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EDITED BY
Miguel Rebollo-Hernanz,
University of Illinois at Urbana-Champaign, United States

REVIEWED BY
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Izmir Institute of Technology, Türkiye
Mojtaba Yousefi,
Semnan University of Medical Sciences, Iran

*CORRESPONDENCE
Disney Ribeiro Dias,
diasdr@ufla.br

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Characterization of antimicrobial and antioxidant performance of dihydroeugenol and its glycoside incorporated into whey protein isolate films

Déborah Braga Resende[1], Luara Aparecida Simões[2], Natalia de Andrade Teixeira Fernandes[3], Lucas Baldo Luvizaro[4], Marali Vilela Dias[4], Diogo Teixeira Carvalho[5], Roberta Hilsdorf Piccoli[4], Rosane Freitas Schwan[1] and Disney Ribeiro Dias[4]*

[1]Department of Biology, Federal University of Lavras, Lavras, Brazil, [2]Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal, [3]Chemistry Department, University of California, Davis, Davis, CA, United States, [4]Department of Food Science, Federal University of Lavras, Lavras, Minas Gerais, Brazil, [5]Laboratory of Pharmaceutical Chemistry, Federal University of Alfenas, Alfenas, Brazil

Bio-based plastics are gaining attention as environmentally friendly alternatives to traditional petroleum-based plastics in food packaging. Biodegradable films made from biocompatible macromolecules like polysaccharides and proteins offer promising solutions. This study investigated the effects of incorporating dihydroeugenol and glycoside into whey protein isolate (WPI) films to enhance their antimicrobial and antioxidant properties for food packaging. The findings showed that the glycoside film surpassed the dihydroeugenol film in inhibiting bacterial growth, forming larger inhibition halos against *Escherichia coli*, *Staphylococcus aureus*, *Listeria* monocytogenes, and *Salmonella* Enteritidis (inhibition halos ≥15 mm). The glycoside film exhibited superior effectiveness against Gram-positive bacteria, indicating enhanced penetration through their cell membranes. Additionally, the synthesized compounds significantly boosted the antioxidant activity of the films compared to the control film without dihydroeugenol and glycoside. With a relatively low solubility (23% ± 2.87% and 24% ± 3.92% for dihydroeugenol and glycoside, respectively), these films are suitable for packaging and safeguarding food products with medium to high humidity. This study concluded that incorporating dihydroeugenol and its glycoside into WPI films holds great promise for developing active packaging solutions in the food industry. These films offer both antibacterial and antioxidant properties, particularly advantageous for food products with medium to high humidity.

KEYWORDS

whey protein isolate, active package, eugenol, antioxidant activity, food-contaminating bacteria

1 Introduction

Due to increasing environmental awareness, the development of bio-based plastics is gaining more and more attention, as for many years, traditional commercial food packaging materials were generally petroleum-based plastics such as polyethylene (PE), polypropylene (PP) and polystyrene (PS) (Kunwar et al., 2016). For this reason, some degradable films composed of biocompatible macromolecules with good film-forming properties, such as polysaccharides and proteins, seem to be promising alternatives for plastic packaging (Zhang et al., 2020; Zhang and Jiang, 2020).

To ensure the effectiveness of biodegradable films, several criteria must be met. These include satisfactory mechanical properties, effective barriers against water vapor, oxygen, and ultraviolet light, biodegradability, thermal stability, resistance to food-borne bacteria, desirable sensory attributes, cost-effective production, and compatibility with the packaged product (Guimarães et al., 2018; Yadav et al., 2020). Proteins have been successfully used to form films and coatings. Well-studied proteins, such as whey protein, casein, wheat gluten, soy protein or zein, have been used to develop films obtained from renewable resources with faster degradability than other polymeric materials (Ramos Ó. L. et al., 2012; Cinelli et al., 2014; Mohammadi et al., 2020).

Whey proteins include different globular proteins such as β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulins, and proteose-peptone polypeptides (Lent et al., 1998). β-lactoglobulin is the main whey protein and dominates gelling and aggregation behavior in whey protein formulations (Hammann and Schmid, 2014). The widespread availability and versatility of whey proteins make them highly suitable for the production of films and coatings. Whey proteins possess the unique ability to undergo conformational changes and interact with one another, forming modified three-dimensional networks. These properties make whey proteins an excellent choice for developing films and coatings with enhanced functionalities (Schmid and Müller, 2019; Alizadeh-Sani et al., 2020).

