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Development and Characterization of Pectin and Beeswax-Based Coatings Enhanced with Anthocyanins and Its Antioxidant and Antifungal Properties

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Abstract: Currently, approximately one third of food is wasted; to counteract this, several novel packaging technologies have been developed to extend its shelf life, among which active packaging stands out. In this research, filmogenic solutions of pectin and beeswax with the addition of bioactive compounds as anthocyanins were developed and characterized to evaluate their potential application as active coatings. The antioxidant and antifungal activity of anthocyanins and coatings were determined, and the rheological properties, pH, color, SEM and FT-IR of the coatings were evaluated. The antioxidant activity of the anthocyanins had IC50 values of 79.52 and 56.14 µg/mL for DPPH$^{\bullet}$ and ABTS$^{\bullet+}$, respectively, and 0.25% (*w*/*v*) for the antifungal activity against *Colletotrichum siamense*, which was inhibited by 32.16% and had morphological affectations in the fungus. The best formulation for coating was obtained with 3% (*w*/*v*) pectin, 1% (*w*/*v*) wax, and 1% (*w*/*v*) Tween 80, and 0.1, 0.25, and 0.5% anthocyanins were added. The rheological properties showed adequate viscosity values (0.08–0.12 Pa·s), and the pH values were acidic (3.05–3.78) and showed reddish tones. FT-IR analysis showed that the interactions between the components included the C=O stretching band being shifted due to intermolecular interactions and SEM micrographs showed that the film coatings presented continuous areas of pectin with embedded wax crystals. Promising results were obtained for antioxidant and antifungal activity for the coatings. The formulations presented suitable characteristics for their use as active coating in food.

Keywords: active coatings; anthocyanins; antioxidant activity; antifungal activity; rheological properties

1. Introduction

Food losses and food waste currently represent a global problem, with one third of the volume of food production being discarded. This occurs throughout the production and supply chain, as well as in households, retail stores, and gastronomic centers. Food losses and wastage result in large economic losses and food shortages [1]. In 2022, 1.05 million tons of waste were generated, almost a fifth of the total amount of food available for consumption [2,3].

To reduce these alarming figures, it is necessary to use technologies that counteract these effects, among which active packaging stands out; these are systems that interact with the food product to improve its quality and prolong its shelf life [4,5]. The use of natural polymers is promising as they have good mechanical properties and are renewable, which could be of use when being employed to displace synthetic polymers, which are not biodegradable and can be environmentally toxic [6–8].

The development of active coatings based on pectin and beeswax, as well as the incorporation of bioactive compounds, could have potential in improving the quality and shelf life of fruit and vegetable products. In this sense, the addition/inclusion of anthocyanins in the formulation could confer antioxidant and antifungal activity to the coating [9–11]. These compounds belong to the group of phenolic compounds called flavonoids and present a flavone nucleus consisting of two aromatic rings in their structure: a benzopyrillium and a phenolic group, both joined by a three-carbon unit [12–14]. Several studies have reported good antioxidant activity for these compounds and a correlation of this biological activity with the content of polyphenolic compounds [12,15–17]. For example, Del-Toro-Sánchez et al. [16] identified that the correlation coefficients between antioxidant activity and phenolic compounds of methanolic and ethanolic extracts of three safflower (*Carthamus tinctorius* L.) by-products ranged from 0.896 to 0.999, while for sorghum stalks, Tian et al. [17] found correlation values between 0.530 and 0.968 for the phenolic compounds and antioxidant techniques evaluated.

On the other hand, the polymers pectin and beeswax act as a structural base for the coating formulations, giving it the desired properties so that it can fulfill its function; it is extremely important that a viscosity value that allows the coating to form a thin layer on the food when applied by immersion is achieved and that its distribution is homogeneous and continuous over the entire surface [18–20]. Pectin, due to its high gelling capacity, will confer this property, while wax will confer hydrophobicity to the material to prevent the food's own humidity from diluting and disintegrating the coating [21–23]. It should be noted that there are few previous reports of blending pectin and beeswax [24,25], so further investigation of this mixture is needed, and there are no previous reports on the addition of anthocyanins.

The combination of pectin and wax, polymers of very opposite natures, is possible through the use of plasticizers that allow the emulsification of the oily phase (beeswax) in the aqueous phase (pectin solution) [26,27]. Polysorbate 80, better known as Tween 80, is a non-ionic surfactant recognized for its high emulsifying power and is also recognized as safe for use in food (GRAS); it exhibits low toxicity, high biocompatibility, and is biodegradable; although, its biodegradability is supported by specific microbial activity, for example, *Fusarium oxysporum* effectively biodegrades this compound [28–31].

