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Rice flour coating supplemented with rosemary essential oil to preserve the internal, microbiological, and sensory quality of quail eggs

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ABSTRACT

Coatings of rice flour (RF), reinforced or not with rosemary essential oil (ROS), were used to evaluate changes in the internal quality of quail eggs stored at room temperature. Quality parameters [egg weight loss (EWL, %), Haugh unit (HU), yolk index (YI), albumen and yolk pH], microbiological (counts of total aerobic mesophilic bacteria and *Enterobacteriaceae*) and sensory (colour, aroma, odour, texture, taste, and general acceptability) parameters were evaluated during the experiment. Compared to the number of total aerobic mesophilic bacteria on the shell of uncoated eggs (2.02 ± 0.49; 1.78 ± 0.20 $\log_{10}$ CFU mL^{-1}), RF/ROS exhibited significant inhibition effects for these bacteria on 0. (1.16 ± 0.25 $\log_{10}$ CFU mL^{-1}) and 21. (0.84 ± 0.11 $\log_{10}$ CFU mL^{-1}) days of storage at room temperature. On days 7, 14, and 21, RF had no effect on HU ($P > 0.05$), but eggs coated with RF/ROS had significantly higher HU ($P < 0.05$) than

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uncoated eggs from day 14, maintaining grade AA (73.88 ± 2.67) on day 21, while uncoated eggs had grade A (68.90 ± 1.55) at that time. The RF coating reinforced with ROS was a bioactive, efficient, and safe formulation for application based on internal quality, microbiological and sensorial aspects of quail eggs.

KEYWORDS

coating, egg microbiology, egg quality, egg storage, essential oils

1. INTRODUCTION

The preservation of egg quality and extension of their shelf life can include refrigerated storage conditions and eggshell coatings (Oliveira et al., 2020; Yüceer and Caner, 2020; Pires et al., 2022). Coatings are more viable options, as the high cost of cooling makes its application unfeasible, especially in poorer countries. Widely available cereals produced on a large scale, such as rice, have been shown to be promising candidates for the preparation of food coatings, including eggs. The rice protein concentrate applied to stored eggs, in Brazil, positively influenced the internal quality of these eggs, increasing their shelf life (Pires et al., 2020). Rice bran oil can also be applied as a coating on stored eggs to preserve their internal quality (Nongtaodum et al., 2013). The development of coatings based on flours of agricultural crops compared to, for example, protein coatings, is due to their positive characteristics in terms of cost, acquisition, and availability (Majzoobi et al., 2015). In addition, the use of rice flour (RF) as a matrix for manufacturing coatings is due to its biodegradability, safety for humans and the environment, and gel-forming potential (Silva-Rodrigues et al., 2020). However, no reports of the use of RF-based coatings for eggs are found.

More efficient coatings for eggs can be obtained by combining them with hydrophobic antimicrobial molecules such as essential oils. When enriched with essential oil (e.g., tea tree, copaiba, thyme, ginger, lemongrass, or Tahiti lemon), the egg coating acts as a shield against internal and external microbial degradation of the egg, as well as minimising physical and chemical deterioration events, preserving egg quality (Pires et al., 2020; Oliveira et al., 2022a, 2022b). Rosemary essential oil (ROS - *Rosmarinus officinalis* L.) has been shown to be a favourable option for inclusion in coatings, as it contains α-pinene, which exhibited a broad antibacterial spectrum against Gram-negative and Gram-positive bacteria, and 1,8-cineole, which showed antibacterial activity against Gram-negative pathogenic bacteria (Ojeda-Sana et al., 2013). Thus, the objective of this research was to evaluate the application of a RF coating supplemented with ROS on quail eggs and its effects on the internal, microbiological, and sensory quality of quail eggs during storage. If effective, the coating can be applied in routine egg processing in the poultry industry, as soon as possible after laying eggs.

2. MATERIALS AND METHODS

ROS was purchased commercially (Phytoterapica, Rio de Janeiro, Brazil). Preliminary antimicrobial testing carried out showed that the 1% ROS inhibited the growth of *Escherichia coli* and *Staphylococcus aureus*, forming inhibition halos of 8.25 ± 0.28 mm and 9.05 ± 0.24 mm, respectively. As the essential oil met the antimicrobial criteria, it was added as an antimicrobial supplement to the coating.