Essential oils, products of plants' secondary metabolism, have been considered safe and effective alternatives for microbial control and can be added to films (Lang and Buchbauer, 2012; Wen et al., 2016). Active packaging refers to the interaction between the packaging material and the food it contains, where the packaging itself serves a functional role. One such function is antimicrobial activity, which can be achieved through the incorporation of essential oils into films. This active packaging approach offers various benefits for stored food, such as extending shelf life and reducing microbial growth. By leveraging the antimicrobial properties of essential oils, these films can contribute to preserving the quality and safety of packaged food products (Raut and Karuppayil, 2014; Catarino et al., 2017; Noori et al., 2018). Furthermore, glycosylation has been widely used as a structural modification method that optimizes the physicochemical and biological properties of the starting compound (Hiroyuki et al., 2002; Kurosu et al., 2002) and has been applied in other eugenol derivatives with the evaluation of antifungal activity where the results showed a significant increase in the activity after glycosylation (De Souza et al., 2014).

Based on this, dihydroeugenol and deacetylated dihydroeugenol glycoside compounds, previously synthesized in a previous work by our group (Resende et al., 2017), were used as antimicrobial additives in the production of whey protein isolate (WPI) films with cellulose nanofiber. Subsequently, the synthesized films were evaluated for its ability to inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes,* and *Salmonella* Enteritidis and its physical and optical properties were evaluated to verify if adding the compounds interferes with the film's characteristics.

2 Materials and methods

2.1 Chemicals

The dihydroeugenol (PubChem CID: 17739) was provided by the Laboratory of Pharmaceutical Chemistry of the Federal University of Alfenas, and the synthesis and characterization of glycoside of dihydroeugenol were synthesized at the same laboratory, according to Resende et al. (2017). Whey protein isolate (WPI 9400) with 90% protein was purchased from Hilmar Ingredients (Hilmar CA, United States). The glycerol and sodium chloride were obtained from Merck (Darmstadt, Germany), bacteriological peptone and yeast extract were bought from Kasvi (Sao Paulo, Brazil). Brain Heart Infusion (BHI) broth/agar, TSA (Tryptic Soy Agar), 2,2-diphenyl-1-picrylhydrazyl (DPPH), cellulose nanofiber, and ethanol were purchased by Sigma Aldrich, (Darmstadt, Germany).

2.2 Microorganisms, standardization, and maintenance of the inoculum

The bacterial strains used were *E. coli* ATCC 25922; *L. monocytogenes* ATCC 19117; *S. aureus* ATCC 8702 and *Salmonella* Enteritidis S64. The stock cultures were stored in freezing medium (15 mL of glycerol; 0.5 g of bacteriological peptone; 0.3 g of yeast extract; 0.5 g of sodium chloride; 100 mL of distilled water, pH 7.0). The cultures were reactivated by inoculating 100 µL aliquots into tubes containing 10 mL of Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 h. The cultures were standardized at 10^8 CFU mL^{-1} through a growth curve (OD 600 nm) with counting on BHI agar plates, incubated at 37°C, for 24 h.

2.3 Development of active packaging films incorporated with dihydroeugenol and its glycoside

The WPI films were prepared according to Zinoviadou et al. (2009) and Azevedo et al. (2015), with adaptations. The control film, labeled as "Control," was produced by dissolving WPI and glycerol in distilled water. In a separate step, cellulose nanofiber was dispersed in the glycerol solution. All solutions were individually subjected to magnetic stirring for 30 min. After stirring, the solutions were poured into each other, and magnetic stirring was continued for 10 min at room temperature. At the end of this stirring, a film-forming solution of 12% (m/v) WPI, 30% (m/m) glycerol and 1% (m/m) cellulose nanofiber in relation to WPI was obtained. Then the pH was adjusted to 8 with 2N NaOH and the final solution was subjected to ultrasonic homogenizer (Sonifier Cell

TABLE 1 Inhibition Halos (mm) formed by the Antibacterial Activity of WPI Films with cellulose nanofiber incorporated with dihydroeugenol or glycoside as antimicrobial agents.

