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Whey Protein Isolate and Garlic Essential Oil as an Antimicrobial Coating to Preserve the Internal Quality of Quail Eggs

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Abstract: The aim of this study was to evaluate the effects of a coating formulation involving whey protein isolate (WPI) and garlic essential oil (GEO) on the internal quality and microbiological and sensory attributes of quail eggs stored for 28 days at room temperature. Unwashed quail eggs (171) were divided into treatments of uncoated eggs (UE), WPI-coated eggs and WPI/GEO-coated eggs and analyzed during the study period. Eggs coated with WPI/GEO had 1.51 $\log_{10}$ CFU/mL^{-1} less aerobic mesophilic bacteria, 2.02 $\log_{10}$ CFU/mL^{-1} less Enterobacteriaceae and 1.34 $\log_{10}$ CFU/mL^{-1} less molds and yeasts on the eggshell when compared to UE. The Haugh unit (HU) in the range of 0–28 days of storage was significantly higher for WPI/GEO- (88.26 ± 4.83, grade AA) coated eggs than WPI-coated (86.55 ± 6.20, grade AA) and UE (85.94 ± 6.46, grade AA). The new coating formulation (WPI/GEO) can be an option to preserve the quality of quail eggs.

Keywords: egg production; food safety; microbiological quality; sustainability

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1. Introduction

The availability of good quality quail eggs to consumers depends on the degree of deteriorative impacts they have suffered before being offered for sale. This degree of deterioration will be determined by the management of the eggs, which mainly includes the environmental conditions to which they are subjected, whereby the degree of internal deterioration of eggs is lower in cool compared to hot environments [1,2]. However, these cool environments will only be fully available for egg storage if new technologies significantly reduce their cost [3].

Alternatives are being tested to extend the shelf life of eggs and reduce their waste. Coatings aim to preserve the eggs without compromising the shell structure [4,5]. For example, protein coatings, such as those made from whey protein isolate (WPI), have previously been applied to eggs, and antimicrobial effects and preservation of internal egg quality have been reported [6–8]. Essential oils have been shown to enhance the preservative and antimicrobial properties of whey protein isolate (WPI) coatings [9]. One example is garlic essential oil (GEO), a volatile and antimicrobial compound extracted from a bulbous plant species called garlic (*Allium sativum* L.; family Liliaceae), which

is cultivated worldwide [10]. Garlic, as a natural product, become more popular in the composition of antibiotic solutions; synthetic commercial formulations can have harmful effects, mainly on the environment, that can last for many years [11]. This essential oil combined with WPI promoted a potential food preservative effect in terms of antimicrobials and antioxidants [12]. In addition, it has been shown to inhibit the in vitro growth of *Escherichia coli* O157:H7 (ATCC 35218), *Staphylococcus aureus* (ATCC 43300), *Salmonella enteritidis* (ATCC 13076), *Listeria monocytogenes* (NCTC 2167) and *Lactobacillus plantarum* (DSM 20174) [13].

These potential coatings stimulated interest for further studies in order to determine their effective application in the industrial food sector, including the egg industry. Thus, the aim of this study was to evaluate the effects of a coating formulation involving WPI and GEO on the internal quality and microbiological and sensory attributes of quail eggs stored for 28 days at room temperature.

2. Materials and Methods

The GEO (commercially purchased; Laszlo, Minas Gerais, Brazil) was extracted from garlic rhizomes by steam distillation. Gas chromatography coupled with mass spectrometry (GC-MS) was used to analyze the chemical composition of GEO. The oil was analyzed in duplicate using a gas chromatograph (GCMS-QP2010 SE, Shimadzu, Kyoto, Japan) equipped with a Quadrex capillary column (DB-5MS) (5% diphenyl dimethylsiloxane) (30 m × 0.25 mm, 0.25 µm film thickness, Agilent Technologies). Prior to analysis, 20 µL of GEO was diluted in HPLC-grade dichloromethane (1:25). The sample was injected with a split ratio of 10 and a volume of 1 µL. The injector temperature was maintained at 240 °C, and the carrier gas was helium (99.999%) at a flow rate of 1 mL min^{-1}. The column oven temperature program was set at 60 °C and increased up to 246 °C at a rate of 3 °C min^{-1}, with an additional ten-minute constant hold, for a total run time of 72 min. The equipment was operated in electron impact ionization mode at 70 eV and scanned at a range of 35 to 500 m/z. The chemical composition of the essential oils was determined by comparing the mass spectra of oil components with the National Institute of Standards and Technology (NIST 14) library and Kovats index. The relative composition was calculated by dividing the peak area of each compound by the sum of all peak areas based on the Total Ion Chromatogram provided by Shimadzu GCMS Postrun Analysis Software. The major compound identified in GEO was di-2-propenyl trisulfide (30.72%) (Table 1).

