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Original Article

Quality of Eggs Covered with Biofilms Containing Different Levels of Andiroba Oil and Stored at Room Temperature

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ABSTRACT

The objective of the current study was to investigate the effects of different levels of andiroba oil (AO) in a bioproduct based on andiroba oil (BBAO) on the physical quality, bacteriological concentration, chemical composition, yolk lipid oxidation, and sensory characteristics when coating eggs and storing them for seven days at room temperature. The eggs were arranged in a completely randomized design, in which treatments consisted of a control group (eggs stored for seven days without the application of BBAO) and eggs covered with biofilms produced with BBAO with different levels of AO (1%, 5%, 10%, and 15%). Each treatment contained 50 eggs, and each egg was considered a replicate. Data collected were subjected to Tukey test and linear or polynomial regression at 0.05 statistical significance. The results indicate that the application of BBAO on eggs stored for 7 days at room temperature resulted in better (p<0.05) conservation of their physical quality and chemical composition, and a reduction (p<0.05) or elimination of bacteriological concentrations. This conservation effect became increasingly pronounced as the concentration of AO in the BBAO increased. However, it is crucial to consider the implications of BBAO on sensory characteristics, as higher concentrations of AO in BBAO lead to a decreased (p<0.05) sensory acceptance of the eggs. Therefore, it was concluded that the use of AO in the bioproduct, especially at high levels, can provide better conservation of the eggs for seven days at room temperature, particularly concerning the physical, chemical, and microbiological characteristics; however, it can also cause significant changes in sensory attributes.

INTRODUCTION

Eggs are some of the most complete foods used in human diets, primarily due to their rich nutritional composition containing vitamins, minerals, fatty acids, and proteins (Kusum *et al.*, 2018; Eddin *et al.*, 2019). In Brazil, eggs comprise the diet of 99% of Brazilian families, with an average consumption of 190 to 200 eggs per capita/year (ABPA, 2021; 2022). However, the egg production chain in Brazil, similarly to other nations with large egg production, has issues related to maintaining the quality of *in natura* eggs during storage, particularly at room temperature in the short term (Carvalho *et al.*, 2003; Pires *et al.*, 2015), which leads to impairments of their physicochemical composition.

Notably, Brazilian laws do not mandate the storage of eggs in a refrigerated environment; merely recommending that eggs be preferably stored in a refrigerated setting to extend their shelf life (Brazil, 1990; 1991; 2003; 2017). In this context, Brazilian egg production for local consumption, as is observed in several other nations, tends to subject

eggs to room temperature conditions from the hens' laying stage to the time they reach the consumer, especially due to storage costs that can become more expensive when producers choose to keep eggs in a refrigerated environment (Lana *et al.*, 2017). However, this can become a significant issue along this chain, since the storage of eggs at room temperature requires the processing and transportation from the farm to the consumer to be extremely expedited in order to prevent a loss of quality within a short period of time (Rêgo *et al.*, 2012).

Several techniques and products have been studied to conserve the internal and external quality of eggs at room temperature, and provide alternatives for these production chains. Coating treatments applied to the eggshell surface have been reported as particularly effective measures, acting like "artificial cuticles" that decrease gas exchange through the eggshell and maintain the egg's quality attributes for an extended duration (Biladeau & Keene, 2009; Pissinati *et al.*, 2014; Brasil *et al.*, 2019). According to Waimaleongora-Ek *et al.* (2009) and Brasil *et al.* (2019), an oil-based coating has demonstrated substantial efficacy in retarding egg weight loss and maintaining internal quality due to its hydrophobic properties.

