Effects of rice protein coatings combined or not with propolis on shelf life of eggs

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ABSTRACT Although eggs are an excellent protein source, they are a perishable product. Many methods exist to extend shelf life of food and one of them is the use of protein coatings that may be combined with antimicrobial substances, as propolis. The effectiveness of rice protein coatings plus propolis on maintaining interior quality and eggshell breaking strength of fresh eggs was evaluated during storage at 20°C for 6 wk. Egg quality was assessed by weight loss, Haugh unit (**HU**), albumen pH, yolk index (**YI**), shell strength, and scanning electron microscopy in uncoated eggs (control treatment) and eggs coated with rice protein concentrate and propolis at 5 or 10%. The HU and YI were higher in coated eggs ($P < 0.001$). Weight loss increased ($P < 0.001$) during long-term storage. Uncoated eggs showed the highest weight loss (5.39%), whereas rice protein (4.27%) and rice protein plus propolis at 5% (4.11%) and 10% (4.40%) solutions were effective in preventing weight lost ($P < 0.001$). Uncoated eggs had the worst ($P < 0.001$) HU (58.47), albumen pH (9.48), and YI (0.33) after 6 wk of storage. The eggs coated with rice protein and rice protein plus propolis presented results with similar intern quality between them during all the storage period. Scanning electron microscopy demonstrated a lower surface porosity in coated eggshell, indicating that the use of the coating may provide a protective barrier against the transfer of gases and moisture. In conclusion rice protein and propolis treatments helped to maintain egg quality for a longer time compared to uncoated eggs. These could be a viable alternative for maintaining the internal quality of fresh eggs during long-term storage at room temperature.

Key words: egg quality, eggshell, natural antimicrobial, protein coating, storage time

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INTRODUCTION

Eggs are an excellent natural source of high-quality protein, antioxidants, carotenoids, vitamins, and phospholipids (Lesnierowski and Stangierski, 2018). Immediately after they are laid, aging processes begin in shell eggs, altering their chemical, physical, and functional characteristics (Lucisano et al., 1996). The porosity of the eggshell allows gas exchange with the external environment, facilitating the loss of water and CO_2. The longer the storage time, the greater is the deterioration of the internal quality, due to the greater CO_2 movement through the shell (Oliveira and Oliveira, 2013). According to the Brazilian legislation (Brasil, 1990), an egg is fresh up to 28 D after being laid and the refrigeration of the eggs in points of sale is optional and, therefore, does not occur in practical conditions. Storage technologies have been developed to extend the shelf life of eggs. For example, promising results have been obtained in the coating of eggshells with natural products such as whey protein, zein (Caner and Yüceer, 2015), rice protein (Pires et al., 2018), and propolis (Copur et al., 2008; Akpinar et al., 2015).

Propolis is a resin containing a complex mixture of substances, produced by honey bees that result from the collection of substances secreted by different plants. During propolis collection, bees mix the beeswax and the collected propolis with their saliva (Park et al., 1998). Bees use the propolis to protect the colony from rain and to provide thermal insulation, as well as to reinforce the structural stability to the hive (Costa and Oliveira, 2011). Propolis also have several properties, such as antibacterial (Silici and Kutluca, 2005), antifungal (Seven and Silici, 2011), antiprotozoan, and antiviral activities (Schhnitzler et al., 2010). The effects observed are complex, due to the wide array of components in its chemical composition, as it may contain more than 300 substances including flavonoids, phenolic acid, esters, terpenes, and sugars (Aygun, 2016). Brazil

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is a great producer and exporter of propolis of *Apis mellifera* and the Brazilian propolis is characterized by the presence of hydroxycinnamic acid (Oldoni et al., 2015). However, the composition and biological activity of the Brazilian propolis vary significantly, depending on the type of sample and geographical area of collection (Machado et al., 2016).

Rice (*Oryza sativa* L.) is a major food crop, with global annual production estimated at about 480 million metric tons (expressed on a milled rice basis) (USDA, 2015). Rice bran is the major by-product generated during milling and the defatted residues of bran contain ranges from 10 to 16% of protein (Cao et al., 2009; Faria et al., 2012). Rice proteins are generally regarded as hypoallergenic (Fiocchi et al., 2006), antioxidant (Faria et al., 2012), and are considered an emulsifier, also showing the ability of blinding oil and water (Chandi et al., 2007). These properties make rice protein suitable for a broad range of industrial food applications.

