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Maintenance of quality of eggs submitted to treatment with propolis extract and sanitizers

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ABSTRACT. This study aimed to evaluate the internal and microbiological quality of eggs submitted to different types of shell treatments. One hundred and forty-four fresh red eggs were distributed in a scheme of 4 treatments (no washing; washing and immersion in chlorine; washing and immersion in peracetic acid; and washing and spraying of propolis extract) x 5 storage periods (7, 14, 21, 28, and 35 days), stored at 25°C, in each period 6 eggs per treatment were analyzed. The parameters to assess were: weight loss; shell weight; yolk weight; albumen weight, yolk diameter; yolk height; albumen height and Haugh unit (HU). The microbiological quality of eggs was evaluated at 35 days through analysis for aerobic mesophilic bacteria, thermotolerant coliforms, *Staphylococcus* spp., *Salmonella* spp. and molds and yeasts. The treatment with propolis extract was the only effective one to maintain the high HU quality of the eggs until 21 days of storage at 25°C and was effective against microbiological contamination of all bacterial groups. The results presented showed greater effectiveness of the propolis extract for maintenance of internal and microbiological quality of eggs, it can be an alternative product to chemical sanitizers.

Keywords: Haugh unit; pulverization; chlorine; peracetic acid; antimicrobial activity.

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Introduction

The egg is a food that has advantageous nutritional and technological properties and low cost. They contain 12.56% of proteins, vitamins A, B-12, K, D, folic, and choline, as well as minerals such as iron, selenium, phosphorus, and potassium, considered indispensable for the human diet, and are useful as raw material for the food industry (Filipiak–Florkiewicz et al., 2017; Oliveira & Oliveira, 2013). The use of these benefits for human health depends on the quality of the egg offered to the consumer.

The loss of egg quality is a natural, continuous process, associated with the pores present in the shell, which allow the loss of carbon dioxide (CO_2) and water to the environment and increase the susceptibility of invasion by microorganisms into the eggs, after egg laying (Alpkinar, Canogullari, Baylan, Alasahan, & Aygun, 2015). The results are structural and biochemical changes in albumen and yolk that cause weight loss, less technological use of egg proteins and shorter shelf life (Oliveira & Oliveira, 2013).

The high storage temperatures and the prolonged time between the collection of eggs and their consumption are the main factors that influence the loss of quality (Pissinati et al., 2014; Guedes et al., 2016; Lana et al., 2017; Santos Henriques, Rodrigues, & Uczay, 2018). Knowing this, the marketing conditions in many regions of Brazil are unfavorable to the conservation of commercial eggs, since there is no obligation to refrigerate and there are long distances between the places of production and commercialization (Fernandes, Mori, Nazareno, Pizzolante, & Moraes, 2015). Therefore, studies of conservation methods and physical barriers applied to eggs that aim to minimize quality losses during storage, providing the shelf life extension become relevant (Galvão, Dos Santos, & Lima Neto, 2018).

Despite the growth in the production of egg products (egg powder and pasteurized egg), egg sold in shell is the most common form of marketing (ABPA, 2019). Thus, the eggshell is a natural packaging and an external aspect that influences the moment of purchase. The integrity and cleanliness of the shell are important parameters used to preserve the quality of the eggs, since the outside of the egg is vulnerable to contamination by microorganisms (Oliveira & Oliveira, 2013). The pathogen *Salmonella* spp. is an important control agent,

due to its impact on public health, and because eggs are considered the main vehicles of food transmission to humans (Seockmo, Eduardo, Thomas, & Ladish, 2016).

Washing and sanitizing eggs before packaging is a method that aims to reduce problems related to microbial shell contamination (Al-Ajeeli, Taylor, Alvarado, & Coufal, 2016). Despite being widely adopted in the United States, the cleaning of "fresh" eggs for consumption in Brazil is a controversial technique, given the prerogative of damaging the natural protection barriers of the egg, allowing microbial and chemical contamination (Al-Ajeeli et al., 2016; Stringhini et al., 2009).