The Brazilian Amazon is home to numerous native species with the potential to contribute to these bioproducts and lead to improvements in egg conservation (Clement *et al.* 2005). Among these, andiroba oil (AO) (*Carapa guianensis* Aubl.) stands out due to its exceptional physicochemical properties, including antibacterial, antioxidant, antiparasitic, antiseptic, antiviral, emollient, and insecticidal attributes, being widely used in the composition of bioproducts in the cosmetics and food industries (Forget *et al.*, 2009; Meccia *et al.*, 2013; Carvalho *et al.*, 2019). Chemically, AO is primarily composed of saponifiable materials, notably fatty acids and non-fatty components, such as triterpenes, tannins, and alkaloids, that confer it a broad biological activity, adding significant commercial and economic value (Oliveira *et al.*, 2018; Sousa *et al.*, 2022).

In light of the above, it was hypothesized that a bioproduct based on andiroba oil (BBAO) could take advantage of such exceptional properties of AO composition to create an artificial cuticle, also be called "biofilm", through a bioproduct, improving the conservation of eggs at room temperature, and slowing down their natural quality loss (Biladeau & Keene, 2009; Pissinati *et al.*, 2014). Therefore, the objective of the current study was to investigate the effects of different levels of AO in a BBAO to coat eggs that were subsequently stored for seven days at room temperature on their physical quality, bacteriological concentration, chemical composition, yolk lipid oxidation, and sensory characteristics.

MATERIAL AND METHODS

The current experiment was conducted at the Faculty of Agrarian Sciences of the Federal University of Amazonas, located in Manaus, state of Amazonas, Brazil. All experimental procedures were conducted in accordance with the guidelines of the Local Experimental Animal Care Committee (protocol number 005/2022), and were approved by the local institutional ethics committee.

AO acquisition and BBAO formulation

AO used in this study was obtained from seeds of the andiroba tree (*Carapa guianensis* Aubl.) through mechanical pressing, yielding approximately 500 g of unfiltered oil per kg of seed (Sousa *et al.*, 2019). Physical-chemical determinations and chemical characterization of the AO were performed according to the methods described by Adams (2017), AOCS (2017; 2022) and Sousa *et al.* (2022). For the production of BBAO, the AO was initially placed in a graduated plastic container with 1L according to the predetermined AO level. Subsequently, a nonionic surfactant (Tween 20) was dripped into the AO while both were stirred. Finally, distilled water was slowly added while the solution was stirred again for better dilution. The BBAO solutions obtained were packaged in plastic containers with a capacity of 1L. The BBAO formulas were calculated according to the following equation:

BBAO = AO + 10NIS + DW

Where:

AO = % of andiroba oil per treatment

10NIS = fixed percentage of 10% of Nonionic surfactant (Tween 20).

DW = % of distilled water necessary to complete the formula

Egg sources, experimental design, and BBAO application

Eggs were sourced from Hissex Brown hens (56 weeks-of-age) housed in cages (1.00x0.45x0.45 m) with a stocking density of 0.16 birds/m². The hens were fed diets formulated based on the recommendations of Rostagno *et al.* (2017). They had access to water

ad libitum and were managed as per the guidelines provided in the breed manual.

The eggs were arranged in a completely randomized design, in which treatments consisted of a control group (eggs stored for seven days without the application of BBAO) and eggs covered with BBAO biofilms with different levels of AO (1%, 5%, 10%, and 15%). Each treatment contained 50 eggs, and each egg was considered a replicate. For the application of BBAO, eggs were immersed in a sterilized container containing the BBAO for 30 seconds. Then, the eggs were stored for seven days at room temperature in a room with controlled environmental conditions, with average temperature and relative air humidity of 22.4°C and 55%, respectively. After the end of this period, the experimental analyzes were performed.

Physical quality

Five eggs from each treatment underwent a physical quality analysis according to the methods described by Brasil *et al.* (2019). Measurements included egg weight; percentages, heights, circumferences and pH values of the yolk and albumen; yolk color on a scale from 1 to 15 using a graduate fan; and eggshell characteristics including weight, percentage and thickness (measured in the basal, meridional, and apical regions). Haugh units were calculated according to the formula described by Eisen *et al.* (1962).