Previous studies already described the use of rice by-products and propolis as feedstocks for the preparation of edible coating (Park et al., 1998; Dias et al., 2010; Das et al., 2013; Akpinar et al., 2015). However, information available on the combined effects of these products is very limited, particularly in eggs. Thus, the aim of the study was to evaluate the internal quality and the resistance of eggshell after application of rice protein coating combined with propolis in eggs after 6 wk of storage

MATERIALS AND METHODS

A total of 300 table eggs, freshly laid (1-day-old) from ISA Brown hens, were supplied by a commercial farm (Rio Grande do Sul, Brazil) and used in the present study. All eggs were obtained from birds of the same age, maintained under similar environment, handling, and feeding conditions. The eggs were randomly divided into 4 treatments. Uncoated eggs were used as a control treatment. The other treatments consisted of coatings based on rice protein concentrate (**RPC**) with different inclusions of *Apis mellifera* propolis (0, 5, or 10%) according to Aygun et al., (2012).

Preparation of Coating Solutions and Coating of Shell Eggs

Rice protein film-forming solution was prepared by dissolving 8% (w/w) RPC (MidWay Labs, FL, USA) in distilled water, and adding 20% (w/w) glycerol (Neon, São Paulo, Brazil) as plasticizer. Propolis solution was prepared by dissolving 5 or 10% of dry extract of propolis (Apis Flora, São Paulo, Brazil) in distilled water. The propolis solution was then mixed into the rice protein solution at concentrations of 0, 5, and 10%. The solutions were kept on a magnetic stirrer for 5 min and heated in a water bath (90°C) for 30 min, following the procedures described by Antunes (Antunes, 2003). Then, the temperature was reduced to 25°C and the pH adjusted to 10 with 1 N NaOH solution, in order to proceed the dissolution of the proteins in the film-forming coating.

All eggs were washed with water at 42°C and chlorine (50 ppm) was used as a sanitizer following the standard practices recommended by the Brazilian legislation (Brasil, 1990). Eggs were divided into 4 treatments: a control uncoated group, a rice protein-coated group, and 2 rice protein-coated groups that were combined with propolis at 5 and 10% solutions. The clean eggs were individually submerged in the coating solutions at 24°C for 1 min, so that the coating visibly covered the entire shell surface. The eggs were then dried for 5 min (Caner and Cansiz, 2008) and stored at a controlled ambient temperature (20°C) and humidity (±65%) for up to 6 wk in plastic trays specific for eggs. The uncoated washed eggs served as a control treatment.

Twelve eggs were immediately submitted for the quality analysis to represent the characteristics of fresh eggs (zero days of storage). Weekly during the study, 12 eggs from each group were randomly separated for quality evaluation (weight loss, Haugh unit (**HU**), yolk index (**YI**), and albumen pH). Breaking strength (12 eggs per treatment), color (6 eggs per treatment), and electron microscopic structure of the shells (3 eggs per treatment) were evaluated at the end of the experiment.

Weight Loss

The eggs were weighed individually using a digital precision (±0.001 g) scale (Bel, Mark M 214A, Milano, Italy). Weight loss (%) during storage was calculated as described by Caner and Cansız (2008), using the following equation:

$$Weight\ loss,\ \% = \frac{(Final\ weight - Initial\ weight)}{Initial\ weight}\ x\ 100$$

Haugh Unit

The albumen height was measured with a digital caliper (TMX PD - 150, China) at a distance of 10 mm from the yolk. After, the HU was obtained through the equation proposed by Haugh (1937):

$$HU = 100\ log\ [h - \frac{\sqrt{(30W^{0.37} - 100)}}{100} + 1.19$$

Where h is the thickness of albumen (mm) and W is the mass of the entire egg (g).

Based on the HU results, the eggs were graded as: Class AA, when HU was higher than 72; Class A, eggs with HU from 71 to 60; Class B, eggs with HU from 59

to 31; or Class C, when HU was lower than 30 (Yuceer and Caner, 2014)

Yolk Index

The width and height of the yolk (mm) were measured with a digital caliper (TMX PD - 150, China). After, the YI was calculated through the equation (Sharp and Powell, 1930):

$$YI = \frac{(Yolk\ height)}{(Yolk\ width)}$$

pH Measurements

After separation of the yolk and albumin, the dense and the fluid albumen were homogenized for 20 s, and then the pH was determined using a digital pHmeter (Kasvi model k39-2014B, Paraná, Brazil) previously calibrated with buffer solutions of pH 7 and 10 (Brasil, 1999).

