 2010 and 2017 (22%), with 190 eggs *per capita* in 2017, mainly due to the accessible price. Eggs are present in the diet of 99% of the Brazilian families.

This increase in egg consumption and its nutritional benefits to the population depends on the quality of the product offered to the consumers, and there are several external and internal egg quality parameters that affect its acceptability. External egg quality is related to the eggshell (shape, strength, and hygiene), and internal quality aspects are related to albumin, yolk, air chamber, color, odor, and flavor (Mendes, 2010). The Brazilian legislation (Brazil, 1997) establishes minimum conditions for internal egg quality; however, only egg weight and eggshell characteristics are considered by the producers.

Research to evaluate techniques and products that may extend egg storage time has been carried out, particularly on coating treatments of the eggshell surface that act as "artificial cuticles," reducing gas exchange through the eggshell and maintaining the egg's natural



quality characteristics for a longer period of time. According to Waimaleongora-Ek *et al.* (2009), an oil-based coating has been very effective in delaying egg weight loss and preserving the internal quality due to its hydrophobicity, good sealing characteristics, and long storage stability.

In this context, the Amazonian region has several native plant species with economic, technological and nutritional potential (Clement *et al.*, 2005). Among these, the genus *Copaifera*, belonging to the subfamily *Caesalpinoideae* (Andrade Junior *et al.*, 2000; Veiga Junior & Pinto, 2002) stands out, with 16 species occurring in Brazil and only four in the North region, where copaiba oil is widely marketed and used in popular medicine (Cascon & Gilbert, 2000).

Copaiba oil has a number of characteristics that make it possible to coat commercial eggs, such as flexible coverage and transparency, as well as anti-inflammatory, antiseptic, antimicrobial, antibacterial, antifungal, disinfectant, antibiotic, antitetanic, and anticancer properties (Mendonça & Onofre, 2009).

Considering the above, the present study aimed at evaluating the efficiency of a biofilm based on copaiba oil for coating eggs stored for different periods.

MATERIAL AND METHODS

This study was conducted at the Laboratory of Poultry Technology, Poultry Sector, Department of Animal and Vegetable Production (DPAV), College of Agrarian Sciences (FCA), Federal University of Amazonas (UFAM), South Sector at the University Campus, Manaus, State of Amazonas, Brazil.

The experimental protocol was approved by the Ethics Committee on the Use of Animals (CEUA) of the College of Agrarian Sciences of the Federal University of Amazonas under protocol n. 015/2016.

A total of 446 large white eggs from Hisex White hens (48 wks of age) were used. These birds (1.421 ± 0.053 kg average body weight) were housed in cages and fed to diets formulated according to Rostagno *et al.* (2017).

The coating solutions (1L) containing copaiba oil (SRC) were manufactured according the formula: SRC = % copaiba oil + solvent (ethyl ether) + 1% BHT (antioxidant). The copaiba oil (*Copaiferamultijuga* Hayne) was obtained from Consórcio de Produtores Sateré Mawé, located in Parintins, state of Amazonas (distant 370 Km from Manaus-AM). For coating application, eggs were immersed in the solution for one minute, and then dried at room temperature.

Storage temperature and air relative humidity were recorded twice a day (09:00 and 15:00 hours) using a digital thermo-hygrometer positioned at egg height, between the eggs, and at center of the storage room, with averages of 28.48 °C and 61.40%, respectively.

For the physical-chemical analysis, 396 eggs were distributed in a completely randomized design in a 6x6 factorial arrangement: control (egg not immersed in any solution) + five copaiba oil coating solutions (4, 8, 12, 16, or 20% copaiba oil) and six storage periods (1, 7, 14, 21, 28, or 35 days), with x replicates of 11 egg each.

Eggs were weighed on an electronic scale to the nearest 0.01 g immediately before storage, and at the end of each storage period. In order to determine egg specific gravity, eggs were placed in wire baskets, and immersed in plastic buckets containing different levels of sodium chloride (NaCl) to obtain densities ranging between 1,075 and 1,100 g/cm³, at 0.005-intervals. Eggs were removed when they floated to the surface, recording the density value of the solution.

Eggs were broken and their contents placed on a flat glass plate to measure albumen and yolk heights at the medial region between the outer border of the albumen and the yolk, using an electronic caliper; the values are expressed in millimeters. To determine albumen and yolk weights, a manual albumen and yolk separator was used. The albumen and the yolk were placed in plastic cups, tared-weight in analytical scale, and their weights were calculated relative to egg weight. Yolk color was evaluated using the colorimetric fan (Roche) with scores between 1 and 15.