Pectin is a high molecular weight complex polysaccharide derived from plants and is found forming about 30% of the cell wall of dicotyledonous plants [32–34]. It is a useful biopolymer in the food industry as it acts as a gelling, stabilizing, emulsifying, and thickening agent. In the context of food packaging, pectin is a viable option due to its high-water solubility, excellent film-forming capacity, and high flexibility [33–35]. In

addition, its wide use in various areas is justified by its recognized biocompatibility and biodegradability [36].

Beeswax, on the other hand, is an effective substance for reducing the level of humidity and permeability due to its high hydrophobicity as its structure is made up of esterified unsaturated fatty acids [21,37,38]. It also contains lactones, flavonoids, alcohols, free acids, esters, and other natural compounds that give it special characteristics, resulting in emulsifying properties, plasticity, compatibility with other natural products, and a pleasant odor [21,38]. It is recognized as non-toxic and environmentally friendly, guaranteeing its biocompatibility, and it degrades in the soil and serves as food for insect larvae, demonstrating its biodegradability [39–41].

The need to develop efficient food packaging and preservation technologies to counteract the high levels of food losses and wastage, together with the scarce information on the development of polymeric matrices combining the proposed components to produce active formulations, is what led to the conceptualization of this study, with the objective of developing and characterizing active coatings of pectin and beeswax with the addition of anthocyanins by evaluating their rheological, structural, antioxidant, and antifungal properties.

2. Materials and Methods

2.1. Reagents

For the formulation of the coatings, commercial pectin (Comercial Zazueta, Hermosillo, Sonora, Mexico), commercial beeswax (Farmacia Dermatológica Cruz Rosa, Hermosillo, Sonora, Mexico), and commercial anthocyanins were obtained from purple carrots (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef) of the LINICOL BCP 12HQT brand. The reagents used were reagent grade and of the SIGMA Aldrich brand (St. Louis, MO, USA), the Czapek agar culture medium was BD Bioxon brand, and the *Colletotrichum siamense* strain isolated and identified was obtained from the Microbiology and Mycotoxins Laboratory, Department of Food Research and Graduate Studies, University of Sonora.

2.2. Development of Active Coatings

For the formulation of the coatings, we proceeded in a stepwise manner, as displayed in Figure 1. Pectin solutions (P) of 2, 3, and 4% (w/v) were first prepared and studied according to their rheological behavior in order to define the concentration with the best properties. Once the pectin concentration was identified, beeswax (W) was added to it, previously melted and mixed with Tween 80 (T), so that the final wax concentrations were 0.5, 1.0, and 2.0% (w/v), establishing the Tween 80 concentration at 1.0% (w/v). These three formulations were mixed with constant agitation for 15 min at 1500 rpm to achieve the emulsification of the wax in the pectin solution, and their rheological behavior was also studied to identify the best formulation, which will be known as the control coating and will have anthocyanin (A) additions of 0.10, 0.25, and 0.50% (w/v) to give rise to the active coatings [21,24,42].

2.3. Physicochemical Characterization of Active Coatings

2.3.1. Analysis of Rheological Behavior

Both the pectin solutions and the other formulations were studied using the MCR-102 rheometer (Anton Paar, Ostfildern, Germany) with concentric cylinder geometry, maintaining a distance of 1 mm between the cylinder and the base at a temperature of 25 °C. Shear stress (Pa) and viscosity (Pa·s) were determined as a function of shear rate (0.1–1000 s^{-1}) and each run lasted 600 s, with 300 points being measured for a 2 s interval. In addition, the behavior of shear stress and viscosity with respect to time was also studied for 180 s at

constant shear rates of 30, 60, and 90 s^{-1}. The results were fitted to the Power Law model to determine the flow behavior, identified as "n" in Equation (1) [21,43,44].

$$\tau = K \times \gamma^n \quad (1)$$

where

- $\tau \rightarrow$ Shear stress.
- $K \rightarrow$ Consistency index.
- $\gamma \rightarrow$ Shear rate.

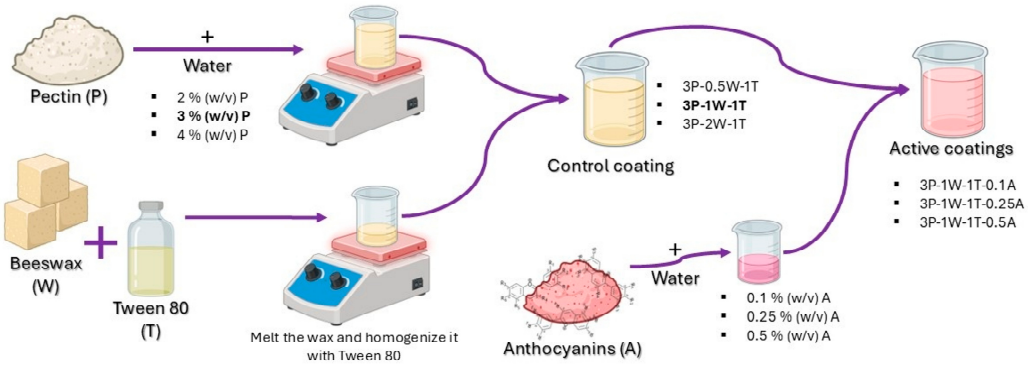

Figure 1. Coating production process.