For in vitro antimicrobial characterization of GEO, inoculum of strains J96 (*Escherichia coli*; ATCC, Manassas, VA, USA) and SA23923 (*Staphylococcus aureus*; ATCC, Manassas, VA) were cultivated in brain heart infusion (BHI) medium at 37 °C for 18 h. After this period, the optical density of the bacterial strains was adjusted to 0.3. This adjustment was made to homogenize the number of bacterial cells per milliliter in the suspension. GEO was diluted in BHI with 5% dimethyl sulfoxide (DMSO) at concentrations of 500, 400, 300, 200 and 100 mg/mL. A 96-well plate was used to perform the assay (duplicate). The oil diluted at a concentration of 500 mg/mL in BHI with 5% DMSO and without the addition of bacterial strains was used for the negative control wells. For the positive control wells, 50 µL of each bacterium was inoculated in BHI medium with 5% DMSO at an optical density of 0.3 and without essential oil addition. To test GEO, 50 µL of each bacterium was inoculated into 150 µL of oil in the wells at each of the concentrations used. After incubation of the plate at 37 °C for 24 h, the reading of the optical density at a wavelength of 600 nm was performed in a spectrophotometer (SpectraMax M3, Molecular Devices, San Jose, CA, USA). GEO inhibited both bacteria with a minimum inhibitory concentration (MIC) of 100 mg/mL. This concentration was selected to compose the coating.

Table 1. Chemical composition of garlic essential oil (GEO) by gas chromatography-mass spectrometry (GC-MS).

Retention Time	Concentration (Peak Area %)	Kovats Index	Kovats Index Literature	NIST 14 Library Similarity (%)	Compound
3.167	4.87	-	835	97	Diallyl sulfide
3.550	0.10	-	889	94	(*E*)-Allyl(prop-1-en-1-yl)sulfane
3.600	0.12	-	889	94	(*E*)-Allyl(prop-1-en-1-yl)sulfane
3.835	0.03	910	905	90	3,4-dimethyl-thiophene
4.027	3.13	922	923	93	methyl-2-propenyl-disulfide
4.283	0.22	936	925	95	(*Z*)-Methyl propenyl disulfide
4.443	0.34	945	964	97	(*E*)-Methyl propenyl disulfide
4.841	0.67	964	997	97	3H-1,2-Dithiole
5.094	0.87	975	982	93	Dimethyl trisulfide
6.424	0.07	1028	1026	93	*p*-cymene
6.532	0.08	1032	1036	92	D-Limonene
8.260	18.95	1087	1085	91	Diallyl disulphide
8.588	1.68	1095	1099	94	(*Z*)-1-Allyl-2-(prop-1-en-1-yl)disulfane
8.803	1.99	1101	1099	94	(*E*)-1-Allyl-2-(prop-1-en-1-yl)disulfane
10.245	11.84	1145	1116	95	allyl methyl trisulfide
10.535	0.04	1152	1130	93	Methyl-propyl-trisulfide
10.599	0.49	1154	1142	92	4-Methyl-1,2,3-trithiolane
11.905	0.32	1186	1156	96	3-Vinyl-1,2-dithiacyclohex-4-ene
12.941	1.06	1212	1148	97	2-Vinyl-4*H*-1,3-dithiine
13.033	0.21	1215	1232	89	Dimethyl-tetrasulfide
16.922	30.72	1307	1292	95	di-2-propenyl-trisulfide
17.122	0.45	1313	-	97	1-allyl-3-propyl-trisulfide
17.471	0.16	1322	1306	95	(*E*)-1-Allyl-3-(prop-1-en-1-yl)trisulfane
17.691	0.19	1327	-	96	(*E*)-1-Allyl-3-(prop-1-en-1-yl)trisulfane
18.937	0.48	1358	1364	95	5-Methyl-1,2,3,4-tetrathiane
22.420	0.24	1441	-	88	Methyl 1-(methylthio)propyl disulfide
26.511	9.65	1542	1531	91	diallyl Tetrasulfide
26.718	0.35	1547	1573	86	6-Ethyl-4,5,7-trithia-2,8-decadiene
27.985	0.12	1579	1581	-	7-Methyl-4,5,8-trithia-1,10-undecadiene
28.361	0.28	1588	1591	94	6-Methyl-4,5,8-trithia-1,10-undecadiene