Eggshell Color

Six eggs from each treatment were evaluated for color using the Colorimeter Konica Minolta Chroma Meter CR-410, (Osaka, Japão) The L^* (lightness), a^* (greenness), and b^* (yellowness) values were obtained after the storage period and values were taken at 3 random locations on each egg. At least 1 value was taken at the blunt or round tip for every egg (Biladeau and Keener, 2009).

Eggshell Breaking Strength

Eggshell breaking strength (puncture strength) was determined at the end of the 6 wk storage period using a texture analyzer (TAXT Texture Analyzer, Stable Micro Systems, Surrey, England). Each egg was mounted on a texture analyzer platform and the eggshell was punctured at the top (small end) using a 3 mm die probe at 5 mms^{-1} constant speed and a distance of 6 mm. The trigger force used was 3 g, following the method described by Oliveira (Oliveira, 2006). The force (N) required to puncture the shell was recorded as the eggshell breaking strength (Yuceer and Caner, 2014).

Ultrastructural Assessment

At the end of the project, 3 eggs from each treatment were randomly selected and lightly broken. After, their eggshells were segmented with scissors in 3 parts corresponding to the apical, equatorial, and basal regions. Residual albumen was removed. Then, fragments of approximately 0.5 cm^2 were removed from each egg region. The samples were mounted on a stub, coated with gold–palladium of 35 nm for 3 min (Sputter Coater - SCD 050 Balzers, Germany), and analyzed through a scanning electron microscope (JEOL 6060, Japan) at a standard magnification of 250 ×.

Statistical Analysis

Statistical procedures were performed using SAS statistical software (9.4, SAS Inst. Inc., Cary, NC, United States). The normality of the data was verified using the Shapiro-Wilks test through the UNIVARIATE procedure. Afterward, the data were submitted for analysis of variance using PROC GLM, considering each egg an experimental unit. Statistical models included the effects of treatments (coating types), storage periods (weeks), and interaction (treatments by storage periods); except for eggshell color and breaking strength, which was evaluated only once at the end of the project and was analyzed considering only the treatment effect. Eventual differences ($P < 0.05$) were assessed with a Tukey multiple comparison test.

RESULTS AND DISCUSSION

The eggs evaluated at day zero presented mean HU value of 82.02, assuring their excellent quality (AA) standard according to the USDA (USDA, 2000) recommendation. The other quality parameters evaluated in the beginning of the trial were also in accordance with the Brazilian legislation (Brasil, 1997), which determine minimum internal quality conditions for yolk (translucent, firm, consistent, and without germ) and albumen (transparent, consistent, limpid, no stain, and intact chalaza).

Weight Loss

The initial egg weight did not differ ($P > 0.05$) between uncoated eggs (68 g) and eggs coated with RPC (69 g), neither those coated with RPC + 5% (68 g) nor RPC + 10% (69 g) of propolis. The weight loss ($P < 0.001$) increased with increasing storage periods, ranging from 4.40 to 5.39% after 6 wk (Table 1). Weight loss during storage has already been reported (Kim et al., 2006; Jones et al., 2018) and is caused primarily by evaporation of water and loss of carbon dioxide trough the pores of shells. This is one of the important measurements to monitor the changes in quality of fresh shell eggs during storage (Caner, 2005). Eggs may be classified by weight. In this case, more profit could be achieved by reducing water loss (Biladeau and Keener, 2009).

Treatment by time interaction ($P < 0.001$) was found for weight loss, with differences ($P < 0.001$) among treatments observed in all studied periods of control group (uncoated) eggs had the highest weight loss during the entire project reaching 5.39% weight

Table 1. Effect of rice protein concentrate and propolis coatings[1] on cumulative weight loss (% in relation to week zero) of egg during 6 wk of storage at 20°C.[1]

Coating	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	*P*-value
Control	$1.05 \pm 0.02^{F,a}$	$1.32 \pm 0.09^{E,a}$	$2.61 \pm 0.11^{D,a}$	$3.45 \pm 0.12^{C,a}$	$4.55 \pm 0.17^{B,a}$	$5.39 \pm 0.17^{A,a}$	0.0001
RPC	$0.79 \pm 0.03^{F,b}$	$1.04 \pm 0.07^{E,b}$	$1.78 \pm 0.10^{D,b}$	$2.40 \pm 0.16^{C,b}$	$3.56 \pm 0.14^{B,b}$	$4.27 \pm 0.19^{A,b,c}$	0.0001
RPC + P5	$0.73 \pm 0.05^{F,c}$	$1.06 \pm 0.07^{E,b}$	$1.66 \pm 0.09^{D,b,c}$	$2.24 \pm 0.12^{C,b}$	$3.43 \pm 0.15^{B,b}$	$4.11 \pm 0.07^{A,c}$	0.0001
RPC + P10	$0.57 \pm 0.04^{E,d}$	$1.07 \pm 0.03^{D,b}$	$1.59 \pm 0.14^{C,c}$	$1.71 \pm 0.12^{C,c}$	$3.43 \pm 0.17^{B,b}$	$4.40 \pm 0.18^{A,b}$	0.0001