Treatment	*E. coli*	*S. aureus*	*L.monocytogenes*	*S.* Enteritidis
Control	0	0	0	0
WPID	7.50 ± 2.00^{b}	15.00 ± 1.00^{b}	9.00 ± 1.50^{b}	7.50 ± 1.00^{b}
WPIG	15.00 ± 1.00^{a}	22.00 ± 2.00^{a}	23.00 ± 1.00^{a}	18.00 ± 2.50^{a}

Control: WPI, film without the addition of dihydroeugenol and its glycoside. WPID: WPI, film with the addition of dihydroeugenol (6% v/v), WPIG: WPI, film with the addition of dihydroeugenol glycoside (6% v/v). Mean values of different letters, in the same column differ significantly ($p < 0.05$) by the Tukey test.

Disruptor Branson–Model 450D, Manchester, United Kingdom) for 10 min, at 80 W and 25°C. The solutions were then heated at 90°C for 30 min in a water bath for protein denaturation and then cooled to room temperature and poured onto plastic plates.

For active treatments, dihydroeugenol and its glycoside were added in a final concentration of 6% (v/v) relative to the WPI after the water bath to maintain the constituent's preventing evaporation or thermal degradation. The solutions were then subjected to ultrasonic homogenizer again. The thickness control was performed by the volume applied to the support, corresponding to 16.36 mL in a 78.53 cm^3 area. The films were dried at room temperature for 24 h ensuring the solvent's slow evaporation and film formation.

2.4 Determination of the antimicrobial activity of packages

The evaluation of antibacterial potential was performed by the disc diffusion method, according to Bauer et al. (1966), with some modifications. After broth growth, aliquots of 200 µL of the standardized culture of *E. coli*; *L. monocytogenes*; *S. aureus* and *Salmonella* Enteritidis were plated on TSA (Tryptic Soy Agar) surface. On the already inoculated TSA, active film discs with a diameter of 15 mm were arranged. The disks were previously subjected to UV light for 30 min on each side and then applied to each quadrant of the plate containing the respective bacteria. The plates were incubated at 37°C for 48 h and after the incubation the halo size was measured using a vernier calipers. As a negative control, films without the addition of dihydroeugenol and its glycoside, were evaluated. The results were expressed in millimeters by averaging the values obtained in three repetitions.

2.5 Antioxidant activity of active films

The method applied, 2,2-diphenyl-1-picrylhydrazyl (DPPH), is based on the ability of the DPPH radical to be scavenged and discolored in the presence of an antioxidant compound, upon reduction, the purple color of the radical changes to yellow at the same time that the absorbance decreases at 515 nm resulting in the reduction of absorbance values (Foti, 2015).

The WPI films (0.1 g) were cut into small pieces and mixed with 2 mL of ethanol 80% for solubilization. The mixture was vigorously vortexed for 3 min and kept at room temperature for 3 h. Then it was stirred again. An aliquot of the extract was mixed with 3.9 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) 0.1 mM in ethanol 80%. The mixture was vigorously vortexed for 1 min and kept in the dark for 30 min. The absorbance was measured at 515 nm using a spectrophotometer (SP 2000UV). Ethanol 80% was used as a negative control and was mixed with DPPH 0.1 mM. The radical scavenging activity (SA) was calculated according to equation (Byun et al., 2010):

$$(\%)SA = (A\ control - A\ sample)/(A\ control)\quad x100 \qquad (1)$$

Wherein:$A_{control}$: negative control absorbance.A_{sample}: sample absorbance.