In this context, natural products have been highlighted in the food industry, with propolis standing out as antimicrobial agent. Propolis is a resinous mixture produced by bees from resins collected from various plants, with antimicrobial, antioxidant and preservative properties. (Akpinar et al., 2015; Gregoris, Fabris, Bertelle, Grassato, & Stevanato, 2011; Kocot, Kielczykowska, Luchowska-Kocot, Kurzepa, & Musik, 2018; Przybylek & Karpinski, 2019). Some studies have proven the effectiveness of propolis extract in the disinfection of embryonated eggs (Aygun, Sert, & Copur, 2012; Vilela et al., 2012) and in the shelf life extension of commercial eggs (Carvalho et al., 2013; Pires et al., 2019), and may be a viable alternative in conservation techniques.

The objective of this work was to evaluate the internal and microbiological quality of red eggs submitted to different types of surface treatment of shell, using active chlorine, peracetic acid and alcoholic extract of commercial propolis.

Material and methods

The research was developed from October to November 2018, at Instituto Federal de Educação, Ciência e Tecnologia do Maranhão, campus São Luís - Maracanã. One hundred forty-four fresh red eggs were collected at random from laying hens of the Rhode Island Red breed, 42 weeks old. The eggs were transported to the Chemistry Laboratory, where they were distributed in a completely randomized design with a factorial 4 (treatments) x 5 (storage periods). The treatments were: eggs not washed or sanitized (control); eggs washed with water and immersed in active chlorine (50 ppm concentration); eggs washed with water and immersed in peracetic acid (50 ppm concentration); and eggs washed with water and sprayed with comercial alcoholic propolis extract from *Appis melifera* L. (30%).

Washing was performed with drinking water at 25°C, using soft bristle brushes. The sanitization step was performed by immersing the eggs in the sanitizer solution according to the treatment, at a temperature of 25°C for 1 minute. Spraying with commercial propolis extract (30%) was carried out homogeneously over the entire surface of the egg using hand sprayers. After spraying, the eggs remained on supports for drying at room temperature for two hours.

After drying, the eggs were identified, weighed individually on a semi-analytical balance and placed, aseptically, in sterile cellulose pulp trays. The samples were stored in incubators of BOD type, at a temperature of 25°C. The internal quality of the eggs was evaluated on the 7th, 14th, 21st, 28th and 35th day of storage by analyzing their weight loss, albumen height and weight; height, diameter and weight of the gem and Haugh unit of internal content (Figueiredo et al., 2011). Six eggs per treatment were analyzed in each evaluated storage period. The data obtained from each one were considered a repetition.

The determination of weight loss consisted of weighing the eggs of each treatment on a semi-analytical scale, on day zero and after each storage period. The weight loss of eggs in grams was determined by the difference between the weight of the sample at the beginning and at the end of storage time.

The eggs were individually broken carefully and the internal content was evaluated on a flat glass table. The height of the albumen was measured at the midpoint between the yolk end and the end of the dense albumen using a 150 mm Zaas Universal Analog Pachymeter. The diameter and height of the yolk were measured at its central point. Then, the yolk was separated from the albumen and the yolk was weighed. The peel was washed and dried for 24 hours at room temperature and after they were weighed. The weight of the albumen was determined by the difference in egg weight in relation to the weight of the yolk and the shell.

Haugh unit values were calculated considering the logarithmic relationship between the height of the dense albumen and the weight of the egg. Equation 1 was applied for the calculation:

$$HU = 100\text{Log}(H + 7{,}57 - 1{,}7W^{0{,}37})$$

Letter "H" is the height of the albumen in millimeters and letter "W" is the weight of the egg in grams (Haugh, 1937).

At 35 days of storage, microbiological analyses of the internal content of an egg sample from each of the treatments were carried out, with the sample unit corresponding to a pool of 6 eggs. The eggs were analyzed for counts of thermotolerant coliforms, aerobic mesophilic microorganisms, molds and yeasts, *Staphylococcus* spp. and research by *Salmonella* spp., following the methodology recommended by the Ministry of Agriculture, Livestock and Supply of Brazil (MAPA) (Brazil, 2003).

The internal quality variables were submitted to analysis of variance and the means compared by the Tukey test, with 5% probability, using the statistical program *InStat* (*Graphpad Instat: GraphPad Software Oberlin*, San-Diego-CA, USA).

Results and discussion

When evaluating the internal quality of eggs submitted to the different shell treatments (Table 1), there were significant differences for the weight loss, albumen weight and yolk diameter variables, in the five storage times evaluated.

Table 1. Internal quality parameters of red eggs submitted to different types of shell surface treatment stored at 25°C for 35 days.