[1]Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).
[a–d]Means in the same column with different lowercase letters are significantly different ($P < 0.001$).
[A–F]Means in the same row with different capital letters are significantly different ($P < 0.001$).
RPC: Rice protein coating; RPC+P5: Rice protein coating with 5% of propolis; RPC+P10: Rice protein coating with 10% of propolis.

loss at the end of the study. Eggs coated with RPC and 5 or 10% of propolis showed weight loss of 4.27, 4.11 and 4.40%, respectively, throughout the experiment.

According to FAO (2003), 2 to 3% loss of egg weight during storage is acceptable. In this study, the egg coating kept the weight loss within the acceptable range up to 4 wk of storage, which was not observed in the uncoated eggs (3.45% at this same time).

Various studies have shown the enhancement effects of using coatings on the moisture loss of the eggs during storage. These effects were associated with the use of protein-based coatings (Caner and Yuceer, 2015; Almeida et al., 2016; Xu et al., 2017) and propolis (Copur et al., 2008; Akpinar et al., 2015). Variations in egg weight loss between studies may be due to different storage times, storage temperatures, egg sizes, or shell porosities (Akpinar et al., 2015). In the current study, eggshells coated with RPC, alone or in combination with propolis, showed a lower surface porosity in the ultrastructural assessment (Figure 1), which may have contributed to a lower weight loss during storage. This demonstrates that the use of coatings may provide a protective barrier against the transfer of gases and moisture through the eggshell (Lee et al., 1996; Kim et al., 2006). Previous study (Wong et al., 1996) also indicated a more porous structure of the uncoated shells, which was evident in the thicker and stronger shells and lower weight loss for the coated eggs compared to the uncoated eggs.

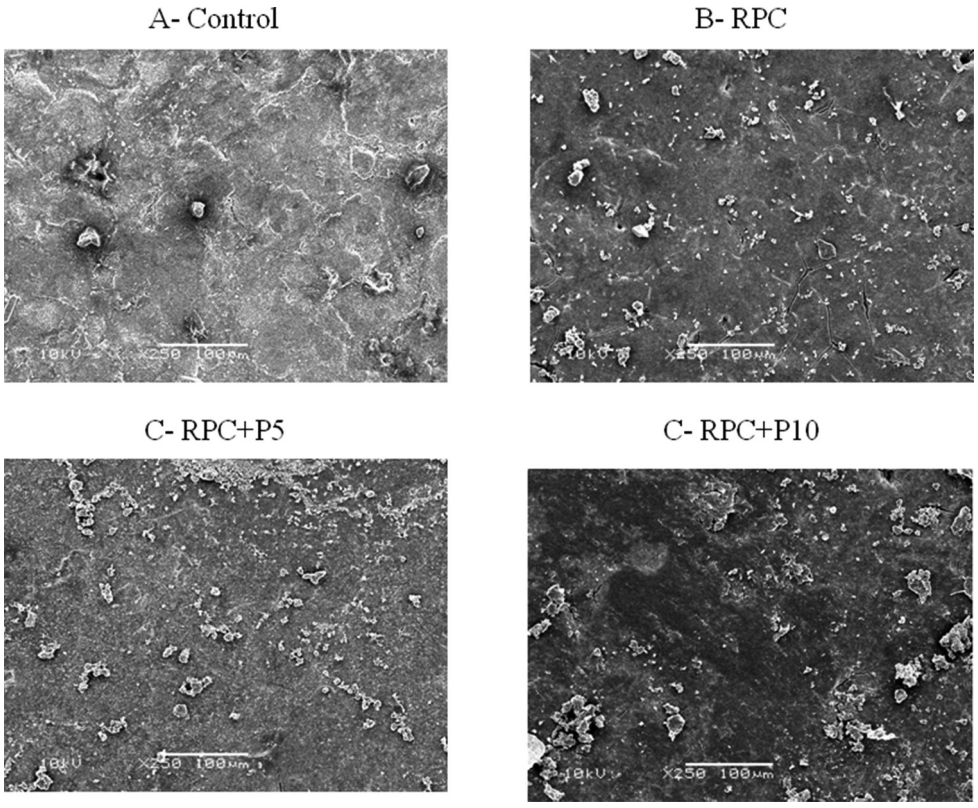

Figure 1. Scanning electron microscopy (× 250) of uncoated eggshell (picture A) and coated eggs (pictures B to D) after 6 wk of storage. RPC: rice protein concentrate coating.