The RF (Bio Mundo, Brasília, Brazil) solution was prepared based on procedures adapted from Silva-Rodrigues et al. (2020). Twenty-four grams of RF were dissolved in 400 mL of distilled water and stirred constantly at 80 °C. After 5 min of stirring, glycerol (50% w/w of RF; Dinâmica, São Paulo, Brazil) was added to the RF solution and stirred continuously for an additional 25 min. Then, the solution was cooled to 40 °C and 1% (w/w) of ROS diluted in Tween 80 (1% v/v; Dinâmica, São Paulo, Brazil) was added. Finally, the solution was stirred for another 10 min. As the RF contained 3.5 g of proteins, the pH of the solution was adjusted to 10.0 with NaOH 1N (Merck, Darmstadt, Germany), to dissolve these.

One hundred and sixty-two eggs were purchased from a quail rearing system (Planaltina, Federal District, Brazil). The quails were 14 weeks old. To measure the conservation potential of the immersion coating with RF, the eggs were randomly divided into three treatments (uncoated eggs, eggs coated with RF, and eggs coated with RF/ROS). The eggs were immersed for 5 min in the coating and then allowed to dry for 3 h at 28.39 ± 0.56 °C and 44.54 ± 0.78% relative humidity before being placed in sterilised plastic trays. The trays were stored on clean benches in a room for 21 days at an average temperature and humidity of 29.75 ± 1.53 °C and 42.40 ± 8.33%, respectively. Temperature and humidity were recorded every 5 min using a HOBO data logger (Onset Computer Corp., Bourne, MA), and the averages are reported here.

Egg quality parameters [(1) egg weight loss (EWL, %), (2) Haugh unit (HU), (3) yolk index (YI), (4) albumen and yolk pH] were obtained from 7 eggs/treatment every 7 days from day zero (with the exception of the EWL that was from the seventh day). The internal quality of the egg was initially characterised as follows: 89.14 ± 4.38 (HU), 0.47 ± 0.06 (YI), 8.70 ± 0.23 (albumen pH), and 6.27 ± 0.45 (yolk pH).

1. EWL: The initial egg weight (IEW) and the final egg weight (FEW) were measured using an analytical scale with 0.0001 g precision (Gehaka, São Paulo, São Paulo, Brazil) to calculate the EWL, where $EWL = (IEW–FEW)/IEW \times 100$.
2. HU: Albumen height (H) and egg weight (W) were measured using a digital calliper with 0.001-mm precision (Mitutoyo, Suzano, São Paulo, Brazil) and an analytical scale with 0.0001 g precision (Gehaka, São Paulo, São Paulo, Brazil), respectively, to calculate the HU, where $HU = 100 \log (H + 7.57–1.7\ W^{0.37})$ (Haugh, 1937).
3. YI: The height (h) and diameter (d) of the yolk were measured using a digital calliper with 0.001-mm precision (Mitutoyo, Suzano, São Paulo, Brazil) to calculate the YI, where $YI = h/d$ (Funk, 1948).
4. Albumen and yolk pH: Albumen and yolk pH were measured individually using a calibrated digital pH meter (206–pH2, Testo, Lenzkirch, Baden-Württemberg, Germany) after the previous analyses.

Adapting the methodology of Wells et al. (2010), nine eggs per treatment were transferred on days 0 and 21 to sterile bags (3 eggs per bag) containing 60 mL of 0.1% peptone saline solution and rubbed manually for 5 min. Rub solutions (not serially diluted with the exception of uncoated egg solutions) were plated on plate count agar (Ionlab, Paraná, Brazil) for total aerobic mesophilic bacteria counts and on violet red bile glucose agar (Ionlab, Paraná, Brazil) for *Enterobacteriaceae* counts. The total count of mesophiles and *Enterobacteriaceae* was determined after incubating the plates at 36 °C for 48 h. Colonies were expressed as $\log_{10}$ CFU mL^{-1}.

Adapting the methodology of Figueiredo et al. (2014), nine eggs per treatment on day 21 (the same eggs that were used for the eggshell microbial count) were sanitised with 70% alcohol and aseptically broken after drying. The contents of a pool of 3 eggs (18 mL; 6.0 mL of each egg) were transferred to a beaker with 162 mL of 0.1% peptone saline solution. Similarly to the previous analysis, dilution, plating, incubation, and colony enumeration steps were performed.

Sensory analysis of the eggs was performed on the last day of egg storage according to a protocol adapted from Nwamo et al. (2021). Ten volunteer panellists aged between 20 and 40 years (50% women and 50% men) from a technical course in the area of Agricultural Sciences received a detailed explanation and signed a Free and Informed Consent Form as required by the Brazilian Legislation. The eggs were boiled at 100 °C for 15 min and then served to the panellists. Each panellist received a coded whole egg and a glass of water to drink between each treatment sample. Colour, aroma, odour, texture, taste, and general acceptability were evaluated using a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely).