Bacteriological concentration

Bacteriological analysis procedures were conducted according to standard Brazilian norms (Brazil, 2018). Standardized samples were prepared by collecting the internal contents of five eggs from each treatment, homogenizing them for 60 seconds, and creating dilutions of 10^{-1} and 10^{-2} with 1% buffered peptone water. For total mesophyll enumeration, selected dilutions were inoculated into Petri plates with Plate Count Agar and incubated at 36°C for 48 hours. The outcome was expressed as Colony Forming Units per 1.0 g of sample (CFU/g). To enumerate *Escherichia coli*, dilutions were inoculated into test tubes and incubated at 36°C. Positive outcomes were confirmed by subsequent testing and expressed as Most Probable Number per gram (MPN/g). *Staphylococcus aureus* counts were determined by inoculating dilutions onto Baird-Parker Agar plates, which were then incubated at 36°C for 48 hours. The outcome was expressed as Colony Forming Units per 1.0 g of sample (CFU/g). *Salmonella* spp. analysis was conducted through pre-enrichment, selective broths, and PCR testing for diagnosis and confirmation.

Chemical composition and yolk lipid oxidation

Five eggs from each treatment underwent chemical content analysis, which included moisture, minerals, fats, and proteins assessments. These analyses were conducted following the methods recommended by the Association of Official Analytical Chemists (AOAC, 2019). Moisture content was determined using AOAC method 925.10 (2019), minerals through muffle calcination following AOAC method 923.03 (2019), fats using AOCS Ba 3-38 method, and proteins analyzed via the Kjeldahl method, according to AOAC methodology 920.87 (2019).

Other five eggs from each treatment were used to conduct a yolk lipid oxidation analysis. The eggs were cracked, and their yolks were separated and frozen. Subsequently, the frozen yolks underwent freeze-drying, where water and other solvents were removed through sublimation, bypassing the liquid state. The dehydrated yolks were then subjected to Thiobarbituric Acid Reactive Substances (TBARS) analysis to measure the degree of lipid oxidation using a modified version of the methodology described by Vyncke (1970), adapted by Ramanathan & Das (1992).

Sensory characteristics

Five eggs from each treatment, totaling 25 eggs, were used for sensory analysis. These eggs were hard-boiled, cooled, and prepared by removing the hot water and placing them in cool water for 3 minutes. The eggs were then peeled, cut into halves, further divided into quarters, and placed on plates with random identification numbers known only to the researchers (Hayat *et al.*, 2010). Twenty untrained judges conducted the sensory analysis after a 3-hour period of not consuming food or smoking. To prevent bias from eggshell colors, the judges did not have visual contact with the eggshells. They assessed the eggs for appearance, yolk color, aroma, and flavor using a continuous unstructured line intensity scale ranging from 0 to 9, with anchor points at both ends for each attribute (Berkhoff *et al.*, 2020).

Statistical analyses

The data were initially assessed for normality, and necessary transformations were applied. Subsequently, a one-way ANOVA was conducted using R software (version 4.1.3) following Logan's (2010) guidelines. Firstly, Tukey's honestly significant difference test was used to test the significant differences among mean values. The results are presented as means and the

significant level for differences was set at $p<0.05$. Secondly, polynomial regression was applied to the variables that showed significance in the ANOVA to analyze the influence of the independent variable on each tested dependent variable. Linear and quadratic models were constructed for each tested dependent variable, with the choice of the model that best suited the dataset being made according to the values of the coefficient of determination (R^2), with the highest value of this coefficient indicating the best model (Chatterjee, 2006; Dormann *et al.*, 2013). The correlation analysis was also applied between independent variable (AO levels) and each dependent variable to determine the intensity and direction of their relation (ranging from -1 to 1) (Dormann *et al.*, 2013).