Eggshells from broken eggs were washed, dried at room temperature for 48 h, weighed in digital scale, and their weight was calculated relative to egg weight. Dry eggshells were used to determine eggshell thickness, measured using a micrometer. The readings were performed in three regions of the eggshell: basal, meridional and apical, and the values were recorded. From the values obtained in the three regions, the average of the eggshell thickness, in micrometer, was calculated.

Haugh units were calculated based on egg weight and albumen height values according to the formula: $UH = 100 \times \log (H + 7.57 - 1.7 \times W^{0.37})$, where H = albumen height (mm); and W = egg weight (g).

Eggshell strength was determined in a machine at the Materials Laboratory of the Superior College of Technology of State of Amazonas University. This machine was connected to a computer, generating the



force levels (in Newtons) required to break the eggshell in the parallel and meridional direction.

For the sensory analysis, eggs were distributed according to a completely randomized design in a 2x2 factorial arrangement: eggs coated or not with copaiba oil solution (20%) and stored for 1 or 18 days at room temperature. These two storage periods and the copaiba oil solution were determined from the egg quality results.

Sensory analysis used a 9-point hedonic test (acceptability), which extremes consisted of the phrases "I liked it very much" (9) and "I disliked it very much" (1), characterizing a preference test (Dutcosky, 1996). In each test, a sample (half of an egg boiled in hot water for 10 minutes) of each treatment was used for evaluation. The panel included 44 untrained and voluntarily tasters who

evaluated the attributes: aroma, color, appearance, and taste.

Statistical analysis was performed using the software Statistical Analysis System (2008) and estimates of treatments were subjected to Tukey test at 5% significance level.

RESULTS

Weight loss results of the entire egg and its main structures are present in Table 1. Differences ($p < 0.05$) among storage periods were observed in egg weight loss, yolk % and albumen %. Longer storage periods resulted in higher egg weight loss, higher yolk percentage, and reduced albumen percentage, but eggshell relative weight was not affected.

Table 1 – Weight loss of the entire egg and its main structures in eggs coated with different copaiba oil solutions and stored at room temperature for different periods.

Factors	Variables			
	Egg weight loss (%)	Yolk (%)	Albumen (%)	Eggshell (%)
Storage				
1 day	0.12 ^d	28.06 ^b	58.79 ^a	9.92
7 days	0.40 ^{cd}	31.26 ^a	56.08 ^{ab}	10.08
14 days	1.43 ^{bc}	32.53 ^a	56.39 ^{ab}	10.00
21 days	1.77 ^b	33.79 ^a	55.05 ^{ab}	9.95
28 days	2.15 ^b	33.92 ^a	55.08 ^{ab}	9.99
35 days	4.35 ^a	34.22 ^a	53.43 ^b	10.11
Coating				
Control	2.73 ^a	32.09	54.36	10.03
4%	2.08 ^{ab}	31.31	54.63	10.05
8%	1.59 ^b	32.04	56.43	9.86
12%	1.44 ^b	32.90	55.61	10.13
16%	1.33 ^b	33.22	55.75	9.99
20%	1.12 ^b	32.18	58.04	9.96
Effect		p-value		
Storage	0.01*	0.01*	0.03**	0.85 ^{ns}
Coating	0.01*	0.06 ^{ns}	0.19 ^{ns}	0.77 ^{ns}
Storage x Coating	0.98 ^{ns}	0.99 ^{ns}	0.95 ^{ns}	0.08 ^{ns}
CV (%)	25.94	5.58	4.63	3.02

CV – Coefficient of variation; * Significant effect ($p < 0.01$); ** Significant effect ($p < 0.05$); ns – non significant effect.

Copaiba oil coating reduced ($p < 0.05$) egg weight loss, but did not influence ($p > 0.05$) the relative weight of main egg structures. However, no interaction was detected ($p > 0.05$) between egg storage period and different copaiba oil solutions.

Internal and external egg quality results are present in Table 2. Longer storage periods reduced ($p < 0.05$) yolk and albumen height, increased ($p < 0.05$) yolk

diameter, and worsen ($p < 0.05$) eggshell quality (lower specific gravity and eggshell strength).

Coating eggs with different copaiba oil solutions preserved ($p < 0.05$) egg internal (yolk and albumen height) and external (specific gravity and eggshell resistance – meridional and parallel) quality. However, no interaction ($p > 0.05$) between the storage period of the eggs and coating with different copaiba solutions was detected.