2.3.2. pH Determination

The determination of pH was carried out using the HANNA Instruments model HI2211 potentiometer (Mexico City, Mexico) by direct immersion of the electrode in 5 mL of the formulations of the filmogenic solutions [45].

2.3.3. Color Determination

For color evaluation, 3 mL of the filmogenic solutions were analyzed and deposited in a Petri dish (diameter of 60 mm) for analysis with a MiniScan XE Plus colorimeter (model 45/0-L, HunterLab, Reston, VA, USA), obtaining the coordinates of L*, a*, and b*. The measurements were performed on the white ceramic piece of the equipment supplied with the equipment. For the measurement, the sample volume was deposited in the Petri dishes and we waited for 1 min for its homogeneous distribution before reading, avoiding lifting the plate from the flat surface [46,47].

2.3.4. Analysis by Fourier Transform Infrared Spectroscopy (FT-IR/ATR)

A total of 50 µL of the control and active coatings were evaluated and their components were analyzed, depositing approximately 8 mg of sample in the equipment. It was performed with the Perkin Elmer equipment, Spectrum Two model (Waltham, MA, USA), using an attenuated total reflectance (ATR) detector, and the reading was performed in the range of 4000 to 400 cm^{-1} [48].

2.3.5. Study by Scanning Electron Microscopy (SEM)

The JEOL 7800F (Pleasanton, CA, USA) was used to study the surface properties of the coatings, now films, by scanning electron microscopy. The coatings were transformed into films by the solvent evaporation technique in Petri dishes in a Thermo Fisher Scientific oven (NY, USA) at 50 °C for 8 h. The films were cut into approximately 2 × 2 mm squares, placed in a specimen holder with carbon tape, and coated with gold [49].

2.4. Antioxidant Activity of Anthocyanins and Coatings

The antioxidant activity of anthocyanins and coatings was determined by DPPH, ABTS, and FRAP methods, and the total phenol and flavonoid content of anthocyanins was also determined. Commercial anthocyanins were analyzed at concentrations of 0.3 to 10 mg/mL for the free radical techniques; for the rest of the determinations, a concentration of 2.5 mg/mL was used [16,50].

2.4.1. DPPH• Free Radical Scavenging Capacity

DPPH• free radical was prepared by dissolving 1.25 mg of 1,1-diphenyl-2-picrylhydrazyl in 50 mL ethanol and brought to an absorbance of 0.7 at 515 mn in the Thermo Fisher Scientific Inc. Multiskan GO microplate reader (New York, NY, USA). A total of 200 µL of this and 20 µL of the sample were combined to measure their absorbance at 515 nm after 30 min rest. The data were transformed to inhibition percentages for anthocyanins solutions using Equation (2), and the inhibition percentages of the concentration inhibiting 50% of the radicals (IC50) were determined by linear regression. For the coatings, the results were expressed as µmol TE/g of coating through the elaboration of a calibration curve using trolox [50].

$$In\ (\%) = \frac{Abs(R) - [Abs(S) - Abs(B)]}{Abs(R)} \times 100 \quad (2)$$

where

- $In\ (\%) \rightarrow$ Percentage of inhibition.
- $Abs(R) \rightarrow$ Absorbance of the radical.
- $Abs(S) \rightarrow$ Absorbance of the sample with the radical.
- $Abs(B) \rightarrow$ Absorbance of the dye blank of the sample.

2.4.2. ABTS•+ Free Radical Scavenging Capacity

To measure the ABTS•+ free radical scavenging capacity, the methodology reported by Robles-García et al. [50] was used. ABTS•+ free radical was prepared by adding 19.3 mg of 2,2′-azinobis(3-ethylbenzothiazolin)-6-sulfonic acid reagent (ABTS•+) to 5 mL of distilled water and 88 µL of 3.78% (w/v) aqueous potassium persulfate solution was also added to this solution. The mixture was allowed to stand in total darkness for radical activation and, after 16 h, 1 mL of the solution was taken and added to 88 mL of distilled water. This new solution was adjusted in a Thermo Fisher Scientific Inc. Multiskan GO, New York, NY, USA, microplate reader to an absorbance of 0.7 at 734 nm. For the determination, 20 µL of the samples and 290 µL of the radical were mixed, allowed to stand in the dark for 30 min, and the absorbance was read at 734 nm. The results were expressed in the same way as for the DPPH• radical assay.