Haugh Unit

The liquefaction of the dense albumen is evidenced by the reduction of HU values. Haugh unit results of uncoated and coated eggs are shown in Table 2. The initial HU value (82.02) decreased with increasing storage time ($P < 0.001$). The reduction of HU value can be attributed to ovomucine proteolysis, cleavage of disulfide bridges, or by the interaction between α and β ovomucines (Oliveira and Oliveira, 2013). During the storage, the enzymes present in the albumen hydrolyse the amino acid chains and, by destroying the protein structure, release the water that was bound to the large protein molecules, which leads to fluidization of the albumen and loss of the viscosity of the denser albumen (Brasil, 1990).

Interaction was observed between storage time and different treatments ($P < 0.001$). At the end of the tested storage time (week 6), the HU means ranged from 58.47 (uncoated eggs) to 62.72 (RPC combined with propolis). The HU of the uncoated eggs decreased more rapidly than in the coated eggs, with the differences among treatments observed early as the first week and maintained up to the end of the project. These results support previous observations that different protein coatings (Caner and Yuceer, 2015; Xu et al., 2017; Pires et al., 2018) were effective in preserving the albumen quality of eggs. Advantages of using coatings containing propolis were observed at the end of the project, when treatments with this substance at 5 and 10% showed better results compared to the treatment that used RPC alone. These results agree with previous observations (Copur et al., 2008; Akpinar et al., 2015)

The HU values indicated that uncoated eggs changed in quality from grade "AA" to "A" after 3 wk, and to grade "B" after 6 wk. Meanwhile, eggs coated with RPC changed from "AA" to "A" after 4 wk of storage and eggs coated with RPC combined with propolis changed from "AA" to "A" only after 5 wk of storage at 20°C. This demonstrated that the use of coatings can preserve the internal egg quality (grade maintenance) for 1 to 2 wk longer compared to uncoated eggs. Advantages of coatings (grade maintenance) were already reported (Caner and Yuceer, 2015; Pires et al., 2018) for stored eggs.

Yolk Index

The YI of uncoated and coated eggs decreased ($P < 0.001$) throughout the storage (Table 3), as already reported in previous studies (Akpinar et al., 2015; Almeida et al., 2016; Xu et al. 2017). During storage, water is transferred from the albumen to the yolk, which increases the weight and makes the yolk membrane less elastic and more susceptible to rupture (Oliveira and Oliveira, 2013). A fresh egg of good quality has a YI of around 0.45, whereas an older egg will have a lower YI. The higher the YI the better is the quality of the yolk (Yuccer and Caner, 2014).

Table 2. Effect of rice protein concentrate (RPC) and propolis coatings on Haugh unit (**HU**) and egg grade[1] (designated after each mean, in the parenthesis) during up to 6 wk of storage at 20°C.[2]

Coating	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	*P*-value
Control	$82.02 \pm 0.39(AA)^{A,a}$	$79.65 \pm 0.45(AA)^{B,b}$	$75.32 \pm 0.34(AA)^{C,c}$	$70.90 \pm 0.55(A)^{D,b}$	$66.49 \pm 0.53(A)^{E,b}$	$63.23 \pm 0.49(A)^{F,b}$	$58.47 \pm 0.52(B)^{G,c}$	0.0001
RPC	$82.02 \pm 0.39(AA)^{A,a}$	$81.14 \pm 0.52(AA)^{B,a}$	$78.40 \pm 0.32(AA)^{C,b}$	$76.96 \pm 0.36(AA)^{D,a}$	$71.81 \pm 0.40(A)^{E,a}$	$68.60 \pm 0.48(A)^{F,a}$	$61.55 \pm 0.62(A)^{G,b}$	0.0001
RPC + P5	$82.02 \pm 0.39(AA)^{A,a}$	$81.17 \pm 0.23(AA)^{B,a}$	$78.89 \pm 0.26(AA)^{C,a,b}$	$77.19 \pm 0.26(AA)^{D,a}$	$72.37 \pm 0.55(AA)^{E,a}$	$68.68 \pm 0.26(A)^{F,a}$	$62.67 \pm 0.35(A)^{G,a}$	0.0001
RPC + P10	$82.02 \pm 0.39(AA)^{A,a}$	$81.46 \pm 0.32(AA)^{A,a}$	$79.09 \pm 0.57(AA)^{B,a}$	$77.35 \pm 0.39(AA)^{C,a}$	$72.31 \pm 0.55(AA)^{D,a}$	$69.10 \pm 0.34(A)^{E,a}$	$62.72 \pm 0.38(A)^{F,a}$	0.0001