A completely randomised design with 7 repetitions was used to evaluate internal egg quality parameters, triplicate for antimicrobial analysis, and 10 repetitions for sensory analysis. PROC UNIVARIATE and PROC GLM, from SAS Studio University Edition software (SAS Inst. Inc., Cary, NC), were used to test normality and perform analysis of variance on the data, respectively. Tukey's test was applied to determine sample means that differed significantly ($P < 0.05$). Non-normal data were compared by the Kruskal–Wallis test using the PROC NPAR1WAY procedure.

3. RESULTS AND DISCUSSION

Figure 1 shows bacterial counts in shells and contents of coated and uncoated eggs. Compared to the number of total aerobic mesophilic bacteria on the shell of uncoated eggs (2.02 ± 0.49; 1.78 ± 0.20 $\log_{10}$ CFU mL^{-1}), RF/ROS treatment exhibited significant ($P < 0.05$) inhibition effects for these bacteria in the eggshell on days 0 (1.16 ± 0.25 $\log_{10}$ CFU mL^{-1}) and 21 (0.84 ± 0.11 $\log_{10}$ CFU mL^{-1}) of storage at room temperature (Fig. 1A). The bactericidal effect of RF/ROS on the eggshell was rapid, as the same day the eggs were coated, the mesophilic bacteria count was reduced and remained continuously low for 21 days. There was also a significant reduction ($P <$ 0.05) for eggs coated with RF/ROS (1.05 ± 0.25 $\log_{10}$ CFU mL^{-1}) in relation to uncoated eggs (2.17 ± 0.11 $\log_{10}$ CFU mL^{-1}) for the count of these bacteria in the egg content at 21 days of storage (Fig. 1B). This is probably due to the fact that, although eggshells are porous and can facilitate microbial penetration, they are equipped with natural chemical and physical antimicrobial barrier systems (Gantois et al., 2009), and that, together with the rapid and significant antimicrobial action of the RF/ROS coating on the eggshell, may have reduced microbial penetration rates. The excellent antibacterial performance of the RF/ROS coating is due to the presence of ROS, which in this study had its *in vitro* antibacterial potential validated against *E. coli* and *S. aureus* bacteria. Rosemary has the potential to inhibit microorganisms due to its bioactive composition (e.g., rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol, and isorosmanol), which can cause combined detrimental damage to microbial survival, such as alterations in the genetic material of the cell and leakage of cellular components after cell membrane disruption (Nieto et al., 2018). The non-contamination of the eggshell and egg contents by bacteria of the *Enterobacteriaceae* family may have been a reflection of strict

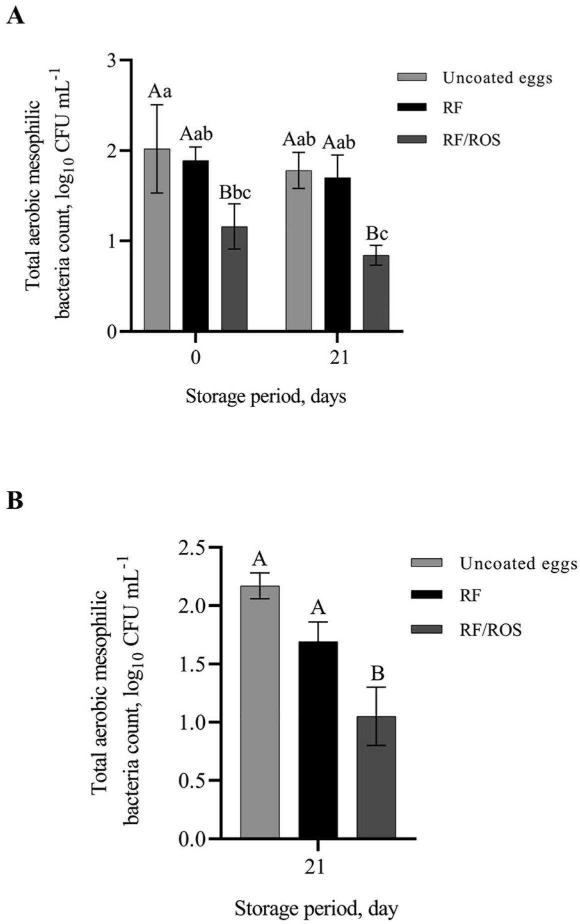

Fig. 1. Counts of total aerobic mesophilic bacteria on eggshell surfaces (A) and egg contents (B) of coated and uncoated eggs at 0 and 21 days of storage at room temperature. Results are mean ± standard deviations of triplicate measurements. $^{A-B}$Means with different capital letters (treatment effect within each day of storage) are significantly different (P < 0.05). $^{a-c}$Means with different lowercase letters (effect of day of storage within each treatment) are significantly different (P < 0.05). RF: Rice flour; RF/ROS: Rice flour plus rosemary essential oil