2.6 Water solubility

The water solubility of films was determined according to the method of Rhim et al. (2005). The samples (3 × 5 cm) were weighed (P1) and placed in beakers containing 30 mL of deionized water. The beakers were covered with parafilm and stored at 25.0°C ± 0.5°C for 24 h. After this period, pieces of undissolved films were recovered using filter paper. The filtrate was dried at 105°C for 24 h and then weighed (P2). The solubility in water was determined from the undissolved dry matter and calculated according to the following equation:

$$(\%)Water\ solubility = ((P1 - P2)x100)/P1) \qquad (2)$$

2.7 Optical properties

2.7.1 Color

For determining the color of WPI films, colorimeter Color Quest XE, Hunter Lab (Reston, VA, United States) and Comission Internationale de L'Éclairage (CIE) system (L*, a*, b*) with a light source D65, an observer angle of 10° and mode reflectance were used. The parameters (L*, a*, b*), hue angle (h°) and chroma saturation index (C*) were analyzed. L* indicates brightness ranging from 0 (black) to 100 (white); a* indicates the color intensity ranging from green (−) to red (+); b* indicates the color intensity ranging from blue (−) to yellow (+) (Sarker and Oba, 2019).

2.7.2 Transparency

To determine the transparency of WPI films, transmittance percentage (% T) at 600 nm was measured using a GBC UV/VIS 918 spectrophotometer (Shimadzu, Tokyo, Japan) according to ASTM (2015) method. The transparency (T600) was calculated according to the following equation:

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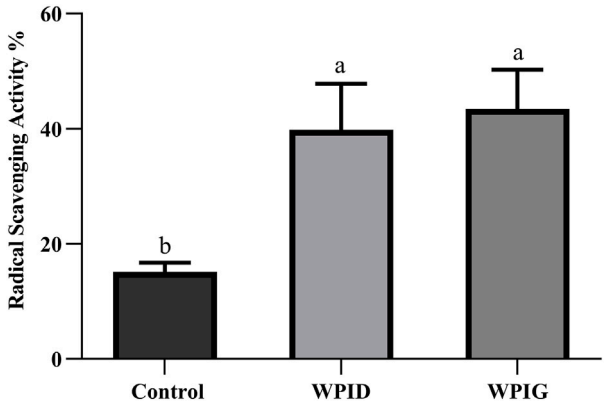

FIGURE 1
Antioxidant assay in the evaluation of DPPH radical intercept of whey protein isolate (WPI). Control: WPI film without the addition of dihydroeugenol and its glycoside. WPID: WPI film with the addition of dihydroeugenol (6% v/v), WPIG: WPI film with the addition of dihydroeugenol glycoside (6% v/v). Each bar represents the means ± SD, considering the results obtained in at three independent experiments. Bars followed by different letters differ significantly ($p < 0.05$) by the Tukey test.

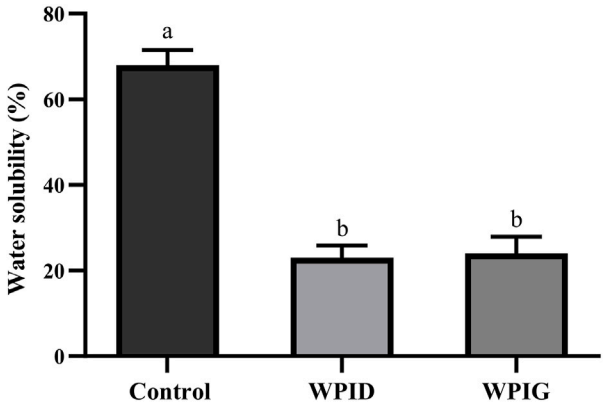

FIGURE 2
Water solubility of whey protein isolate (WPI). Control: WPI film without the addition of dihydroeugenol and its glycoside. WPID: WPI film with the addition of dihydroeugenol (6% v/v), WPIG: WPI film with the addition of dihydroeugenol glycoside (6% v/v). Each bar represents the means ± SD, considering the results obtained in at three independent experiments. Bars followed by different letters differ significantly ($p < 0.05$) by the Tukey test.

$$T600 = ((\text{Log}\,\%T))/\partial \quad (3)$$

Where ∂ is the film thickness (mm).

2.7.3 Statistical analysis

The results obtained for the WPI films incorporated with dihydroeugenol and glycoside of dihydroeugenol were submitted to analysis of variance (ANOVA), and the averages were compared through the Tukey test at a 5% significance level, using SISVAR 5.1 software (Ferreira, 2011). Results were expressed as mean values ± standard deviation ($n = 3$).