RESULTS

The physicochemical analysis of the AO used in the production of the BBAO revealed average results of 2.37±0.28% free fatty acid content, acidity value of 3.26±0.55 mg NaOH/g, peroxide value of 1.53±0.32 meq/1000g, saponification value of 186.92±0.24 mg KOH/g, iodine value of 87.68±0.53 g I2/100g, pH of 4.63±0.02 (mg/100g), and moisture value of 0.001±0.11%. The AO composition analysis using GC/MS identified eight chemical constituents that accounted for 99.98% of the oil. Saturated fatty acids comprised 42.47%, monounsaturated fatty acids made up 45.43%, polyunsaturated fatty acids constituted 8.65%, and triterpenoids accounted for 3.43%. The primary fatty acids identified in AO were oleic (45.43%), palmitic (28.44%), stearic (10.05%), and linoleic acids (8.65%).

Table 1 shows the average results for the physical quality of eggs. The use of BBAO had a significant ($p<0.05$) impact on egg weight and yolk, albumen, and shell percentages. Increased levels of AO in the BBAO resulted in higher egg weights, yolk percentages, and shell percentages. However, it also caused a linear reduction ($p<0.05$) in the percentage of albumen. Additionally, higher levels of AO in BBAO led to significantly better ($p<0.05$) results in yolk and albumen height, yolk color, yolk and albumen pH values, shell thickness, and Haugh unit in eggs stored for seven days compared to those stored without BBAO. Nevertheless, yolk diameter exhibited a linear increase ($p<0.05$) with increasing AO levels in the BBAO.

Table 1 – Physical quality of eggs covered using bioproduct based on andiroba oil (BBAO) with different levels of andiroba oil (AO).

Variables[1]	AO levels into the BBAO (%)					*p*-value[2]	CV[3] (%)	Correlation	Math model[4]	R^2
	0	1	5	10	15					
EW	55.52^{c}	56.25^{c}	56.55^{c}	58.20^{b}	59.27^{a}	0.04	7.99	0.30	Y = 55.6699 + 0.2403x	0.49
Y	29.88^{c}	30.08^{b}	31.41^{b}	32.13^{a}	33.26^{a}	0.04	9.26	0.44	Y = 29.9769 + 0.2227x	0.39
A	58.15^{a}	56.55^{a}	52.89^{b}	49.85^{b}	45.74^{c}	0.05	14.14	-0.61	Y = 57.5683 - 0.7948x	0.38
S	10.19^{b}	10.58^{b}	10.91^{ab}	11.17^{a}	11.56^{a}	0.03	12.85	0.33	Y = 10.38363 + 0.08082x	0.41
YH	16.33^{c}	16.25^{c}	16.73^{bc}	17.00^{b}	18.00^{a}	0.03	8.08	0.45	Y = 16.1928 + 0.10804x	0.40
AH	5.33^{c}	5.38^{c}	5.75^{b}	5.75^{b}	6.00^{a}	0.05	11.08	0.39	Y = 5.38079 + 0.04224x	0.45
YD	41.55^{c}	44.25^{b}	45.00^{b}	46.00^{a}	46.00^{a}	0.03	5.41	0.39	Y = 43.53942 + 0.16461x	0.35
AD	108.14	113.00	112.00	101.75	114.33	0.77	15.29	-0.01	-	-
YC	6.00^{c}	6.14^{c}	6.50^{b}	6.50^{b}	7.66^{a}	<0.01	11.88	0.49	Y = 6.14753 + 0.06674x	0.44
YPh	6.99^{c}	7.06^{bc}	7.09^{b}	7.16^{b}	7.27^{a}	<0.01	1.84	0.36	Y = 7.067056 + 0.008351x	0.33
APh	6.95^{c}	7.13^{c}	7.33^{b}	7.35^{b}	7.47^{a}	0.05	3.86	0.29	Y = 7.219389 + 0.004534x	0.38
ST	0.37^{c}	0.38^{c}	0.41^{b}	0.48^{a}	0.50^{a}	<0.01	4.42	0.63	Y = 0.387952 + 0.006836x	0.40
HU	73.12^{b}	73.23^{b}	76.00^{a}	75.38^{a}	76.88^{a}	0.75	5.92	0.44	Y = 74.88784 + 0.01822x	0.56

[1] EW – Egg weight (g); Y – yolk (%); A – albumen (%); S – shell (%); YH – Yolk height (mm); AH – Albumen height (mm); YD – Yolk diameter (mm); AD – Albumen diameter (mm); YC – Yolk color; YPh – Yolk pH; APh – Albumen pH; ST – Eggshell thickness (μm); HU – Haugh unit.