2.4.3. Ability to Reduce Ferric to Ferrous Iron (FRAP)

Three solutions were prepared: 1. sodium acetate buffer (300 mM) at pH 3.6 in distilled water, 2. ferric chloride ($FeCl_3$) (20 mM) in distilled water, and 3. 2,4,6-tripyridyl-triazine (TPTZ) (10 mM) in hydrochloric acid (40 mM). The solutions were mixed at a ratio of 10:1:1, respectively, yielding the working solution (FRAP). The procedure consisted of combining 280 µL of FRAP with 20 µL of the sample and leaving the mixture to rest in darkness for 30 min. Subsequently, the absorbance of the sample was read at 638 nm in a Thermo Fisher Scientific Inc. Multiskan GO, New York, NY, USA, microplate reader. For the expression of the results, a calibration curve was made using trolox as standard, and the results were reported as µmol TE/g sample [50].

2.4.4. Total Phenol Content

The Folin–Ciocalteu method was used to determine the total phenol content. In a microplate, 10 µL of the anthocyanin solution and 25 µL of the 1 N Folin solution were added. After 5 min, 25 µL of the 20% sodium carbonate and 140 µL of the distilled water were added. Subsequently, the microplate was kept at room temperature and in the dark for 30 min, and then absorbance was measured at 760 nm using a Thermo Fisher Scientific Inc. Multiskan GO, New York, NY, USA, microplate reader. The results were reported in mg GAE/g sample by constructing a calibration curve with gallic acid [16].

2.4.5. Total Flavonoid Content

The methodology employed by Del-Toro-Sánchez et al. [16] was used to quantify the total flavonoid content. In the first step, 80 µL of the anthocyanin solution was combined with 80 µL of aluminum trichloride dissolved in ethanol at a concentration of 20 g/L. The mixture was then gently stirred for 30 s and kept in the dark for 1 h. After that, it was shaken again to measure the absorbance at 415 nm using the Thermo Fisher Scientific Inc. Multiskan GO, New York, NY, USA, microplate reader. For the expression of the results, a calibration curve was constructed with quercetin, and the total flavonoid content was reported in mg QE/G of sample.

2.5. Antifungal Activity of Anthocyanins and Coatings

Radial mycelial growth was measured to determine the percentage of inhibition. The spore suspension of *C. siamense* was obtained by adding 6 mL of Tween 80 to the fungus grown 7 days before (1X Czapek Agar, 27 °C). The concentration of this suspension was determined by counting the spores in a Neubauer chamber using a ZEISS optical microscope (Jena, Germany) to determine the inoculation volume for an inoculum load of 1×10^4 spores. The inoculation volume was 15.2 µL of spore suspension. For radial mycelial growth, 1X Czapek Agar enriched with anthocyanin solutions of concentrations from 0.06 to 1.00% (w/v) (0.06, 0.13, 0.25, 0.50, 1.00% w/v) mixed in a 5:1 ratio, respectively, was used. The enriched culture medium was sterilized with UV light for 3 min and placed in Petri dishes, being well inoculated and incubated at 27 ± 2 °C (Felisa incubator). Every 24 h, radial growth was measured by determining the diameter in millimeters with a ruler, and the measurement of each sample was carried out in 2 directions. This was repeated until the *C. siamense* control reached the edge of the plate. The results were expressed in terms of percentage inhibition, considering the growth of the controls in comparison with that of the treatments, as shown in Equation (3) [51,52].

$$In\ (\%) = \frac{G(C) - G(S)}{G(C)} \times 100 \quad (3)$$

where

- $In\ (\%) \rightarrow$ Percentage of inhibition.
- $G(C) \rightarrow$ Growth of the *C. siamense* control.
- $G(S) \rightarrow$ Growth of *C. siamense* with sample.

Once the growth evaluation was concluded, the plates were placed under refrigeration at 4 °C to stop the development. Afterwards, observations of mycelial morphology and measurements of spore dimensions were carried out. Samples of *C. siamense* were taken from the ends of the colonies with forceps and deposited on a slide with a drop of sterile water and covered with coverslips. The slides were placed in the specimen holder of the Olympus CX31 optical microscope (Olympus, Tokyo, Japan) where they were observed at 40× magnification. This microscope had an Olympus U-CMAD3 microscope camera (Me-

dia Cybernetics, Rockville, MD, USA) adapter connected to a computer for data collection and processing using Image-Pro Plus version 6.3 software. For the measurement of spores, measurements were made at 10 spores per field in 10 fields [51,52].

2.6. Experimental Design and Statistical Analysis

This study consists of a completely randomized single-factorial experimental design for the characterization of the coatings and their antioxidant and antifungal activities, the factor being the different formulations, while the same completely randomized single-factorial design was used for the antioxidant activity, with the difference being that the factor was the different concentrations studied. The statistical analysis was performed by analysis of variance (ANOVA) with comparisons by Tukey's test at $\alpha = 0.05$ in InfoStat 2020. With the results of the antioxidant techniques by DPPH and ABTS, the IC50 was determined by linear regression in Excel version 2411. For each sample, 3 replicates were performed for each determination.