Treatment	Storage time (days)				
	7	14	21	28	35
Weight loss (%)					
Control	3.39 [a]	6.58 [a]	9.09 [a]	10.30 [a]	13.27 [a]
Chlorine	3.36 [a]	6.45 [a]	9.96 [a]	12.99 [ab]	15.58 [a]
Peracetic acid	3.49 [a]	6.82 [a]	10.52 [a]	13.56 [b]	17.48 [a]
Propolis extract	1.67 [b]	1.93 [b]	3.33 [b]	4.23 [c]	5.00 [b]
Shell weight (g)					
Control	6.21 [a]	5.49 [a]	6.55 [a]	6.00 [a]	6.28 [a]
Chlorine	6.05 [a]	6.07 [a]	5.91 [a]	6.01 [a]	5.95 [a]
Peracetic acid	5.92 [a]	6.04 [a]	6.04 [a]	6.00 [a]	6.00 [a]
Propolis extract	5.94 [a]	6.32 [a]	6.00 [a]	5.97 [a]	6.25 [a]
Yolk weight (g)					
Control	16.13 [a]	16.64 [a]	17.80 [a]	17.15 [a]	17.26 [a]
Chlorine	16.67 [a]	29.88 [b]	16.60 [a]	16.57 [a]	17.30 [a]
Peracetic acid	16.69 [a]	26.55 [b]	17.80 [a]	16.50 [a]	17.19 [a]
Propolis extract	17.22 [a]	18.05 [a]	16.98 [a]	17.39 [a]	17.96 [a]
Albumen weight (g)					
Control	38.99 [a]	33.72 [a]	35.83 [a]	30.96 [a]	31.75 [a]
Chlorine	37.24 [a]	21.04 [b]	35.51 [a]	29.19 [a]	29.13 [a]
Peracetic acid	36.80 [a]	23.28 [b]	33.27 [a]	31.06 [a]	28.01 [a]
Propolis extract	35.74 [a]	37.50 [a]	35.36 [a]	35.02 [b]	35.05 [a]
Yolk diameter (mm)					
Control	42.92 [a]	44.52 [a]	48.59 [a]	52.58 [a]	51.23 [a]
Chlorine	43.58 [a]	46.46 [a]	47.98 [ab]	50.70 [a]	51.32 [a]
Peracetic acid	43.58 [a]	44.49 [a]	48.80 [a]	50.74 [a]	49.63 [a]
Propolis extract	42.92 [a]	43.93 [a]	45.63 [b]	48.19 [a]	47.81 [a]

Averages with the same letters in the column do not differ by Tukey's Test ($p > 0.05$).

The eggs submitted to the shell treatment with propolis extract showed less weight loss, with significant differences ($p > 0.05$) in relation to the other treatments, in all storage periods. These results presented the effectiveness of coating with propolis extract at 30% in preserving weight throughout the storage period. The greatest loss of egg weight was observed at 35 days, with 5% this tratament (Table 1).

From an economic point of view these results are important, since egg weight loss of up to 3% is expected in commercial eggs and it is barely noticeable to the consumer (FAO, 2003). Egg weight loss is related to water evaporation to the external environment. The removal of the protective cuticle from the shell through brushing in the egg washing process is described as an influencing factor in the evaporation speed during storage (Carvalho et al., 2013; Mendes et al., 2014).

Eggs submitted to washing and sanitizing had higher values in weight loss, however, these did not present significant differences in relation to unwashed eggs (control). It can be inferred that the washing of eggs did not influence the acceleration of weight loss. Other factors such as temperature and storage time are recorded as factors of greatest influence on this variable (Guedes et al., 2016; Lana et al., 2017).

The lower weight loss of eggs treated with propolis extract may be related to the resinous composition of the propolis that lines the pores of the shell, preventing the loss of water and CO_2 and preserving the weight of the egg during the storage period (Aygun et al., 2012).

As for the bark weight variable (Table 1), there was no significant difference between treatments in all storage periods. These results are expected, since this variable is not influenced by environmental factors and the use of sanitizers (Pissinati et al., 2014).