[2] Averages followed by lowercase letters in the line demonstrate a significant effect on the variable analyzed by the Tukey test at 0.05 of significance.

[3] CV – Coefficient of variation.

[4] Model adjusted according to the influence of the independent variable on the dependent variable.

Bacteriological concentrations in the eggs are presented in Table 2. Eggs stored for seven days without the use of BBAO exhibited significantly higher ($p<0.05$) bacterial concentrations. Increased levels of AO in BBAO proved effective in linearly reducing or completely eliminating concentrations of mesophiles, *Escherichia coli*, and *Staphylococcus aureus* in eggs stored for seven days at room temperature, especially when higher levels of AO were used. Regarding the presence of *Salmonella* spp., eggs stored for seven

days at room temperature without BBAO showed instances of *Salmonella* spp. presence. *Salmonella* spp. was absent when BBAO was used, regardless of the AO level tested.

Table 2 – Bacteriological concentrations of eggs covered using bioproduct based on andiroba oil (BBAO) with different levels of andiroba oil (AO).

Variables[1]	AO levels into the BBAO (%)					*p*-value[2]	CV[3] (%)	Correlation	Math model[4]	R^2
	0	1	5	10	15					
Mesophiles	6.69[a]	0.51[b]	0.04[c]	0.01[c]	0.00[c]	0.04	2.02	-0.59	Y = 3.1737 - 0.2777x	0.35
Escherichia coli	5.27[a]	0.01[b]	0.00[b]	0.00[b]	0.00[b]	0.03	2.22	-0.55	Y = 2.3361 - 0.2063x	0.30
Staphylococcus aureus	0.27[a]	0.33[a]	0.01[b]	0.00[b]	0.00[b]	0.03	1.32	-0.83	Y = 0.2563 - 0.0214x	0.68

[1] All presented values are to be multiplied by 10^3.

[2] Averages followed by lowercase letters in the line demonstrate a significant effect on the variable analyzed by the Tukey test at 0.05 of significance.

[3] CV – Coefficient of variation.

[4] Model adjusted according to the influence of the independent variable on the dependent variable.

The chemical composition of the eggs, as shown in Table 3, revealed that the mineral content remained unaffected ($p>0.05$) by the application of BBAO. However, an increase in AO levels within BBAO resulted in a positive linear ($p<0.05$) conservation of fat contents in eggs stored for seven days at room temperature compared to those stored without BBAO (control). Despite the BBAO coating, there was a linear ($p<0.05$) reduction in protein contents in eggs treated with the product as compared to the control. Additionally, moisture content linearly increased ($p<0.05$) in eggs treated with increased AO levels in BBAO.

The lipid oxidation of the yolks, as also shown in Table 3, indicates that as the levels of AO in BBAO increased, there was a linear ($p<0.05$) decrease in yolk lipid oxidation in eggs stored for seven days at room temperature compared to those without BBAO. This is further supported by the higher negative correlation observed as the AO level increased.

Table 3 – Chemical composition of eggs covered using bioproduct based on andiroba oil (BBAO) with different levels of andiroba oil (AO).