3. Results

3.1. Development and Physicochemical Characterization of Coatings

3.1.1. Rheological Behavior

Pectin is the viscosifying agent in the formulation, so its rheological behavior was studied individually, at concentrations of 2, 3, and 4% (w/v), as shown in the graphs in Figure 2a,b. These results were used to determine the concentration of pectin used in the development of the filmogenic solutions. Figure 2a shows the graph of shear stress versus shear rate where the higher the concentration of the solution, the greater the shear stress for the same value of shear rate. The 2% pectin solution showed the lowest rate of increase in shear stress versus shear rate, being higher than the highest concentration of pectin studied (4%). Figure 2b shows that solutions with concentrations of 2 and 3% pectin showed the smallest variations in viscosity with respect to shear rate. The decrease in viscosity was smaller at the 2% concentration and more considerable at the 4% concentration. The results of the rheological analysis of the pectin solutions showed a non-Newtonian flow behavior of the pseudoplastic type (Table 1).

Table 1. Ostwald-De Waele model for the solutions that gave rise to the coatings and for the coatings.

Solution	Flow Index (n)	R^2	Fluid Type
Pectin 2%	0.9823	0.999	Pseudoplastic
Pectin 3%	0.9388	0.994	Pseudoplastic
Pectin 4%	0.8562	0.993	Pseudoplastic
3P-0.5W-1T	0.9976	0.962	Newtonian
3P-1W-1T	0.9127	0.996	Pseudoplastic
3P-2W-1T	0.9164	0.996	Pseudoplastic
3P-1W-1T-0.1A	0.8808	0.998	Pseudoplastic
3P-1W-1T-0.25A	0.9366	0.999	Pseudoplastic
3P-1W-1T-0.5A	0.9026	0.998	Pseudoplastic

Adding beeswax and 1% (w/v) Tween 80 to the 3% (w/v) concentration pectin solution yielded the following formulations: 3% pectin, 0.5% wax, and 1% Tween 80 (3P-0.5W-1T); 3% pectin, 1% wax, and Tween 80 (3P-1W-1T); and 3% pectin, 2% wax, and 1% Tween 80 (3P-2W-1T). Figure 2c,d shows the rheological behavior of these formulations. The difference between the formulations is given by the wax concentration, which shows a slight effect on the shear stress and viscosity of the mixture. However, at higher shear rates, the difference in rheological behavior increases, with the 0.5% wax formulation differing the most among the three formulations. The wax concentration slightly increases

the shear stress for the same shear rate (Figure 2c) and decreases the viscosity (Figure 2d). In addition, viscosity decreases with respect to shear rate in all formulations, resulting in shear thinning, a characteristic behavior of pectin. For its part, the fluid type (Table 1) is pseudoplastic, except for formulation 3P-0.5W-1T, where the value is 0.9976 (≈ 1), being a Newtonian fluid.