hygiene practices in the quail shed and during egg collection and handling, including coating preparation and egg storage. Our results are in line with previously published reports (Oliveira et al., 2021, 2022b).

At the end of storage, eggs coated with RF/ROS (7.27 ± 1.44%) inhibited higher (P < 0.05) EWL than eggs in the control treatment (10.04 ± 1.03%) (Fig. 2); there was no significant effect (P > 0.05) on EWL between RF (8.87 ± 1.20%) and RF/ROS coated eggs, while RF did not differ (P > 0.05) from control. The better result for RF/ROS is possibly due to its pore sealing action and small shell deformities against the loss of water vapours and gases. Mixing RF (hydrophilic

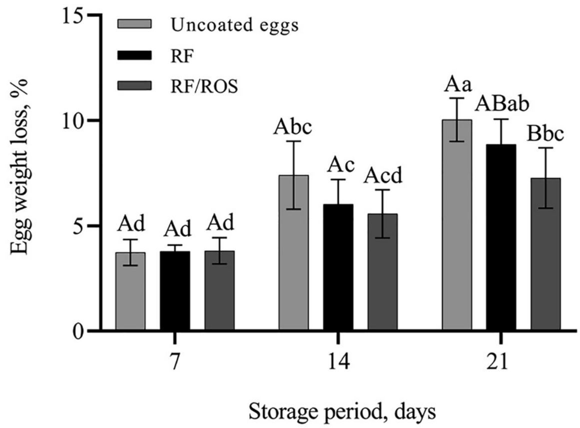

Fig. 2. Weight loss of coated and uncoated eggs during 21 days of storage at room temperature. Results are mean ± standard deviations of measuring 7 eggs. A−BMeans with different capital letters (treatment effect within each day of storage) are significantly different ($P < 0.05$). a−dMeans with different lowercase letters (effect of day of storage within each treatment) are significantly different ($P < 0.05$). RF: Rice flour; RF/ROS: Rice flour plus rosemary essential oil

product) and ROS (hydrophobic product) efficiently met the barrier characteristics required for eggs (supported by reduced EWL), probably because of the combination of these materials with different properties (Sánchez-González et al., 2011).

The HU decreased for coated and uncoated eggs with increasing storage time (Fig. 3). On days 7, 14, and 21, RF had no effect ($P > 0.05$), but eggs coated with RF/ROS had a significantly higher HU ($P < 0.05$) than eggs uncoated from day 14, maintaining grade AA (73.88 ± 2.67) on day 21, while uncoated eggs averaged a grade A (68.90 ± 1.55). These findings corroborate

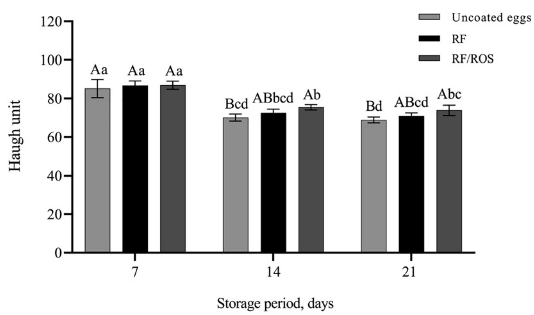

Fig. 3. Haugh unit (HU) of coated and uncoated eggs during 21 days of storage at room temperature. Egg quality score based on HU: AA, excellent (≥72); A, high quality (71–60); B, average quality (59–31); and C, low quality (≤30) (Yüceer and Caner 2014). Results are mean ± standard deviations of measuring 7 eggs. A−BMeans with different capital letters (treatment effect within each day of storage) are significantly different ($P < 0.05$). a−dMeans with different lowercase letters (effect of day of storage within each treatment) are significantly different ($P < 0.05$). RF: Rice flour; RF/ROS: Rice flour plus rosemary essential oil

Oliveira et al. (2022b). The considerable reduction of HU values caused by albumen liquefaction (process that occurs due to depolarisation of ovomucin α and β subunits, which are responsible for the thick nature of albumen) (Obianwuna et al., 2022) leads to considerable economic losses, as it can make the eggs unacceptable to the consumer due to sensory perception, or unviable for sale and consumption due to quality criteria. Preventing loss of albumen quality by coating the egg shell with RF/ROS could be the way forward to minimise egg waste and economic damage in industry, markets, and homes.