Variables[1]	AO levels into the BBAO (%)					*p*-value[2]	CV[3] (%)	Correlation	Math model[4]	R^2
	0	1	5	10	15					
Moisture	77.12[c]	77.55[c]	78.97[b]	78.53[b]	79.13[a]	0.03	10.81	0.49	Y = 77.53205 + 0.11948x	0.25
Minerals	0.89	0.80	0.66	0.76	0.80	0.07	11.23	-0.24	-	-
Fats	6.93[c]	8.01[b]	8.03[b]	8.48[b]	9.64[a]	0.05	12.97	0.79	Y = 7.33289 + 0.14351x	0.63
Proteins	12.35[a]	11.31[b]	11.28[b]	10.98[b]	9.64[c]	0.05	9.70	-0.77	Y = 11.98871 - 0.14057x	0.59
TBARS values	0.242[a]	0.221[b]	0.209[b]	0.201[bc]	0.189[c]	0.01	9.53	-0.92	Y = 0.23088 - 0.0029x	0.86

[1] Moisture, Mineral, Fats and Proteins values are expressed in %. TBARS values are the degree of lipid oxidation expressed in mg MDA/kg dried egg.

[2] Averages followed by lowercase letters in the line demonstrate a significant effect on the variable analyzed by the Tukey test at 0.05 of significance.

[3] CV – Coefficient of variation.

[4] Model adjusted according to the influence of the independent variable on the dependent variable.

The Table 4 shows the average results for the sensory characteristics of the eggs. A linear ($p<0.05$) reduction in the results of all sensory characteristics evaluated was observed as the AO level within the BBAO increased. In general, it is shows a reduction in the sensory acceptance of the eggs covered with BBAO by the judges, especially in terms of appearance and taste, when compared to eggs from the control.

Table 4 – Sensory characteristics of eggs covered using bioproduct based on andiroba oil (BBAO) with different levels of andiroba oil (AO).

Variables	AO levels into the BBAO (%)					*p*-value[1]	CV[2] (%)	Correlation	Math model[3]	R^2
	0	1	5	10	15					
Aroma	6.50[a]	5.80[b]	5.60[b]	4.90[c]	5.30[c]	0.03	3.12	-0.13	Y = 6.13816 - 0.03841x	0.39
Color	6.60[a]	6.30[a]	6.40[a]	5.50[b]	5.00[b]	0.04	2.77	-0.26	Y = 6.23753 - 0.08992x	0.59
Appearance	6.80[a]	6.20[a]	6.70[a]	5.70[b]	4.30[c]	<0.01	3.01	-0.34	Y = 6.65479 - 0.11851x	0.31
Taste	7.50[a]	6.90[b]	6.50[b]	5.80[c]	5.80[c]	<0.01	2.98	-0.26	Y = 7.0166 - 0.0801x	0.47

[1] Averages followed by lowercase letters in the line demonstrate a significant effect on the variable analyzed by the Tukey test at 0.05 of significance.

[2] CV – Coefficient of variation.

[3] Model adjusted according to the influence of the independent variable on the dependent variable.

DISCUSSION

The results observed in the AO composition are very consistent with previous descriptions in the literature (Silva, 2018; Sousa *et al.*, 2022; Dias *et al.*, 2023), especially oleic acid as the major unsaturated fatty acid and palmitic acid as the major saturated fatty acid. Furthermore, the presence of triterpenoids identified through chromatographic analysis conducted in this study is also in accordance with previous studies. It emphasizes that the biological activities of AO are widely associated with both fatty acids content and triterpenoids, as well as other minor unsaponifiable components.

In the results of the physical quality of the eggs, it was observed that higher levels of AO in the BBAO may have created a more consistent additional protective film on the eggshell, leading to better conservation of most evaluated physical attributes. According to the literature, this protective film covers the entire shell surface, causing a safeguard to pore openings and hindering the passage of water and bacteria, consequently conserving the egg quality for a longer period (Muñoz *et al.*, 2015; Eddin *et al.*, 2019; Pires *et al.*, 2020).

The increase in shell thickness and percentage of shell in BBAO-treated eggs supports this observation, due to the formation of a more consistent additional film with increasing levels of AO in the BBAO, as do the improvement in the results of the yolk and albumen percentage, yolk and albumen height, yolk color, yolk and albumen pH values, Haugh units, and the lower weight loss (or higher egg weight retention) in eggs stored for seven days with BBAO. These findings are consistent with the studies from Mendonça *et al.* (2013) and Salgado *et al.* (2018), that also reported coated eggs undergoing less weight loss when compared to uncoated eggs., As suggested by Biladeau & Keener (2009), lipophilic coatings prevent water from penetrating the eggshell, supporting the conservation of internal egg contents.