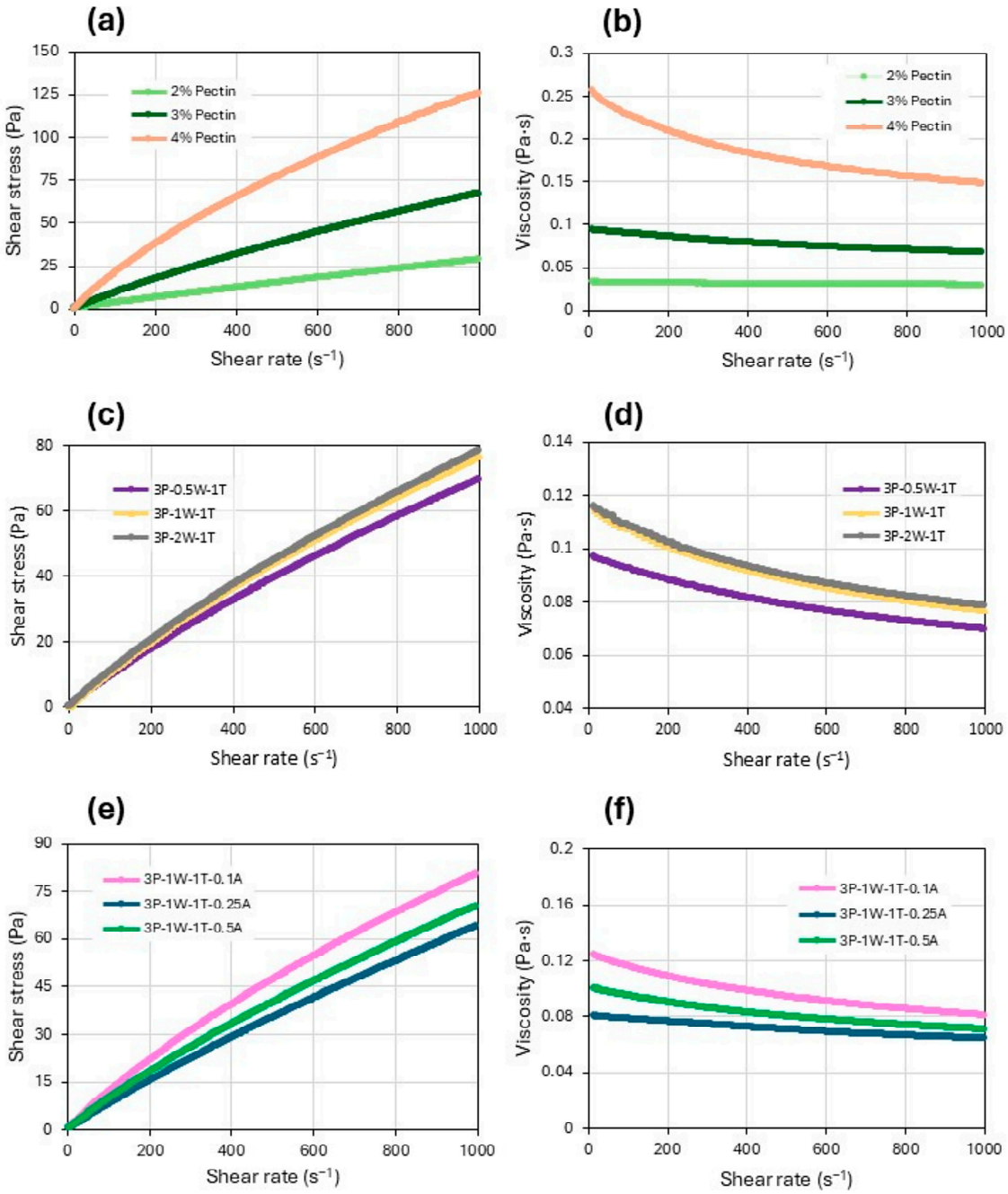

Figure 2. Plots of shear stress and viscosity vs. shear rate for the solutions that gave rise to the coatings and for the coatings. (**a**) Shear stress vs. shear rate for 2, 3, and 4% pectin solutions. (**b**) Viscosity vs. shear rate for 2, 3, and 4% pectin solutions. (**c**) Shear stress vs. shear rate for pectin-wax-Tween 80 mixtures. (**d**) Viscosity vs. shear rate for pectin-wax-Tween 80 mixtures. (**e**) Shear stress vs. shear rate for the active coatings. (**f**) Viscosity vs. shear rate for the active coatings.

For these formulations, stability over time was also evaluated at three constant shear rates (30, 60, and 90 s^{-1}), evaluating shear stress and viscosity versus time (Figure 3a,b). In both graphs, there was stability in the rheological behavior of the formulations at all shear

rates studied during the 180 s analyzed. However, the 3P-0.5W-1T formulation was the one that showed the greatest variation in its shear stress at a shear rate of 90 s^{-1} (Figure 3a), suffering a slight decrease in shear stress at approximately 70 s. Figure 3b shows stability in viscosity for the three formulations at all shear rates studied, with the exception of the 3P-0.5W-1T formulation, which, at a shear rate of 30 s^{-1}, showed a slight increase in viscosity throughout the time studied.