3 Results

3.1 Evaluation of antimicrobial activity of active films

The results of measuring the inhibition halos (mm) formed by the antibacterial activity of WPI films incorporated with dihydroeugenol or glycoside are shown in Table 1. The obtained results show that there was no inhibition of bacterial growth when control film was used without addition of synthesized compounds. The addition of compounds dihydroeugenol and its glycoside was effective against all bacteria tested (halos ranging from 7.5 to 23 mm). Significant difference ($p < 0.05$) in treatments were seen for all the evaluated microorganisms, and WPI film added with dihydroeugenol glycoside was able to form inhibition halos larger than WPI film added with dihydroeugenol.

3.2 Antioxidant activity of WPI active films

The results showed that there was not statistically significant ($p > 0.05$) difference between the antioxidant activity of the films made with the addition of dihydroeugenol (39.79% ± 8.05%) and to those made with glycoside (43.42% ± 6.82%) (Figure 1). The control sample showed that the antioxidant activity of the film prepared from WPI, without other additives, presents a value of 15.1% ± 1.63%. Based on this result, the compounds synthesized significantly ($p < 0.05$) increased the antioxidant activity.

3.3 Films solubility

The results of the films solubility are showed in Figure 2, relatively a low solubility ($p < 0.05$) was observed of the produced films, with averages of 23% ± 2.87% and 24% ± 3.92% for the film with dihydroeugenol and the film with glycoside, respectively, with no significant difference ($P > 0.05$) in solubility between them. The solubility of control film presented an average value of 68% ± 3.49%, indicating that the synthesized compounds added to the film decreased the solubility of films, due to their hydrophobic character.

3.4 Optical properties

3.4.1 Color

The attributes of color are important because they influence the acceptance of products by consumer, All parameters were compared according to Minolta (2007) and are presented in Table 2. The L * attribute (luminosity) had no significant ($p < 0.05$) variation between the two treatments with the addition of compounds. This parameter reflects how clear the sample is, the use of the dihydroeugenol (87.91 ± 0.59) and dihydroeugenol glycoside (89.43 ± 1.41) additives decreased the L* value in relation to the control film (97.20 ± 0.04), but visually are clear enough for food packaging.

TABLE 2 Color parameters of WPI films produced with addition of dihydroeugenol and glycoside.

Treatment	L*	a*	b*	c*	h°
Control	97.20 ± 0.04[a]	−0.97 ± 0.02[a]	9.49 ± 1.69[a]	9.54 ± 0.31[a]	99.17 ± 0.27[a]
WPID	87.91 ± 0.59[b]	−1.47 ± 0.04[b]	28.17 ± 0.98[b]	28.20 ± 0.97[b]	92.99 ± 0.14[a]
WPIG	89.43 ± 1.41[b]	−2.36 ± 0.24[c]	24.22 ± 4.90[b]	24.30 ± 4.96[b]	95.82 ± 1.88[a]

Control: WPI, film without the addition of dihydroeugenol and its glycoside. WPID: WPI, film with the addition of dihydroeugenol (6% v/v), WPIG: WPI, film with the addition of dihydroeugenol glycoside (6% v/v). Mean values of different letters, in the same column differ significantly ($p < 0.05$) by the Tukey test.

Values of a*, which may range from −90 to 70, were significantly higher for the glycoside containing film (−2.36 ± 0.24), showing its better coloring ability in relation to dihydroeugenol (−1.47 ± 0.04). However, the values of b*, may range from −80 to 100, did not vary significantly ($p > 0.05$) among the dihydroeugenol (28.17 ± 0.98) and its glycoside films (24.22 ± 4.90), but showed a tendency to yellow (highest value) when compared to the control film (9.49 ± 1.69).

The saturation index, chroma (C*), defines color intensity, and values close to zero are indicative of neutral colors (gray) and values around 60 indicate vivid and intense colors (Minolta, 2007), there is not vary significantly ($p > 0.05$) among the dihydroeugenol (28.20 ± 0.97) and its glycoside films (24.30 ± 4.96), both presented intermediate values at interval of 0–60, characterizing a medium intensity in the colors, however, the control film showed a lower ($p < 0.05$) value of c* (9.54 ± 0.31). The Hue angle (h°) represents the color tone, and, through it, the sample position can be estimated in color solid (Minolta, 2007). All films analyzed presented values close to 90°, with no significant difference ($p > 0.05$) between them, this value was presented in a shade of yellow color.