The significant decline in physical quality observed in eggs without BBAO (control) and those treated with BBAO containing lower levels of AO can be attributed to their increased vulnerability to microorganisms, which affected their bacteriological concentrations. On the other hand, the gradual reduction in bacterial concentrations with increasing levels of AO in BBAO highlights the biofilm's efficiency in creating an additional protective barrier over the eggs, conserving their contents (Caner, 2005; Eddin *et al.*, 2019; Pires *et al.*, 2020).

According to Meccia *et al.* (2013) and Conde *et al.* (2015), AO presents an inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*, in addition to a possible inhibition of microbial adherence. In light of the above, the use of BBAO can establish an efficient barrier that can both prevent natural water loss and reduce the entry of external microorganisms into the eggs, conserving their internal contents. This process delays the natural quality decline in the eggs, extending their storage period, especially at room temperature.

In this sense, the effectiveness of the barrier established by BBAO in conserving the internal content of the eggs also influenced their chemical composition. It was observed that this conservation primarily impacted the fats predominantly present in egg yolks. As reported by Cindric *et al.* (2007), coatings based on lipids exhibit hydrophobic properties that prevent moisture loss and the infiltration of external oxygen into the egg. As a result, this protective shield against oxygen is more effectively at safeguarding the lipids in the internal contents of the eggs from oxidation.

Moreover, it was observed that the protective barrier established by BBAO significantly impacted the oxidation of lipids in eggs. The literature reports that coatings based on lipids create a hydrophobic shield, effectively preventing moisture loss and the infiltration of external oxygen into the egg (Rêgo *et al.*, 2012; Pissinati *et al.*, 2014; Salgado *et al.*, 2018). This barrier plays a crucial role in safeguarding the lipids predominantly found in the egg yolks, contributing to the reduction in lipid oxidation activity during storage (Pissinati *et al.*, 2014; Salgado *et al.*, 2018). As a result, eggs treated with BBAO, especially with higher levels of AO, display lower levels of yolk lipid oxidation, which may ensure that the quality and freshness of egg lipids are conserved, making BBAO an effective tool in mitigating lipid oxidation during egg storage at room temperature.

Nevertheless, as eggs are stored, the proportion of liquid albumen naturally increases at the expense of the denser portion, with this fluidization and loss of viscosity in the dense albumen being attributed to the hydrolysis of amino acid chains. The degradation of these chains leads to the release of water bound to large protein molecules, such as the albumin present in eggs (Rêgo *et al.*, 2012; Pissinati *et al.*, 2014; Salgado *et al.*, 2018), which leads to a reduction in protein contents through a process called protein denaturation. This process was observed in this study, with a gradual reduction in proteins even with the use of the bioproduct.

Furthermore, eggs treated with BBAO containing higher levels of AO had impaired sensory attributes as compared to those of the control, stored without the use of BBAO. This reduction could be attributed to two factors: the transfer of organoleptic properties from AO to the eggs, and the influence of the BBAO film on the egg's internal content interactions (Biladeau & Keener, 2009; Eddin *et al.*, 2019). This shows that, despite improving content conservation, the use of BBAO on eggs may lead to a significant reduction in sensory acceptance due to the transfer of sensory characteristics from the active ingredient of the bioproduct to the eggs. This potential issue has also mentioned by Pires *et al.* (2015) and Eddin *et al.* (2019).

CONCLUSIONS

It was concluded that the use of AO in a bioproduct, especially at high levels, can provide better conservation of the eggs for seven days at room temperature, resulting in a more effective conservation of their physical quality and chemical composition, in addition to reducing or eliminating bacteriological concentrations. However, it is important to consider the implications of this bioproduct with AO on sensory characteristics, since higher levels of AO in the bioproduct lead to a decreased sensory acceptance of the eggs.

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