Measured in every 7 days, no significant differences ($P > 0.05$) were recorded between treatments for YI (Fig. 4A). However, at the 7–21 day interval, the highest value ($P < 0.05$) of YI was observed for eggs coated with RF/ROS (0.21 ± 0.03), while the lowest were recorded for coated eggs with RF (0.19 ± 0.05) and uncoated ones (0.19 ± 0.04) (Fig. 4B). Overall, RF/ROS coated eggs had better yolk quality. This probably occurred because the quality of the albumen was less impaired due to this coating, promoting less movement of part of the albumen content (resulting from its liquefaction) to the yolk (Obanu and Mpieri, 1984). The present

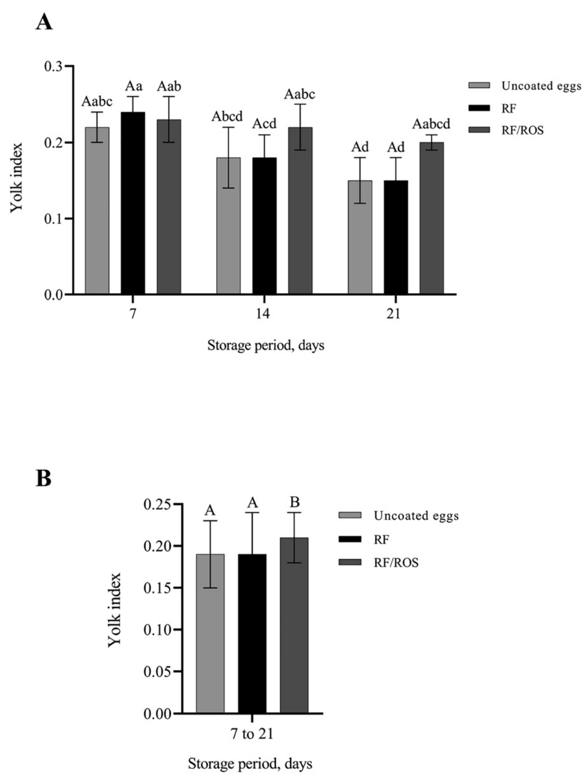

Fig. 4. Yolk index (YI) of coated and uncoated eggs during 21 days of storage at room temperature. Results are mean ± standard deviations of measuring 7 eggs. [A–B]Means with different capital letters (treatment effect within each day of storage) are significantly different ($P < 0.05$). [a–d]Means with different lowercase letters (effect of day of storage within each treatment) are significantly different ($P < 0.05$). RF: Rice flour; RF/ROS: Rice flour plus rosemary essential oil

observations corroborate with Pires et al. (2020). Yolk quality losses attributed to unfeasible or ineffective egg preservation mechanisms can be efficiently minimised using RF/ROS coating.

There was no significant difference ($P > 0.05$) between coated and uncoated eggs for albumen pH on days 7, 14, and 21 or considering the entire storage period (Fig. 5A and 5B). Similar changes on days 7, 14, and 21 ($P > 0.05$) for yolk pH between treatments were also observed (Fig. 5C). On the other hand, it was possible to notice smaller and significant variations ($P < 0.05$) in the yolk pH of eggs coated with RF/ROS when compared to uncoated eggs considering the entire storage period (Fig. 5D). This was likely because less albumen water penetrated the yolk due to improved preservation of the albumen by RF/ROS, resulting in reduced changes in yolk pH.