3.4.2 Transparency

The transparency of the WPI films studied in our work are shown in Figure 3. The control film had an average transparency of 11.63 ± 1.3 log (%T)/mm, while the film with the addition of the dihydroeugenol and glycoside compounds obtained an average transparency of 9.95 ± 0.76 and 8.92 ± 0.98 log (%T)/mm, respectively. It was observed that there was no difference ($P > 0.05$) in transparency for the treatments performed, and compared to WPI films without additives, the transparency was reduced, probably due to the characteristic staining of the synthesized compounds.

4 Discussion

To confirm any effect of the incorporation of dihydroeugenol and its glycoside components in whey protein films, the antimicrobial, antioxidant, and physicochemical properties were evaluated in our work. The efficacy of essential oils as antimicrobial agents when added to films has been reported (Gomes et al., 2017; Noori et al., 2018; Pavlátková et al., 2023).

The choice to work with dihydroeugenol and its synthesized glycoside was made according to the results previously found in the work of our research group Resende et al. (2017), in which dihydroeugenol showed to be more active than eugenol beyond the tested bacteria *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Staphylococcus aureus*. The results are also in agreement with those found in our work, where the glycoside presented a lower Minimal Bactericidal Concentrations when compared to dihydroeugenol, showing a higher antimicrobial potency of glycoside.

To the best of our knowledge, this is the first study of the incorporation of eugenol or dihydroeugenol in WPI films for comparative purposes. However, a study evaluated the antimicrobial activity of polyhydroxybutyrate films incorporated with eugenol. In contrast to *S. aureus*, the zone of inhibition formed was 14 ± 1 mm and was compared to *E. coli*. This value was 19 ± 1 mm and the diameter of film discs was 6 mm (Narayanan et al., 2013). In the WPI films incorporated with dihydroeugenol, the observed effect on *E. coli* was lower compared to the findings reported in the referenced study. However, against *S. aureus*, the effect was higher than what was observed in the cited work. On the other hand, in the WPI films incorporated with the glycoside, the antimicrobial activity was higher against both bacteria compared to the film incorporated with eugenol.

Sanla-Ead et al. (2012) developed cellulose films and used them as additives with eugenol and cinnamaldehyde, both in a concentration of 1% v/v. Among other tests, the researchers evaluated the antibacterial activity of the films, by the same method used in the present study, except for the disc diameter of 5 mm. The results obtained by the researchers showed no activity on tested microorganisms, among them *L. monocytogenes, S. aureus, E. coli* and *Salmonella* Enteritidis. The researchers attributed the results to low concentration and solubility of compounds in films.

Thus, it can be verified that, based on the few works available in literature, it is possible to make a brief comparison of these results with eugenol analog incorporated into other types of films. In the evaluated cases, the WPI film incorporated with dihydroeugenol glycoside proved to be more efficient against the tested bacteria. Thus, the films produced can be evaluated for application in food, increasing their shelf life, and benefiting consumers in the search for natural preservatives and sustainable packaging.

Based on the results of the antimicrobial activity of the active film produced with glycoside, a comparison was made between the activity against the different microorganisms evaluated. It is possible to verify that the mean inhibition zones across the Gram-negative bacteria *E. coli* and *S.* Enteritidis were significantly similar. Likewise, it occurred to the middle front of the Gram-positive *S. aureus* and *L. monocytogenes*. In general, it is possible to realize a significantly higher activity against Gram-positive bacteria, suggesting a better membrane permeability of these cells by the active agent. According to Dorman and Deans (2000), Gram-negative bacteria have an outer membrane that is rich in polysaccharides that forms a hydrophilic surface. This hydrophilic character may present a barrier to the permeability of lipophilic substances, as derivatives of essential oils, thus explaining the greater resistance of Gram-negative bacteria to these compounds.