Table 1. *Cont.*

Retention Time	Concentration (Peak Area %)	Kovats Index	Kovats Index Literature	NIST 14 Library Similarity (%)	Compound
36.463	1.38	1808	-	96	1-Allyl-3-(2-(allylthio)propyl)trisulfane
44.374	0.23	2049	-	94	1-Allyl-3-(2-(allyldisulfanyl)propyl)trisulfane
61.610	2.80	-	-	91	3-(Octanoyloxy)propane-1,2-diyl bis(decanoate)
-	7.34	-	-	-	Unidentified

The antimicrobial coating based on WPI (Bio Mundo, Brasília, Brazil) and GEO was prepared by adapting the method by Caner [14]. The coating solution was formulated by adding 8% (*w*/*w*) WPI, 20% (*w*/*w*) glycerol (plasticizer; Dinâmica, São Paulo, Brazil) and 1% (*w*/*w*) GEO diluted in Tween 80 to a concentration of 100 mg/mL in 400 mL of distilled water. Each substance was added to distilled water at different times. The distilled water was stirred in a magnetic stirrer at 80 °C, first adding the WPI to the water and, after 5 and 30 min, the glycerol and GEO were added, respectively, and stirring ended 10 min later. For the addition of essential oil, the solution temperature was reduced to 40 °C. To finish manufacturing the coating, the protein was dissolved, adjusting the pH of the solution to 10.0 with NaOH 1N (Merck, Darmstadt, Germany).

Of a total of one hundred and seventy-one unwashed eggs from age-matched quails (24 weeks), 57 eggs were uncoated eggs (UE), 57 eggs were immersed in WPI coating and 57 eggs were immersed in WPI/GEO. The eggs were immersed for 45 s in the coating and dried at room temperature. All eggs were stored in sterilized plastic trays at room temperature (average of 26.95 ± 1.28 °C) and humidity (average of 65.11 ± 3.98%) was monitored every 5 min using a HOBO data logger (Onset Computer Corp., Bourne, MA, USA) for 28 days. The eggs were evaluated every 7 days, always at the same time, to find how the deterioration of the eggs behaved in each treatment, until the last day of storage.

The weight of the eggs was measured using a precision scale (Gehaka, São Paulo, São Paulo, Brazil) and expressed in egg weight loss (EWL), considering the initial (IEW) and final (FEW) weight of the egg according to the following calculation: EWL (%) = (IEW−FEW)/IEW × 100. After weighing (W), the eggs were broken and the height (H) of the albumen measured using a digital caliper (Mitutoyo, Suzano, São Paulo, Brazil). The results were presented as Haugh unit (HU) according to the following calculation: $HU = 100 \log (H + 7.57 - 1.7\ W^{0.37})$ [15]. After separating the albumen, the diameter (d) and height (h) of the yolk were measured using a digital caliper (Mitutoyo, Suzano, São Paulo, Brazil). The results were presented as yolk index (YI) according to the following calculation: YI = h/d [16]. With the albumen and yolk separated, the pH of both was individually measured using a calibrated digital pH meter (Kasvi, Campina São José do Pinhais, Paraná, Brazil).

The sensory attributes of eggs after 28 days of storage were evaluated by cooking the eggs in an aluminum pot (Caçarola Paris—28524620, Tramontina, Brasília, Brazil) containing 1.5 L of water at 100 °C for 15 min and based on a hedonic scale from 1 to 9, adjusting the protocol in the method used by Nwamo et al. [17], where 1 = dislike extremely and 9 = like extremely. After signing the Free and Informed Consent Form, ten volunteer panelists from the biology, agricultural and agroindustry courses were invited to evaluate color, aroma, odor, texture, taste and general acceptability. The results obtained for each panelist were recorded on a form. These panelists had already participated in the food quality discipline at the Federal Institute of Brasília, Campus Planaltina, Brasília, Brazil. The evaluation process was divided into three sessions and, in each of them, ten cooked eggs were coded with three letters and a number and served on plastic trays that were also coded. In addition, disposable, biodegradable, transparent cups with 100 mL of water were served for the panelists to clean their palate between one egg and another.