Figure 6 summarises the average sensory scores assigned by the judges to uncoated, coated eggs with or without essential oil cooked on day 21 of storage at room temperature. The sensory scores considered in this study seem not to have been affected by the coatings, as no significant difference ($P > 0.05$) was observed for scores among treatments. Although statistically similar, the RF/ROS coated eggs tended to have higher scores than the uncoated eggs. The essential oil concentration used in coating preparation was safe, and the RF and cooking of the eggs likely blocked or minimised any undesirable effects of the essential oil (e.g., odour), preserving the sensory profile

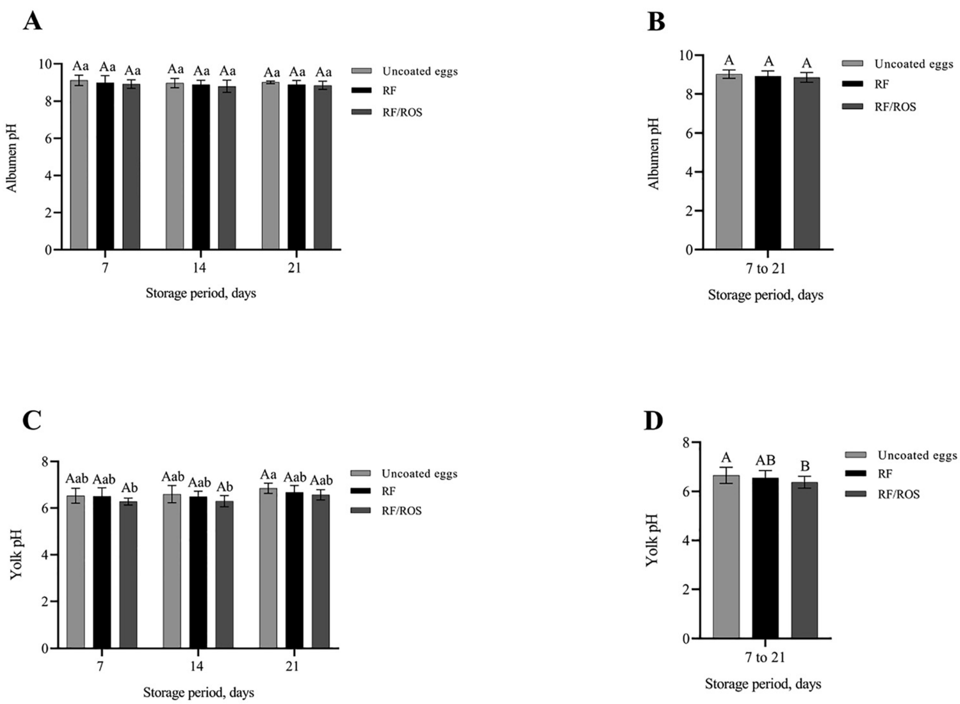

Fig. 5. Albumen (A and B) and yolk (C and D) pH of coated and uncoated eggs during 21 days of storage at room temperature. Results are mean ± standard deviations of measuring 7 eggs. $^{A-B}$Means with different capital letters (treatment effect within each day of storage) are significantly different ($P < 0.05$). $^{a-d}$Means with different lowercase letters (effect of day of storage within each treatment) are significantly different ($P < 0.05$). RF: Rice flour; RF/ROS: Rice flour plus rosemary essential oil

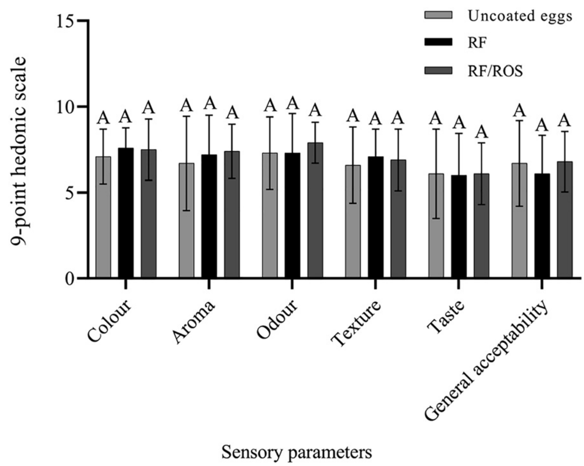

Fig. 6. Sensory parameter scores of coated and uncoated eggs on the 21st day of storage at room temperature. Results are mean ± standard deviations of measuring 10 eggs. ^{A}Means with the same capital letters are statistically similar (P > 0.05). RF: Rice flour; RF/ROS: Rice flour plus rosemary essential oil

of the eggs. It is suggested that the minimal change in the internal quality of eggs coated with RF/ROS and the low microbial loads in the shell and egg contents, even in uncoated eggs, were also key points in not altering sensory factors of the eggs and not posing risks to human health.

4. CONCLUSIONS

The RF coating reinforced with ROS, which has a sustainable, safe and biodegradable profile, proved to be a bioactive and efficient formulation for application in quail eggs for delaying the loss of internal quality, protecting the sensorial aspects and suppressing the presence of micro-organisms in the shell and egg contents during storage at room temperature, which can maximise food safety and minimise food loss.

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