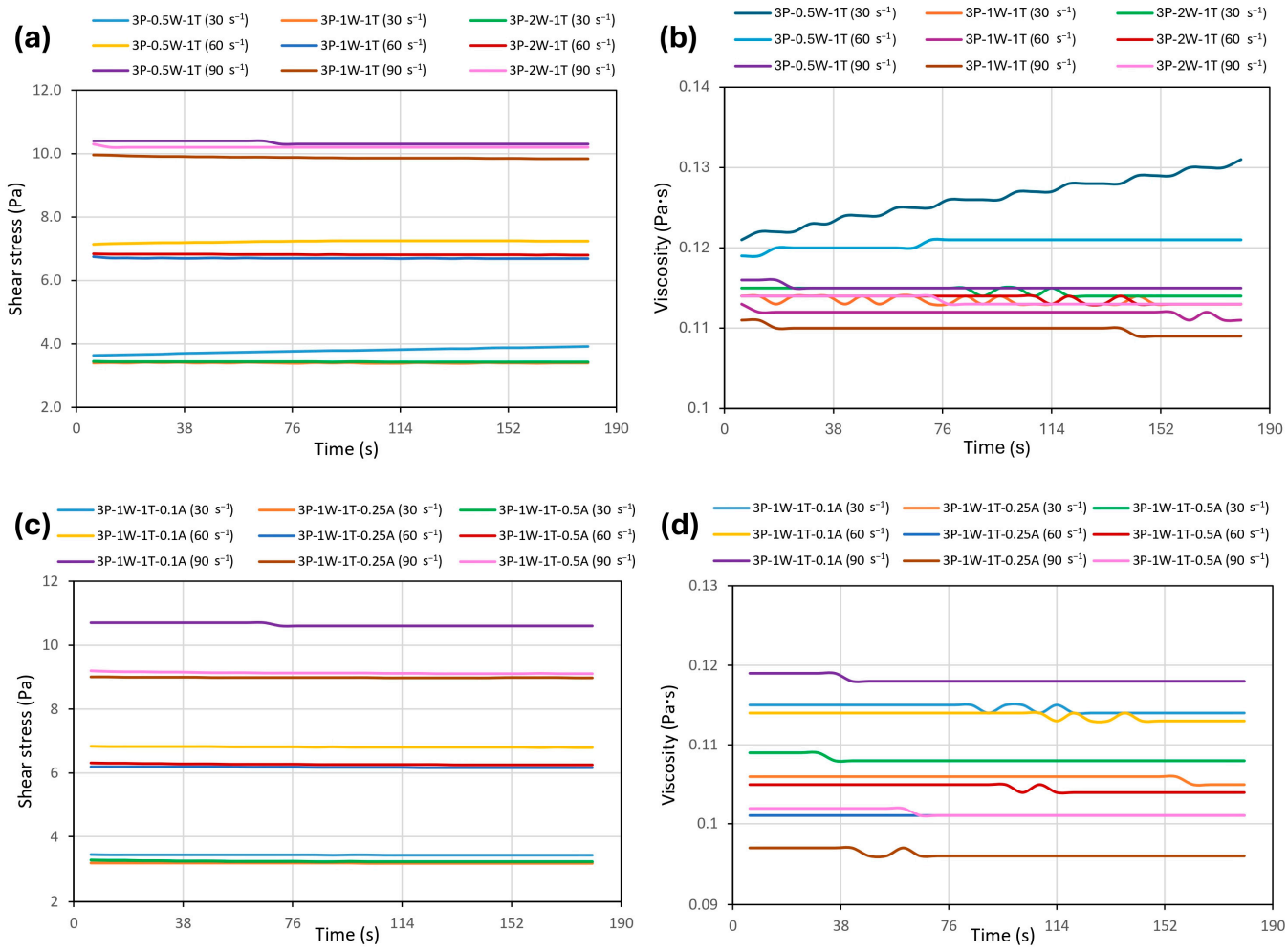

Figure 3. Plots of shear stress and viscosity versus time for the different blends of coating components at shear rates of 30, 60, and 90 s^{-1}. (**a**) Shear stress vs. time for pectin-wax-Tween 80 blends. (**b**) Viscosity vs. time for pectin-wax-Tween 80 blends. (**c**) Shear stress vs. time for active coatings. (**d**) Viscosity vs. time for active coatings.

To the control coating formulation (3P-1W-1T), commercial anthocyanin amounts of 0.1% (3P-1W-1T-0.1A), 0.25% (3P-1W-1T-0.25A), and 0.5% (3P-1W-1T-0.5A) were added, thus formulating the active coatings whose rheological behaviors are shown in Figures 2e,f and 3c,d. The graph in Figure 2e shows very similar behavior with respect to shear stress for the three solutions; however, it can be seen that the greater the addition of anthocyanins, the more the shear stress decreases, exerting a more pronounced effect with respect to that exerted by the emulsification of the pectin with the wax (Figure 2c). The coating with the addition of 0.5% of anthocyanins barely exceeds 60 Pa, being 20 Pa below the control coating. Figure 2f shows that the addition of anthocyanins decreased the viscosity of the solutions. The control coating started its behavior at a viscosity close to 0.12 Pa·s, the same value for the coating with 0.1% of anthocyanins; however, the coatings with 0.25 and 0.5% of anthocyanins start from values of 0.1 and 0.08 Pa·s, respectively,

while the three active coatings behave as pseudoplastic fluids (Table 1). Analyzing stability, Figure 3c,d shows that both shear stress and viscosity are stable over time, with no major variations in values.

3.1.2. pH Analysis

The pH values of the coatings are shown in Figure 4. The results obtained correspond, in all cases, to acid pH (pH < 7), which indicates that H^+ ions are present in the solutions. These results are closely related to the chemical structure of the pectin and anthocyanin molecules in addition to the fact that the label of commercial anthocyanins specifies that they contain citric acid.