The protocols used by Wells et al. [18] and Figueiredo et al. [19] were adjusted and used for bacterial and fungal counting of eggs at 28 days of storage. For shell analysis, the eggs (3 eggs per bag) were placed in sterile bags with a volume of 60 mL of 0.1% peptone saline solution. For the analysis of the egg content, the eggs were broken and the content (3 eggs per bag—6 mL of each egg) was homogenized in a beaker with a volume of 162 mL of 0.1% peptone saline solution. Serial dilutions were performed for uncoated eggshell rinse solution only. A total of 1 mL of each final shell rinse solution and final egg content solution were plated as follows: (1) Plate count agar to count total aerobic mesophilic bacteria; incubation of the plates at 36 °C for 48 h; (2) Violet red bile glucose agar for counting Enterobacteriaceae; incubation of the plates at 36 °C for 48 h; (3) Potato dextrose agar to count molds and yeasts; incubation of the plates at 29 °C for 5 days. Colonies were counted and presented as $\log_{10}$ CFU/mL. Analyzes were performed in triplicate.

The experiment was conducted in a completely randomized design. Initially, data normality was tested. PROC GLM from SAS Studio University Edition software (SAS Inst. Inc., Cary, NC, USA) was used for analysis of variance of the obtained data. Tukey's test was used to compare means ($p < 0.05$). The Kruskal–Wallis test was used to analyze non-normal data.

Parameters were analyzed according to the following statistical model:

$$Yijk = \mu + di + wk + dwjk + eijk$$

where μ: mean overall; di: effects of treatments; wk: effect of storage periods; dwjk: effect of the interaction between treatment and storage periods; eijk: random error.

3. Results and Discussion

The surface preservative and sanitizing properties of essential oils alone or as active ingredients in biopolymer coatings are gradually being explored [5,20–25]. Studies may expand researchers' understanding of how these essential oils can be effectively added to egg coatings, as well as their potential benefits and harms in the preservation and microbiological safety of eggs. In this study, the effectiveness of the WPI/GEO coating was evaluated for quail eggs.

Eggs coated with WPI/GEO (5.24 ± 1.28%) had less weight loss ($p < 0.05$) than UE (8.93 ± 3.79%) in the final stage of storage, while eggs coated with WPI had no effect ($p > 0.05$) weight loss (Figure 1). The water-resistant portion (hydrophobic) of the WPI/GEO coating probably did not facilitate the movement of water from the internal microenvironment of the egg to the external macroenvironment, making egg weight loss less pronounced than UE. GEO is a solution to reduce the WPI water barrier deficit, as eggs coated only with WPI showed similar weight loss to UE. The synergistic action between biopolymers and essential oils in delaying the weight loss of eggs coated with starch-essential oil, protein-essential oil, chitosan-essential oil and flour-essential oil is being increasingly documented [22,26–28].

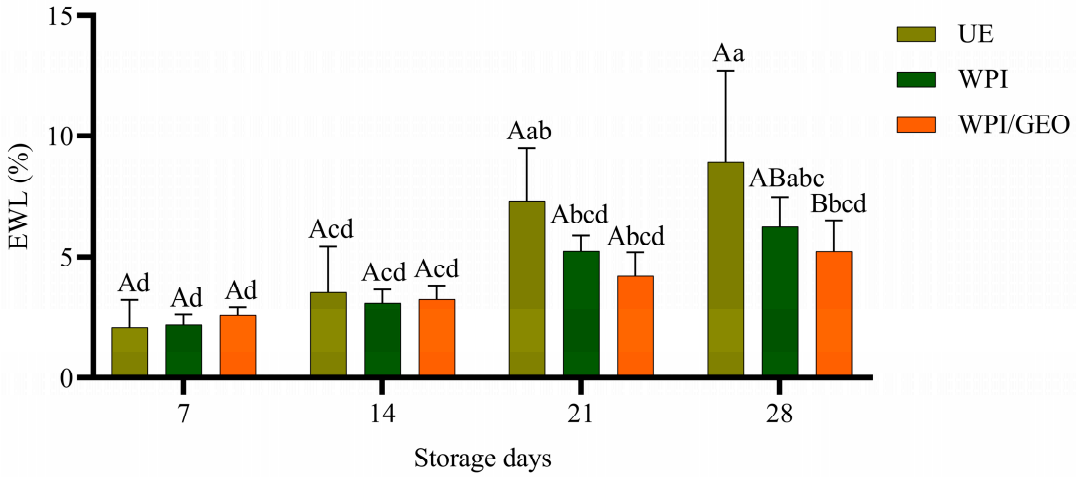


Figure 1. Comparison of egg weight loss (EWL, %) between coated eggs and UE on the 28th day of storage at room temperature. ^{A,B} Means with different capital letters (treatment effect within each day of storage) are different ($p < 0.05$). $^{a–d}$ Means with different lowercase letters (effect of day of storage within each treatment) are different ($p < 0.05$). UE, uncoated eggs; WPI, whey protein isolate; WPI/GEO, whey protein isolate plus garlic essential oil.