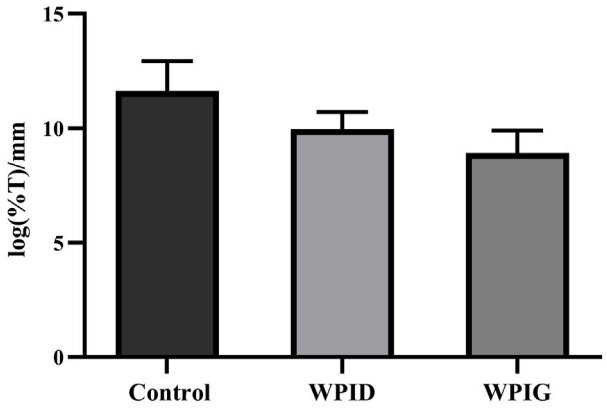

FIGURE 3
Transparency of whey protein isolate (WPI). Control: WPI film without the addition of dihydroeugenol and its glycoside. WPID: WPI film with the addition of dihydroeugenol (6% v/v), WPIG: WPI film with the addition of dihydroeugenol glycoside (6% v/v). Each bar represents the means ± SD, considering the results obtained in at three independent experiments.

Studies have indicated that eugenol possesses inhibitory effects on the growth of *E. coli*, *L. monocytogenes*, and *S. aureus* (Sanla-Ead et al., 2012). It has been observed to disrupt their cellular membranes and induce leakage of potassium ions (Walsh et al., 2003; Gill and Holley, 2006). In the work by Devi et al. (2010), was demonstrated the significant potential of eugenol as an effective antibacterial compound against *Salmonella typhi*. At bactericidal concentrations, eugenol primarily acts by disrupting the cytoplasmic membrane, leading to increased permeability. This disruption causes the leakage of ions and loss of cellular contents, including intracellular proteins, ultimately resulting in cell death. Furthermore, the findings reveal that eugenol possesses chemo-attractant properties toward *S. typhi*.

Films composed of ethylene-vinyl acetate (EVA) blended with eugenol was studied in the work of Nostro et al. (2013), following a 24–48 h incubation period, the film with eugenol at a concentration of 7 wt% exhibited promising activity. The results showed that the combination of EVA with eugenol exhibited activity against *S. aureus*, S. epidermidis, and *E. coli*, with a decrease in optical density of 20%–30%. Additionally, the EVA + eugenol combination displayed weak activity against *S. aureus* and *P. aeruginosa* strains, decreasing optical density by 15%–20%.

The nanocomposite film with sodium alginate, containing 1.5% of clove essential oil, in which the major compound is eugenol, effectively inhibited the growth of *L. monocytogenes* and *S. aureus* during the first 7, 6 days of the storage period, respectively. For *E. coli*, the highest concentration completely inhibited its growth during the first 6 days of the study period, although the antimicrobial effectiveness decreased afterward (Alboofetileh et al., 2014).

In theory, hydrophilic glycosyl groups have the ability to interact with starch molecules and effectively permeate between polysaccharide chains. This interaction acts as a pseudo-plasticizer, leading to improved physical properties of biodegradable films. Consequently, glycosides have the potential to serve as a valuable tool for solubilizing hydrophobic bioactive compounds in aqueous solutions, while simultaneously enhancing the performance of polysaccharide-based films (Wang et al., 2023). Additionally, the formation of micelles by glycosides can further contribute to enhanced antimicrobial activity. On the other hand, the primary way in which eugenol exerts its antimicrobial effects is through its free hydroxyl group. This hydroxyl group has the ability to hinder certain enzymes by binding to proteins and it can also disrupt the cell membrane, thereby increasing its permeability (Souza et al., 2022).

Among the different approaches, the antioxidant effect can also be exploited by adding dihydroeugenol and its synthesized glycoside in biodegradable films. Radical species act as initiators in the lipid oxidation chain, causing undesirable effects on food (López-De-Dicastillo et al., 2012). Based on this result, the compounds synthesized significantly increased the antioxidant activity, when compared with the control sample, agreeing with Moradi et al. (2012), since they are derived from eugenol that has antioxidant activity already elucidated. Additionally, the addition of phenolic compounds, such as the products used, greatly increases antioxidant activity, as reported by Siripatrawan et al. (2013) and Chang-Bravo et al. (2014).