The variables yolk weight, albumen weight and yolk diameter (Table 1) had no differences between the treatments evaluated, at the end of the experiment. However, differences were observed at 14 days of evaluation, in which eggs submitted to cleaning with chlorine and peracetic acid registered a higher yolk weight and a lower albumen weight ($p > 0.05$), indicating inferior quality when compared to eggs that have not been washed and those that have been sprayed with propolis extract. At 21 days of storage, there was a difference between the propolis treatment and the control and peracetic acid treatments, recording the smallest diameter (45.63 mm) of the yolk for the eggs sprayed with propolis.

The reactions that occur naturally in the albumen during storage are directly related to the percentage of yolk and albumen (Pissinati et al., 2014). The transformation of ovalbumin into S-albumin and the dissociation of ovomucin-lysozyme cause the albumen's viscosity to decrease and its greater transfer of water from the albumen to the yolk, determining the increase in its volume and weight, making it larger and more flattened when the egg is observed after being broken on a flat surface (Huang et al., 2012; Pissinati et al., 2014).

The yolk and albumen height were higher for eggs sprayed with propolis extract in relation to the other treatments (Table 2), with significant differences from the 14th day of storage. In this treatment, the yolk height showed higher values until the 35th day of storage, ensuring a better quality to the eggs. The higher values of albumen height can be observed for the treatment with propolis extract, with a significant difference on the 14th to 28th day of storage, suggesting this treatment is more efficient for quality conservation (Table 2).

Table 2. Results of the height of the yolk and albumen and of the Haugh unit (HU) of red eggs submitted to different types of surface treatment of shell stored at 25°C.

Treatment	Storage time (days)				
	7	14	21	28	35
			Yolk height (mm)		
Control	15.08 [a]	12.93[a]	11.46 [a]	10.27 [ab]	9.93[ab]
Chlorine	15.42 [a]	12.58[a]	11.69[ab]	10.27 [ab]	9.44 [b]
Peracetic acid	15.25 [a]	12.88[a]	11.37 [a]	9.76 [a]	9.48 [b]
Propolis extract	15.75 [a]	15.84[b]	13.61 [b]	12.38 [b]	11.78 [ac]
Average	15.38	13.56	12.03	10.67	10.16
			Albumen height (mm)		
Control	5.58[a]	3.11 [a]	2.28 [a]	2.41 [a]	1.92 [a]
Chlorine	4.75 [a]	3.48 [a]	2.42 [a]	2.48 [a]	2.62 [a]
Peracetic acid	4.25 [a]	3.69 [a]	2.68 [a]	2.27 [a]	1.98 [a]
Propolis extract	5.75 [a]	5.73 [b]	5.05 [b]	3.83[b]	2.66 [a]
Average	5.08	4.00	3.11	2.75	2.29
			Haugh unit (UH)		
Control	72.42[a]	47.88 [a]	31.61 [a]	39.87 [a]	29.63 [a]
Chlorine	65.48 [a]	53.65 [a]	35.77 [a]	42.36 [a]	43.39 [a]
Peracetic acid	61.24 [a]	56.42[ab]	42.27 [a]	38.27 [a]	34.76 [a]
Propolis extract	72.11 [a]	72.44 [b]	68.46 [b]	56.53[b]	39.01 [a]
Average	67.81	57.60	44.53	44.26	36.70

Averages with the same letters in the column do not differ by Tukey's Test ($p > 0.05$).

The Haugh unit (HU) was a parameter used to assess changes in albumen quality, correlating dense albumen height and egg weight, the higher the HU value the better the egg quality (USDA, 2000). Thus, it was observed that the most efficient treatment was sprayed with propolis extract, since the values were higher than the HU (Table 2), showing significant differences between the treatments from the 14th to the 28th day of storage.

According to the USDA manual (2000), eggs are classified by HU value in type AA - excellent quality (100 to 72), A - high quality (71 to 60), B - medium quality (59 to 30), and C - low quality (29 to 0). It was observed that in the first two weeks, the eggs sprayed with propolis were classified in type AA (72.11 and 72.44, respectively), in the third week they were classified in type A (68.46), in the fourth in type B (56.53). Only in the last week, regardless of the treatment used, were all classified as type B, however, eggs treated with

propolis registered the highest HU. It is possible to observe that, in the second week, the treatment with propolis maintained the excellent quality, while the others presented inferior quality.