Weekly HU was similar ($p > 0.05$) among treatments. However, the HU covering the entire period (average of 28 days of storage) was higher ($p < 0.05$) for WPI/GEO- (88.26 ± 4.83, grade AA) coated eggs than WPI-coated (86.55 ± 6.20, grade AA) and UE (85.94 ± 6.46, grade AA) (Figure 2). Published reports have shown that the higher the HU, the greater the amount of ovomucin in the albumen [29]. Thus, the coatings may

have preserved more of the ovomucin, as this is a protein that plays an important role in maintaining the viscosity and height of the same [29]. Protein coatings with essential oils can be efficient tools to keep the HU within the ideal range for grading the quality of eggs (AA) stored at room temperature [22,30].

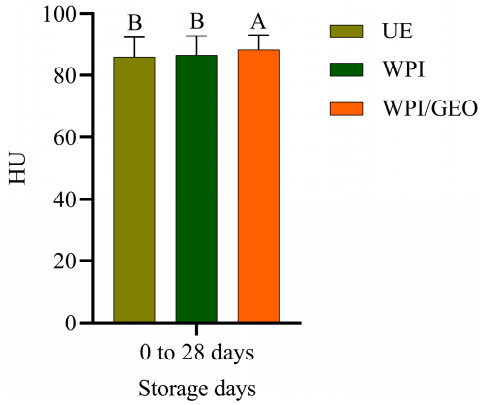


Figure 2. Comparison of the Haugh unit (HU) between coated eggs and UE on the 28th day of storage at room temperature. ^{A,B} Means with different capital letters are different ($p < 0.05$). UE, uncoated eggs; WPI, whey protein isolate; WPI/GEO, whey protein isolate plus garlic essential oil. Egg quality score based on HU: AA, excellent (≥72); A, high quality (71–60); B, average quality (59–31); and C, low quality (≤30) [31].

YI gradually reduced and reached similar values ($p < 0.05$) at the end of storage for eggs coated with WPI and UE, which were significantly lower than eggs coated with WPI/GEO (Figure 3). The preservation of the yolk promoted by the WPI/GEO coating may have occurred due to minimal adverse reactions (reduction of the hydrolysis process of fats and proteins) that compromise the viscosity of the yolk and maximum positive reactions (better preservation of the albumen structure) that minimize the circulation of water from the albumen to the yolk [32]. These reactions may be due to the barrier action of the WPI/GEO coating against water and gases. Other essential oils, such as basil, have shown satisfactory behavior when compounding biopolymer coatings to preserve egg yolk quality (measured by YI) [25].

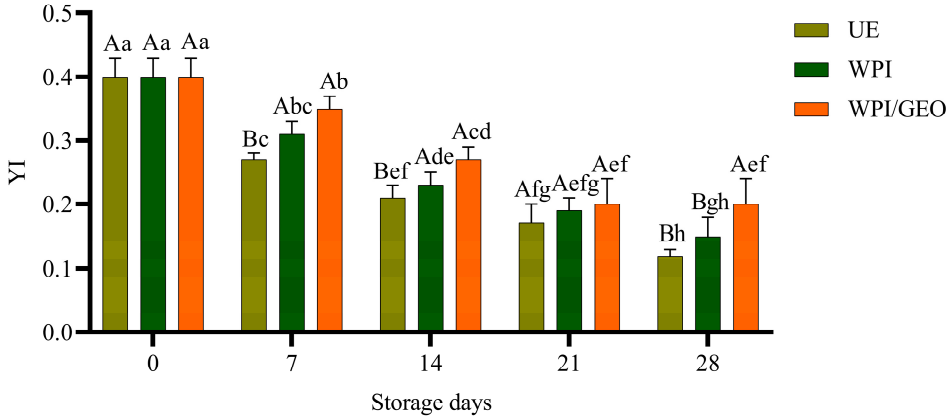


Figure 3. Comparison of yolk index (YI) between coated eggs and UE on the 28th day of storage at room temperature. ^{A,B} Means with different capital letters (treatment effect within each day of storage) are different ($p < 0.05$). $^{a–h}$ Means with different lowercase letters (effect of day of storage within each treatment) are different ($p < 0.05$). UE, uncoated eggs; WPI, whey protein isolate; WPI/GEO, whey protein isolate plus garlic essential oil.