Therefore, the elimination of these radicals by the packaging that will wrap food, by the incorporation of antioxidant species, shows itself as an innovative and interesting alternative for food preservation. In addition to radical scavenging ability, it is important that the antioxidant agent does not affect the overall properties of the film, a fact observed in the present study, where the addition of compounds did not negatively affect the characteristics of the film.

Among the physicochemical properties of the films, despite the hydrophilic character of the glycosidic subunit, the dihydroeugenol glycoside did not increase the film solubility because, besides its aglycone subunit being hydrophobic, hydrogen bonds between the glycosidic subunit and the WPI matrix may occur, preventing the breakdown of the structure by water molecules. So, the solubility of control film presented a higher solubility value, indicating that the synthesized compounds added to the film decreased the solubility of films, due to their hydrophobic character, poor water-soluble biodegradable films are desirable for packaging and protecting food with medium/high humidity. Ramos et al. (2012) and Fairley et al. (1996) reported in their work the partial insolubility of WPI films and attributed this characteristic to the presence of strong intermolecular bonds (disulfide bonds) between the protein molecules inside the polymeric matrix of the WPI films.

The transparency of a film is important so that it allows the consumer to view the product stored there. In this way, it becomes an attribute that influences the purchase of the product. In the work of Al-Salem (2009), in which polyethylene films with UVA additives and light transformers (Tinuvin 494 FB, Tinuvin NOR 371, Chimasorb 81 and Irgastab) were evaluated, not essential oils, the values of transparency calculated by the equation used in this work, were around 19.5, being significantly more transparent than the films synthesized with the addition of dihydroeugenol and its glycoside. On the other hand, the transparency value of the control, only the WPI film was similar to that reported in other studies (Qazanfarzadeh and Kadivar, 2016; Sukyai et al., 2018). Sothornvit et al. (2010) found a transparency of 14.38 in WPI films,

and the addition of other compounds was able to significantly reduce this transparency.

The visual appearance of films is often an important factor in consumer acceptance (Kurek et al., 2014). In general, the color of films with the addition of dihydroeugenol and its glycoside showed a tendency to yellow. The presence of fats and phospholipids is responsible for the tendency of these films to turn into a yellowish color (Lorenzen and Schrader, 2006; Ramos et al., 2013). In the work of Gohargani et al. (2020), the incorporation of *Zataria multiflora* essential oil in chitosan-whey protein-based film containing bionanocomposite tio2, caused a slight reduction in lightness (L*) and an increase in e yellowness, the same was observed in our work.

5 Conclusion

The present study showed that WPI film added with dihydroeugenol glycoside was able to form inhibition halos larger than WPI film added with dihydroeugenol, proving the efficacy of glycoside against *B. cereus, E. coli, S. aureus* and *S.* Enteritidis, with significantly higher activity against Gram-positive bacteria, suggesting a better membrane permeability of these cells by the active agent evaluated. Furthermore, the compounds synthesized significantly increased the antioxidant activity when compared to the control (without dihydroeugenol and glycoside). The results also showed a relatively a low solubility of the produced films (23% ± 2.87% and 24% ± 3.92%, dihydroeugenol and glycoside, respectively), presenting a possible applicability, since poor water-soluble biodegradable films are desirable for packaging and protecting food with medium/high humidity. Overall, this study demonstrated that Incorporation of dihydroeugenol and its glycoside in whey protein isolate film could effectively be considered promising candidates to be used as active packaging for food products as an antibacterial and antioxidant film for packaging food with medium/high humidity.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author.

Author contributions

DD, DC, MD, and RP contributed to the conception and design of the study, DC, MD, RP contributed to the methodology, DR and LL contributed to the investigation, DD and RS provided the resources, DR, LS, and NT contributed to the data curation, DR wrote the first draft of the manuscript. DD, RS, DC, MD, LS, NT, and DC wrote the review and editing, DR and LS contributed to the visualization, DD, DC, RP, and MD were the supervisors of the study, DD contributed to the project administration. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The author(s) RS declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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