[1]Egg grades: AA, HU > 72; A, HU = 71–60; B, HU = 59–31; C, HU < 30.

[2]Data are expressed as means (egg grades) ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).

[a–d]Means in the same column with different lowercase letters are significantly different ($P < 0.001$).

[A–D] Means in the same row with different capital letters are significantly different ($P < 0.001$).

RPC: Rice protein coating; RPC+P5: Rice protein coating with 5% of propolis; RPC+P10: Rice protein coating with 10% of propolis.

Table 3. Effect of rice protein concentrate (RPC) and propolis coatings on yolk index during up to 6 wk of storage at 20°C.[1]

Coating	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	*P*-value
Control	$0.49 \pm 0.01^{A,a}$	$0.45 \pm 0.01^{B,b}$	$0.40 \pm 0.01^{C,c}$	$0.38 \pm 0.01^{D,b}$	$0.36 \pm 0.01^{E,c}$	$0.36 \pm 0.01^{F,b}$	$0.33 \pm 0.01^{G,c}$	0.0001
RPC	$0.49 \pm 0.01^{A,a}$	$0.46 \pm 0.01^{B,a}$	$0.42 \pm 0.01^{C,b}$	$0.40 \pm 0.01^{D,a}$	$0.38 \pm 0.01^{E,b}$	$0.37 \pm 0.01^{E,F,a}$	$0.36 \pm 0.01^{F,b}$	0.0001
RPC + P5	$0.49 \pm 0.01^{A,a}$	$0.46 \pm 0.01^{B,a}$	$0.42 \pm 0.01^{C,b}$	0.41 ± 0.01^{Da}	$0.39 \pm 0.01^{E,a}$	0.37 ± 0.01^{Fa}	$0.36 \pm 0.01^{F,b}$	0.0001
RPC + P10	$0.49 \pm 0.01^{A,a}$	0.46 ± 0.01^{Ba}	$0.43 \pm 0.01^{C,a}$	$0.41 \pm 0.01^{D,a}$	$0.40 \pm 0.01^{E,a}$	$0.38 \pm 0.01^{F,a}$	$0.37 \pm 0.01^{F,a}$	0.0001

[1]Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment. Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).
[a–d]Means in the same column with different lowercase letters are significantly different ($P < 0.001$).
[A–C]Means in the same row with different capital letters are significantly different ($P < 0.001$).
[A–G]RPC: Rice protein coating; RPC+P5: Rice protein coating with 5% of propolis; RPC+P10: Rice protein coating with 10% of propolis.

Interaction was observed between storage time and different treatments ($P < 0.001$). The effect of the coating was observed from the first week of storage, when all coatings tested had a higher YI ($P < 0.001$) compared to the control treatment. At the end of the project, the best YI mean was observed in the treatment that combined RPC and propolis at 10% solution, followed by the other coated treatments. This study demonstrated that the use of coating was able to preserve the yolk quality for a longer time than uncoated eggs, which agree with previous studies (Torrico et al., 2010; Caner and Yuceer, 2015; Pires et al., 2018).

pH Measurement in Albumen and Yolk

The albumen pH varied ($P < 0.001$) over the storage period (Table 4). The average initial albumen pH of the eggs was 8.05 and this value increased to 9.40 at the end of 6 wk in the uncoated eggs. Coated eggs differed ($P < 0.001$) from uncoated treatments in terms of albumen pH from the first week up to the end of the project. The results agree with previous studies (Caner and Yuceer, 2015; Biladeau and Keener, 2009), which reported that different coatings were able to extend the shelf-life of eggs in relation to albumen pH. This implies that the use of rice protein and propolis coatings act as barrier and help diffuse gases less rapidly through the shell.