The eggs had an initial albumen pH of 9.41 ± 0.36, reaching similar values ($p > 0.05$) of 9.97 ± 0.33 and 9.59 ± 0.10 at 28 days of storage for UE and WPI-coated eggs, respectively

(Figure 4). On the other hand, eggs coated with WPI/GEO showed lower ($p < 0.05$) albumen pH (9.34 ± 0.24) than UE at 28 days of storage (Figure 4). Albumen pH increases due to dissociation of carbonic acid and the escape of CO_2 out of the egg [33]. It is assumed that this chemical phenomenon was delayed in eggs coated with WPI/GEO because they had a less alkaline albumen than UE on the last day of storage. Similarly, eggs coated with an emulsion of sweet potato starch and thyme essential oil had significantly less alkaline albumen than UE by the fifth week of storage at 25 °C [21].

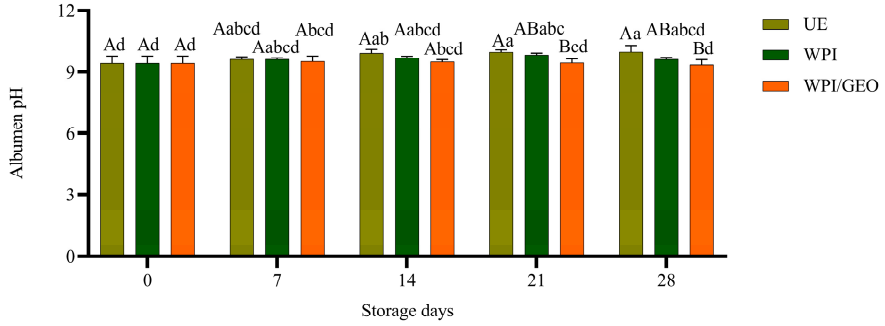


Figure 4. Comparison of albumen pH between coated eggs and UE on the 28th day of storage at room temperature. ^{A,B} Means with different capital letters (treatment effect within each day of storage) are different ($p < 0.05$). $^{a–d}$ Means with different lowercase letters (effect of day of storage within each treatment) are different ($p < 0.05$). UE, uncoated eggs; WPI, whey protein isolate; WPI/GEO, whey protein isolate plus garlic essential oil.

Eggs had an initial yolk pH of 6.89 ± 0.44, and this increased ($p < 0.05$) to 7.76 ± 0.15 for UE on the last day of storage (Figure 5). This increase in yolk pH of UE was greater ($p < 0.05$) than the yolk pH of eggs coated with WPI (7.14 ± 0.24) and WPI/GEO (7.09 ± 0.21) on the same day (Figure 5). Yolk pH depends on the level of deterioration of the albumen [26]. The more deteriorated, the more alkaline the albumen will be [34], the more water resulting from its deterioration will pass into the yolk and the higher the pH of the yolk will be [35]. This explains why WPI- and WPI/GEO-coated eggs had a lower yolk pH than UE. Other results on the use of protein coating plus essential oils are in line with our findings [23].

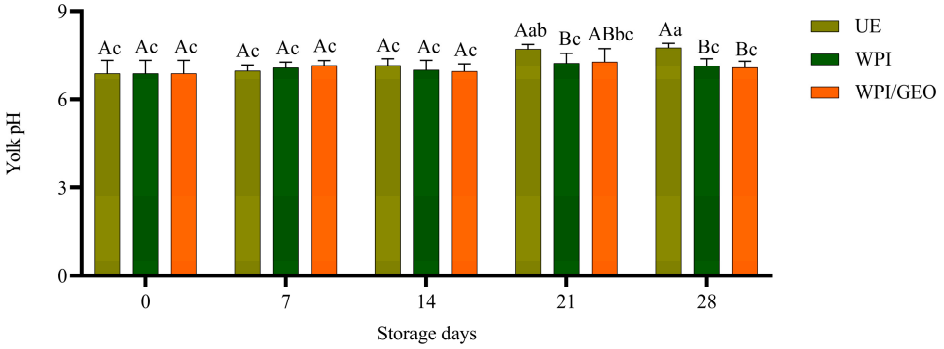


Figure 5. Comparison of yolk pH between coated eggs and UE on the 28th day of storage at room temperature. ^{A,B} Means with different capital letters (treatment effect within each day of storage) are different ($p < 0.05$). $^{a–c}$ Means with different lowercase letters (effect of day of storage within each treatment) are different ($p < 0.05$). UE, uncoated eggs; WPI, whey protein isolate; WPI/GEO, whey protein isolate plus garlic essential oil.