Table 2 – Yolk Height (YH), Albumen Height (AH), Yolk Diameter (YD), Yolk Colour (YC), Eggshell Thickness (ST), Specific Gravity (SG), Haugh Unit (HU) and Eggshell Resistance Meridional (SRM) and Parallel (SRP) of eggs coated with solutions of copaiba oil stored at room temperature in different periods.

Factors	Variables								
	YH (mm)	AH (mm)	YD (mm)	YC	ST (µm)	SG (g/ml ³)	HU	SRM (N)	SRP (N)
Storage									
1 day	14.39 ^a	8.55 ^a	39.49 ^d	6.00 ^a	46.34	1,093.33 ^a	78.77	39.74 ^a	43.98
7 days	13.94 ^a	6.15 ^b	44.88 ^c	5.25 ^b	47.63	1,091.67 ^{ab}	78.24	33.08 ^b	42.55
14 days	11.93 ^{ab}	6.62 ^b	46.94 ^{bc}	5.87 ^a	45.44	1,087.67 ^{bc}	78.09	32.90 ^b	43.72
21 days	12.45 ^{ab}	4.15 ^c	49.78 ^{ab}	6.25 ^a	45.37	1,085.00 ^c	78.32	32.83 ^b	43.86
28 days	12.96 ^{ab}	4.42 ^c	48.83 ^{ab}	5.87 ^a	46.50	1,083.33 ^c	78.35	32.32 ^b	44.72
35 days	11.00 ^b	4.10 ^c	52.38 ^a	6.08 ^a	46.23	1,083.33 ^c	78.59	32.10 ^b	45.59
Coating									
Control	10.30 ^b	4.71 ^c	48.34	5.96	46.07	1,083.33 ^b	78.23	27.28 ^b	38.61 ^c
4%	12.12 ^{ab}	5.20 ^{bc}	47.44	5.96	46.75	1,083.33 ^b	78.55	34.85 ^a	43.95 ^{ab}
8%	12.64 ^{ab}	5.91 ^{ab}	47.25	5.83	46.38	1,085.00 ^b	78.62	35.70 ^a	41.04 ^b
12%	13.57 ^a	5.89 ^{ab}	47.00	5.71	46.14	1,088.33 ^{ab}	77.59	36.99 ^a	43.73 ^{ab}
16%	13.80 ^a	6.51 ^a	46.40	6.00	46.40	1,091.67 ^a	78.77	36.16 ^a	47.76 ^a
20%	14.22 ^a	6.76 ^a	45.86	5.87	45.77	1,091.67 ^a	78.58	31.97 ^{ab}	49.31 ^a
Effect									
	<i>p</i> -value								
Storage	0.01*	0.01*	0.01*	0.01*	0.08 ^{ns}	0.01*	0.95 ^{ns}	0.01*	0.68 ^{ns}
Coating	0.01*	0.01*	0.45 ^{ns}	0.72 ^{ns}	0.89 ^{ns}	0.01*	0.66 ^{ns}	0.01*	0.01*
Storage x Coating	0.99 ^{ns}	0.98 ^{ns}	0.99 ^{ns}	0.99 ^{ns}	0.99 ^{ns}	0.98 ^{ns}	0.08 ^{ns}	0.28 ^{ns}	0.23 ^{ns}
CV (%)	11.60	9.88	4.50	5.93	3.12	0.32	1.67	9.11	7.16

CV – Coefficient of variation; * Significant effect ($p < 0.01$); ** Significant effect ($p < 0.05$); ns – non significant effect.

Results of sensory analysis are present in Table 3. Longer storage periods worsened ($p < 0.05$) egg aroma and taste. Copaiba oil coating solutions worsened ($p < 0.05$) egg aroma, appearance and taste. Yolk

color was not influenced ($p > 0.05$) by the treatments. However, no interaction ($p > 0.05$) between the storage period of the eggs and coating with different copaiba solutions was detected.

Table 3 – Sensory analysis of eggs coated with solutions of copaiba oil stored at room temperature in different periods.

Factors	Variables			
	Aroma	Color	Appearance	Taste
Storage				
1 day	6.65 ^a	6.78	6.98	7.05 ^a
18 days	6.10 ^b	7.01	6.81	6.37 ^b
Coating				
No coating	6.78 ^a	7.01	7.11 ^a	7.27 ^a
Copaiba solution(20%)	5.96 ^b	6.78	6.68 ^b	6.15 ^b
Effect				
	<i>p</i> -value			
Storage	0.01*	0.29 ^{ns}	0.37 ^{ns}	0.01*
Coating	0.01*	0.29 ^{ns}	0.03**	0.01*
Storage x Coating	0.22 ^{ns}	0.21 ^{ns}	0.16 ^{ns}	0.28 ^{ns}
CV (%)	12.33	19.78	18.07	17.55

CV – Coefficient of variation; * Significant effect ($p < 0.01$); ** Significant effect ($p < 0.05$); ns – non significant effect.