No differences among the treatments in terms of yolk pH were observed up to the second week (Table 5). From week 3 to 5, the pH of the yolk in coated eggs was lower than of the uncoated eggs. However, at week 6, there was no difference among the pH of the yolk in control and any coated eggs. The pH of the yolk in uncoated eggs increased ($P < 0.001$) from pH 6.24 at week zero to pH 7.00 at week 6. Few variations in pH of egg yolk were expected because the pH of the albumen increases during storage due to CO_2 loss and migrations of water from the albumen into the yolk during storage (Biladeau and Keener, 2009).

Eggshell Color

The coloration is an important shell quality parameter and has a positive influence on consumer preference (Samiullah et al., 2015). Discoloration of products may lead to dissatisfaction for consumers (Caner, 2005). The L^* values, an indication of lightness or brightness of the shell, ranged from 80.68 to 85.10, indicating light-colored shells (Table 6). Eggs coated with propolis had the lowest L^* values, which could be explained by the presence of the yellow pigment, probably present in the propolis. Similar relationship was described (Wong et al., 1996) for eggs coated with corn zein, which could be explained by the presence of the yellow pigment (xanthophyll) in the coating. In addition, other study (Biladeau and Keener, 2009) found that wax-coated eggs had a decreasing in L^* over time and soy protein isolate had a yellowing effect over time. In this project, eggs coated with whey protein isolate, soy protein isolate, and wax-coated eggs were darker (less glossy) than the uncoated (lower L^*).

There was no difference in a^* values among treatments. However, the uncoated eggs showed higher b^* values than RPC-coated eggs, whereas the propolis-coated eggs were more yellow than the control and RPC. Other studies (Biladeau and Keener, 2009; Caner, 2005) reported that there was no difference in yellow color between protein-based coating eggs and uncoated ones.

The coatings altered the egg's visual appearance. Color values such as L^*, a^*, and b^* provide an objective evaluation of the appearance of coated shell eggs. Even though all proteins will not serve as consumer-acceptable coatings, processors may still be willing to purchase colored shell eggs because they have enhanced mechanical and barrier properties (Wong et al., 1996).

Eggshell Breaking Strength

Reducing egg breaking is important in the poultry industry. Thus, improving shell resistance would result in economic savings due to the reduced incidence of breakage or downgraded eggs (cracks) during handling and storage (Caner and Yuceer, 2015). However, in this study, eggshell breaking strength did not differ ($P > 0.05$) among uncoated eggs (4.22 kgf) and eggs coated with RPC alone (4.55 kgf), or in combination with 5% (4.64 kgf) or 10% (4.79 kgf) of propolis after 6 wk of storage. Although previous studies have described improvements in shell quality and reduction of eggshell breakage after coating application (Caner and Yuceer,

Table 4. Effect of rice protein concentrate (RPC) and propolis coatings on albumen pH during up to 6 wk of storage at 20°C.[1]

Coating	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	*P*-value
Control	$8.05 \pm 0.02^{E,a}$	8.35 ± 0.02^{Da}	8.70 ± 0.05^{Ca}	9.08 ± 0.04^{Ba}	9.21 ± 0.06^{Ba}	9.46 ± 0.16^{Aa}	9.48 ± 0.11^{A}	0.0001
RPC	$8.05 \pm 0.02^{E,a}$	$8.14 \pm 0.05^{E,b}$	$8.37 \pm 0.06^{D,b}$	$8.49 \pm 0.10^{C,b}$	$9.09 \pm 0.07^{B,b}$	$9.17 \pm 0.06^{AB,b}$	$9.20 \pm 0.04^{A,b}$	0.0001
RPC + P5	$8.05 \pm 0.02^{D,a}$	$8.10 \pm 0.04^{D,b}$	$8.28 \pm 0.07^{C,c}$	$8.41 \pm 0.10^{B,b}$	$9.10 \pm 0.08^{A,b}$	$9.12 \pm 0.05^{A,b}$	$9.19 \pm 0.10^{A,b}$	0.0001
RPC + P10	$8.05 \pm 0.02^{D,a}$	$8.09 \pm 0.05^{CD,b}$	$8.19 \pm 0.08^{C,c}$	$8.46 \pm 0.08^{B,b}$	$9.08 \pm 0.07^{A,b}$	$9.11 \pm 0.05^{A,b}$	$9.13 \pm 0.06^{A,b}$	0.0001

[1]Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment.
Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).
[a–d]Means in the same column with different lowercase letters are significantly different ($P < 0.001$).
[A–C]Means in the same row with different capital letters are significantly different ($P < 0.001$).
RPC: Rice protein coating; RPC+P5: Rice protein coating with 5% of propolis; RPC+P10: Rice protein coating with 10% of propolis.