On the 28th day of storage, sensory parameter scores did not differ ($p > 0.05$) between UE (overall egg score = 7.55 ± 1.79) and eggs coated with WPI (overall egg score = 7.97 ± 1.47) and WPI/GEO (overall egg score = 8.23 ± 1.04) (Figure 6). Sensory factors, e.g., color, aroma, odor, texture, flavor and, of course, general acceptability, must be strongly considered in the development and application of coatings supplemented with essential oil for quail eggs due to the strong and characteristic odors of most essential oils, which are not always accepted by

the consumer [36]. Thus, sensory analysis is an important step to validate the use of edible coatings coupled with essential oils. The WPI and WPI/GEO coatings did not cause any undesirable perceptions in the sensory parameters of the eggs that would lead the judges to reduce the egg acceptability scores. It is suggested that, in the case of the WPI/GEO coating, the synergistic action of these two compounds and cooking were important in ensuring that no perceptible odor of oil remained in the egg. Likewise, no effect of the coating based on rice flour and rosemary essential oil was observed on the sensory characteristics of quail eggs at 21 days of storage [26]. It seems that coatings that have essential oils in their formulation can be used without major concerns of making the egg unacceptable to the consumer.

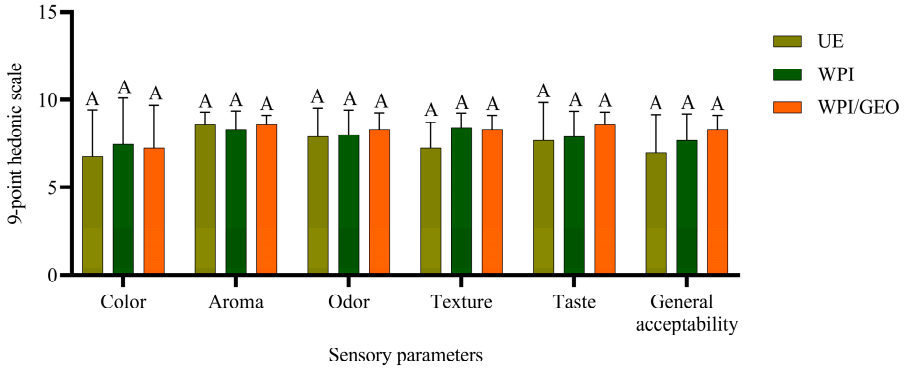


Figure 6. Comparison of sensory parameter scores between coated eggs and UE on the 28th day of storage at room temperature. [A] Means with the same capital letters are statistically similar ($p > 0.05$). UE, uncoated eggs; WPI, whey protein isolate; WPI/GEO, whey protein isolate plus garlic essential oil.

Eggs coated with WPI/GEO showed a lower total count of aerobic mesophilic bacteria (1.84 ± 0.16 $\log_{10}$ CFU/mL^{-1}), Enterobacteriaceae (1.38 ± 0.22 $\log_{10}$ CFU/mL^{-1}) and molds and yeasts (1.49 ± 0.13 $\log_{10}$ CFU/mL^{-1}) on the eggshells ($p < 0.05$) when compared to WPI-coated eggs (2.37 ± 0.10; 2.04 ± 0.32; 2.23 ± 0.29 CFU/mL^{-1}) and UE (3.35 ± 0.13; 3.40 ± 0.06; 2.83 ± 0.34 CFU/mL^{-1}), respectively (Figure 7A). Eggs coated with WPI/GEO had 1.51 log less aerobic mesophilic bacteria, 2.02 log less Enterobacteriaceae and 1.34 log less molds and yeasts on the eggshell when compared to UE. The WPI coating had an antimicrobial action that was potentiated by GEO, confirming the findings of Tokur et al. [9]. GEO has antimicrobial potential, as confirmed in this study. Likewise, Benkeblia [37] reported the antimicrobial potential of GEO against *Staphylococcus aureus* (ATCC 11522), *Salmonella Enteritidis* (ATCC 13076), *Aspergillus niger* (ATCC 10575), *Penicillum cyclopium* (ATCC 26165) and *Fusarium oxysporum* (ATCC 11850). The antimicrobial effect of essential oils may be due to structural and uncontrolled damage to the cell membrane of microorganisms [38]. The detection of bacteria of the Enterobacteriaceae family in the eggshells gives suggests the possibility of poor sanitary conditions in the quail shed.