DISCUSSION

The results of egg storage conditions in the present study showed a strong influence the environmental on egg quality. According to Brandão (2014), there is legislation in Brazil requiring the refrigeration of commercial eggs from storage to retail, and in general, eggs are stored at room temperature until final

distribution. In the present study, the highest weight loss was detected in the eggs for 35 days. Araújo *et al.* (2015), studying the effect of temperature and storage period on egg quality also observed weight reduction in eggs stored at room temperature (± 28 °C) for a long period. According Seker *et al.* (2005), although water loss from the egg to the environment is a natural process that occurs by diffusion through



the eggshell, the reduction of egg internal quality is directly associated with the loss of water and carbon dioxide during the storage period.

On the other hand, the eggs coated with 20% copaiba oil solution lost less weight (up to 41.02% less water loss than the control eggs. Biladeau & Keener (2009) stated that lipophilic coatings hinder the passage of water molecules through the eggshell. The results of the present study agree with those of Mendonça *et al.* (2013), who evaluated the quality of eggs coated with solutions containing 10% propolis or mineral oil, and observed a linear egg weight loss after five weeks of storage, with more pronounced weight loss in non-coated eggs. Salgado *et al.* (2018) also verified that non-coated eggs lost more weight compared with those coated with mineral oil.

According Pires *et al.* (2015), internal egg quality is directly affected by storage time and temperature. Short storage times and low temperature are essential to preserve the biological value of eggs. However, in the present study, eggs were stored at room temperature, increasing the importance of the environmental effect on the maintenance of the quality of the egg and its main structures.

Copaiba oil coating solutions did not influence the weight of main egg structures (yolk, albumen and eggshell). However, there was an effect of the storage period on these structures. According to Barbosa *et al.* (2008), the aging of the eggs promotes albumen liquefaction due to water loss, and the free water binds to proteins and passes to yolk by osmosis, compromising yolk quality.

Internal and external egg quality worsened as storage time increased. According to Staldelman *et al.* (1988), this is a natural behavior of egg components submitted to increasing storage times, especially due the increase in membrane permeability at elevated temperatures, promoting the migration of the water present in the albumen to the yolk. This excessive water results in an increase in yolk volume, weakening the yolk membrane, which makes the yolk appear larger and flattened on a flat surface when the egg is broken (Santos *et al.*, 2016). Figueiredo *et al.* (2011), Pissinati *et al.* (2014), and Giampietro-Ganeco *et al.* (2015) also reported that long storage periods at room temperature promote great changes in egg quality, especially in its internal content and resident microbial load.

The sensory analysis results showed the influence of storage period on egg aroma and flavor. Fresh eggs obtained higher acceptability scores than those stored for 18 days. This may be a result of the characteristic

odor and sour taste of foods stored for long periods, possibly due to changes in egg protein and fat over the storage period (Scatolini-Silva *et al.*, 2013). According to Pissinati *et al.* (2014), CO₂ loss during storage has a direct effect on egg flavor, especially due to the increase in alkalinity, increasing pH from 7.6 to 9.5.

The coating of copaiba oil solution also influenced the sensory attributes (aroma, appearance and taste). The best scores were assigned to the non-coated eggs. This perception may be explained by the strong characteristic odor of copaiba oil (Griswold, 2000). In this case, the eggs coated with copaiba oil absorbed its characteristic odor, which was perceived by the tasters. Possible solutions for this problem would be to reduce the time the eggs are immersed in the coating solution, to change the coating method, or to use some flavor that modifies this odor.

As for egg appearance, it was observed that coating with copaiba solutions maintained the physical-chemical quality of the eggs by efficiently sealing eggshell pores, reducing water and CO₂ losses (Salgado *et al.*, 2018).

According to the preference test, 25% of tasters preferred eggs stored for 1 day and not coated; 35%, eggs with 18 days storage and not coated; 5% eggs, stored for 1 day and coated; and 35% eggs stored for 18 days and coated. Therefore, although the results of sensorial attributes favored the non-coated eggs, there was no consensus in the preference test.

CONCLUSIONS

The present study indicates that solutions containing 16 and 20% copaiba oil can be used as a biofilm to coat eggs, preserving its internal and external quality, and may affect their sensory characteristics. Long storage times significantly impair egg quality (internal, external and sensory).

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