Table 5. Effect of rice protein concentrate (**RPC**) and propolis coatings on yolk pH during up to 6 wk of storage at 20°C.[1]

Coating	0 wk	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	*P*-value
Control	$6.24 \pm 0.15^{C,a}$	$6.45 \pm 0.14^{B,a}$	$6.58 \pm 0.17^{B,a}$	6.92 ± 0.45^{Aa}	$6.95 \pm 0.04^{A,a}$	6.97 ± 0.03^{Aa}	$7.00 \pm 0.04^{A,a}$	0.0001
RPC	$6.24 \pm 0.15^{C,a}$	$6.30 \pm 0.12^{C,a}$	$6.50 \pm 0.19^{B,C,a}$	$6.46 \pm 0.23^{C,b}$	$6.45 \pm 0.10^{C,b}$	$6.73 \pm 0.21^{A,B,b}$	6.79 ± 0.06^{Aa}	0.0001
RPC + P5	$6.24 \pm 0.15^{C,a}$	$6.28 \pm 0.16^{C,a}$	6.41 ± 0.23^{Ca}	$6.48 \pm 0.19^{B,C,b}$	$6.46 \pm 0.11^{B,C,b}$	$6.68 \pm 0.13^{A,B,b}$	$6.84 \pm 0.10^{A,a}$	0.0001
RPC + P10	$6.24 \pm 0.15^{B,a}$	6.28 ± 0.11^{Ba}	$6.46 \pm 0.19^{A,B,a}$	$6.48 \pm 0.21^{A,B,b}$	$6.51 \pm 0.14^{A,B,b}$	$6.68 \pm 0.19^{B,b}$	$6.72 \pm 0.39^{A,a}$	0.0001

[1]Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment.
Statistical models included the effects of treatments ($P < 0.001$), storage periods ($P < 0.001$), and interaction (treatments by storage periods, $P < 0.001$).
[a–d]Means in the same column with different lowercase letters are significantly different ($P < 0.001$).
[A–C]Means in the same row with different capital letters are significantly different ($P < 0.001$).
RPC: Rice protein coating; RPC+P5: Rice protein coating with 5% of propolis; RPC+P10: Rice protein coating with 10% of propolis.

Table 6. Effect of rice protein concentrate (**RPC**) and propolis on the lightness (L^*), greenness (a^*), and yellowness (b^*) values of eggshell after 6 wk of storage at 20°C.[1]

	L^* value	a^* value	b^* value
Control	85.10 ± 3.59^{a}	0.44 ± 0.02	1.69 ± 0.06^{c}
RPC	$83.86 \pm 2.87^{a,b}$	0.41 ± 0.01	1.82 ± 0.10^{b}
RPC + P5	81.43 ± 2.38^{b}	0.39 ± 0.02	1.93 ± 0.09^{a}
RPC + P10	80.68 ± 3.36^{b}	0.42 ± 0.02	2.00 ± 0.05^{a}
P-value	0.0001	0.0880	0.0001

[1]Data are expressed as means ± standard deviations. Information was collected in 12 eggs per treatment.
[a–d]Means in the same column with different lowercase letters are significantly different ($P < 0.001$).
RPC: Rice protein coating; RPC+P5: Rice protein coating with 5% of propolis; RPC+P10: Rice protein coating with 10% of propolis.

2015; Biladeau and Keener, 2009), this characteristic seems to be associated with specific properties of the coatings used in the studies and was not observed in this trial.

Coating Effects

The RPC coating exhibited sufficient hydrophobicity and sealing properties required to effectively retard water loss during the storage at room temperature for up to 6 wk. Propolis is a hydrophobic compound that contributes to improve some properties of coatings, such as the water vapor barrier that reduces the loss of mass by transpiration, which naturally occurs in foods during storage (Pastor et al., 2010). The loss of albumen and yolk quality can be influenced by the capacity of the coating to block the pores on the surface of the shell. In general, the effects of the tested coatings on albumen and yolks are favorable, indicating that the use of RPC-based coating may be a viable alternative to maintain functional properties (HU, YI, and pH) of the eggs, which are adversely affected by storage period.

CONCLUSIONS

Coatings based on rice protein and propolis has been successfully used for extending shelf life of the egg when stored. These properties may help the egg industry in decreasing economic losses during storage at room temperature. Future studies are needed to verify if the use of rice protein coatings associated with propolis can also minimize the contamination of the shell by microorganisms.

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