Carvalho et al. (2013) studied the extension of the shelf life of eggs by covering with propolis and observed that the value of HU differed significantly between treatments "with propolis" and treatments "without washing" and "washed" during the 42 days of storage, indicating better HU results for propolis treatments. In this study, the eggs treated with propolis remained in the AA and A classifications (USDA, 2000), during the storage period, corroborating the results found in this study.

The better quality of eggs sprayed with propolis extract can be explained by its composition predominantly formed by resinous and waxy substances, originally used protectively by bees to seal cracks, reduce the entry and exit of the hive, in addition to repair and fix the comb (Sun, Wu, Wang, & Zhang, 2015). In addition, it is used as a mummifier, covering the body of dead pests, contributing to an aseptic environment (Bonamigo et al., 2017). Thus, the effectiveness of the propolis extract may be associated with the sealing of the pores of the eggshell, reducing gas exchange and the speed of chemical changes that significantly modify the structure of the yolk and albumen during storage.

The results for higher albumen height for treatment with spraying propolis extract indicated less albumen liquefaction, promoting a higher HU, greater height and smaller yolk diameter and, consequently, less weight loss, resulting in the better quality egg when compared to other treatments.

The microbiological characteristics of the internal content of eggs at 35 days of storage was observed that the analyzed eggs had a low microbiological contamination (Table 3). Through Resolution n° 12 of January 2, 2001 (BRASIL, 2001), National Health Surveillance Agency establishes as microbiological standards, the absence of Salmonella sp./25g, for raw whole eggs and values below 1 UFC g^{-1} for coliforms a 45°C/mL in albumen, yolk and mixtures. Thus, the results found (Table 3) demonstrated that the eggs were within the legal parameters, therefore, suitable for consumption.

Table 3. Microbiological analysis of internal content of eggs subjected to different types of surface treatment of shell, stored for 35 days.

	Contamination levels UFC g^{-1}			
Microorganisms	Control	Chlorine	Peracetic acid	Propolis extract
Aerobic mesophilic bacteria	110	1,540	1,600	0
Staphylococcus spp.	20	2,000	0	0
Molds and yeasts	2,515	1,000	530	550
Thermotolerant coliforms,	0	0	0	0
Salmonella spp.	0	0	0	0

The chlorine treatment showed high counts for aerobic mesophilic bacteria, *Staphylococcus* spp., molds and yeasts. Possibly, these results may be related to the removal of the protective cuticle of the peel by disinfectant, compromising its natural protection and allowing the entry of microorganisms through the pores of the peel (Stringhini et al., 2009).

The spraying of the propolis extract was effective against all groups of bacteria important in controlling the quality of the internal content of eggs (Table 2). These results indicate that the application of this product had a prolonged effect on its antimicrobial activity, inhibiting bacterial growth during the storage period. Furthermore, this effect may be related to the protective property of propolis as a physical barrier, preventing the entry of bacteria from the shell to the internal part of the egg (Sun, Wu, Wang, & Zhang, 2015).

When testing the use of propolis as a disinfectant for embryonated eggs, Vilela et al. (2012) found that the peel treatments with propolis in different concentrations showed a lower level of contamination when compared to the control group (without disinfection). Aygun et al. (2012) evaluated the antimicrobial activity of different concentrations (5, 10, and 15%) of the propolis extract sprayed under the shell of embryonated quail eggs, finding a lower count of mesophilic bacteria in all groups using propolis.

The efficiency of the antimicrobial activity of the propolis extract of Brazilian origin is proven against Gram positive and Gram negative microorganisms of animal origin (Gomes, Ítavo, Leal, Ítavo, & Lunas, 2016; Klahr et al., 2019; Souza, Inoue, Fernandes Júnior, Veiga, & Orsi, 2014). Among the components associated with this property, phenolic compounds and flavonoids stand out, which possibly damage the cellular structure of these microorganisms, inhibiting their growth or even eliminating them (Przybylek & Karpinski, 2019). It is important to note that propolis's antimicrobial action varies according to its chemical composition, related to the place, time and harvesting techniques (Pinto, do Prado, & de Carvalho, 2011; Souza et al., 2014).

Conclusion

The treatment of egg shells with propolis extract is effective in preserving the internal quality of the eggs up to 21 days of storage at a temperature of 25ºC, since it maintains high quality for HU, less weight loss and lower level of microbiological contamination than eggs not washed or sanitized with chlorine or peracetic acid.

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