Eggs coated with WPI/GEO showed a lower total count of aerobic mesophilic bacteria (1.59 ± 0.13 $\log_{10}$ CFU/mL^{-1}), Enterobacteriaceae (1.64 ± 0.24 $\log_{10}$ CFU/mL^{-1}) and molds and yeasts (1.55 ± 0.15 $\log_{10}$ CFU/mL^{-1}) on the contents ($p < 0.05$) when compared to UE (2.77 ± 0.36; 2.91 ± 0.24; 2.89 ± 0.36 CFU/mL^{-1}), respectively (Figure 7B). The difference between WPI (2.21 ± 0.14 CFU/mL^{-1}) and WPI/GEO (1.55 ± 0.15 CFU/mL^{-1}) was significant only for molds and yeasts, where the count was lower for WPI/GEO (Figure 7B). Eggs coated with WPI/GEO had 1.18 $\log_{10}$ CFU/mL^{-1} less aerobic mesophilic bacteria, 1.27 $\log_{10}$ CFU/mL^{-1} less Enterobacteriaceae and 1.34 $\log_{10}$ CFU/mL^{-1} less molds and yeasts on the egg content compared to UE. The presence of an antimicrobial coating on eggshells may partially explain the lower load of microorganisms in the content of the coated eggs. It is also suggested that the coatings may have created an anaerobic microenvironment in the eggshells, affecting aerobic microbial growth. The present results support the previous findings reported by Upadhyaya et al. [39], Araújo et al. [40] and Oliveira et al. [41]. For example, Upadhyaya et al. [39] reported the antimicrobial efficiency

of coatings formulated from pectin or gum arabic plus carvacrol or eugenol in reducing *Salmonella enteritidis* on eggshells.

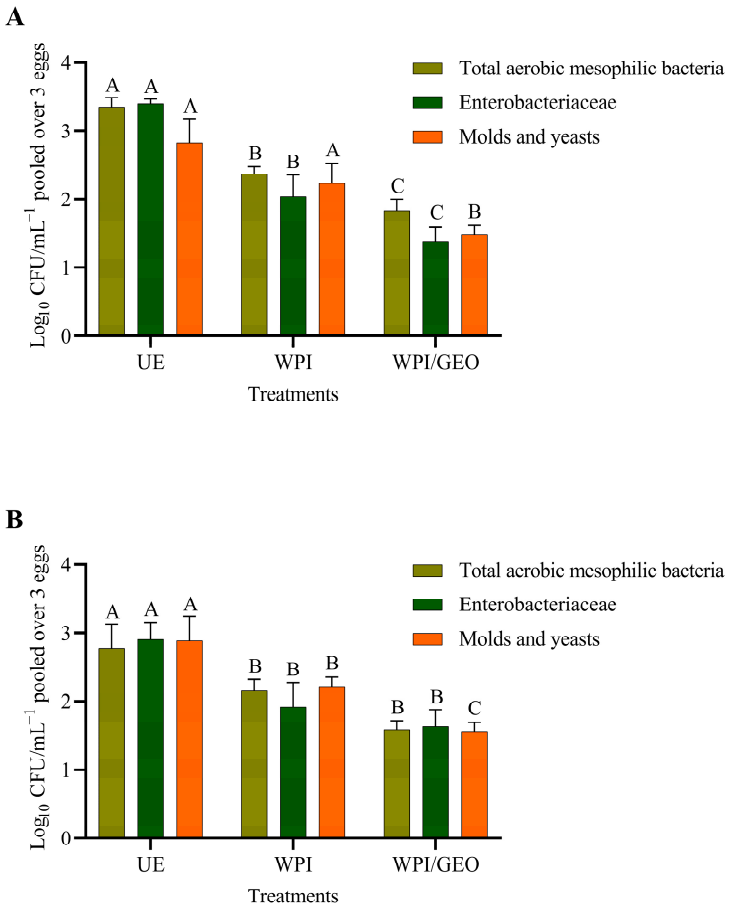


Figure 7. Counts of total aerobic mesophilic bacteria, Enterobacteriaceae and molds and yeast on eggshell surfaces (**A**); and (**B**) egg contents of coated and uncoated eggs at 28 days of storage at room temperature. $^{A–C}$ Means with different capital letters for the same group of enumerated microorganisms are different ($p < 0.05$). UE, uncoated eggs; WPI, whey protein isolate; WPI/GEO, whey protein isolate plus garlic essential oil.

4. Conclusions

The coating developed with WPI/GEO efficiently preserved the internal quality of quail eggs for 28 days at room temperature. In addition, it promoted the lower microbial loads of the shell and egg contents without changing the sensory properties of the eggs. Therefore, coatings composed of hydrophilic, hydrophobic and antimicrobial substances such as WPI/GEO are recommended for quail eggs. GEO shows remarkable potential for composing successful egg coating solutions.

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