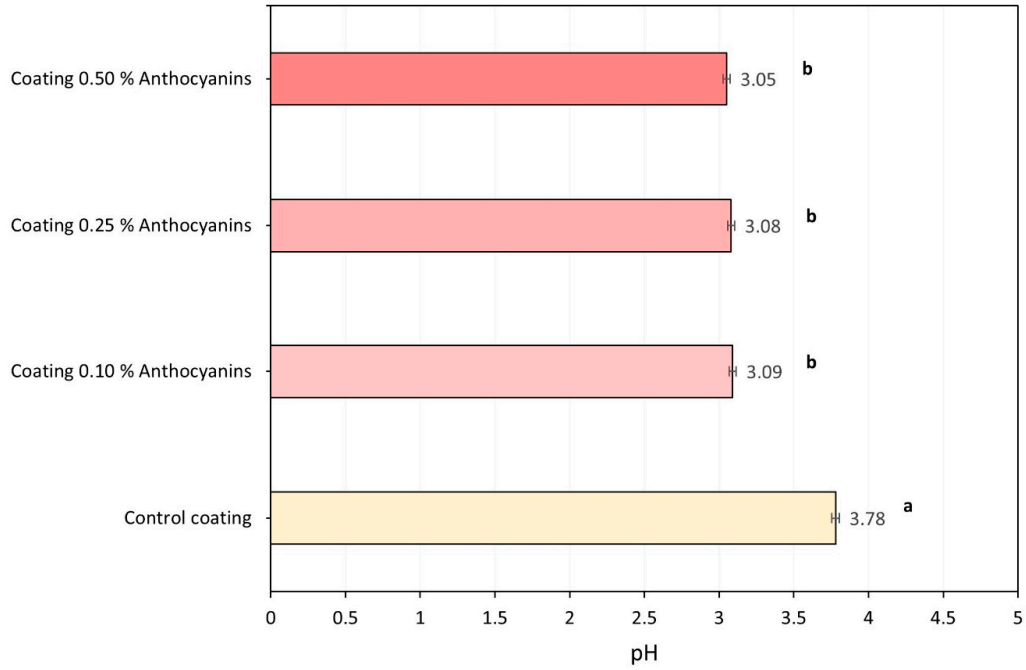

Figure 4. pH of control and active coatings. Different letters indicate significant differences by Tukey's test ($p < 0.05$). Error bars represent the standard deviation. Determination was performed in triplicate.

The active coatings presented a significantly lower pH (Tukey, $p < 0.05$) than the pH of the control while there were no significant differences between them, and the effect of anthocyanin concentration in the range of 0.1 to 0.5% (w/v) was not appreciated.

3.1.3. Color Analysis

Table 2 depicts the values of the L*, a*, and b* parameters of the coatings, as well as the total color difference in the active coatings with respect to the control. Significant differences were detected by Tukey's test ($p < 0.05$) in all the color parameters analyzed for the different formulations, except for the b* coordinate, in the coatings with the addition of 0.1 and 0.25% of anthocyanins, and for the total color difference in the coatings with 0.25 and 0.5% of anthocyanins. For brightness (L*), a higher value was present in the control coating and, as the anthocyanin concentration increased, it decreased. On the other hand, the parameter a* showed that the control coating presented a slight inclination towards green shades, while the incorporation of anthocyanins caused a change in this coordinate towards reddish tones; this tendency being more pronounced the higher the anthocyanin concentration. The b* coordinate showed positive values, indicating a predominance of yellow over blue tones, and the three additions of anthocyanins in the formulation caused a gradual increase in the b* coordinate.

Table 2. Color parameters and total color difference (ΔE) for control and active coatings.

Parameter		Coatings			
		Control	Anthocyanins 0.10%	Anthocyanins 0.25%	Anthocyanins 0.50%
Color	L*	82.86 ± 1.00 [a]	76.38 ± 0.66 [b]	69.22 ± 0.59 [c]	48.65 ± 1.40 [d]
	a*	−0.87 ± 0.02 [a]	8.45 ± 0.70 [b]	19.28 ± 0.56 [c]	37.69 ± 0.76 [d]
	b*	4.55 ± 0.16 [a]	7.88 ± 0.51 [b]	8.60 ± 0.43 [b]	19.88 ± 1.24 [c]
ΔE		---	11.74 ± 0.89 [a]	24.58 ± 0.74 [b]	53.68 ± 1.65 [c]

Determination was performed in triplicate. ΔE: total color difference in the active coatings with respect to the control coating. Results expressed as mean ± standard deviation. Different letters in the row indicate significant differences by Tukey's test ($p < 0.05$).

3.1.4. Fourier Transform Infrared Spectroscopy (FT-IR/ATR)

The study of the functional groups of each molecule, together with the study of the mixtures of compounds in the coating formulations, helps us understand the structural characterization of the coatings, as shown in Figure 5. All the components of the formulations (pectin, beeswax, Tween 80, and anthocyanins) presented their characteristic spectra, previously documented and associated with their multiple functional groups in multiple studies [53–58]. As can be seen in the spectra of the coatings, the addition of anthocyanins had no significant influence on the spectra, resulting in the spectra of the three active coatings being very similar to each other and to the control coating.

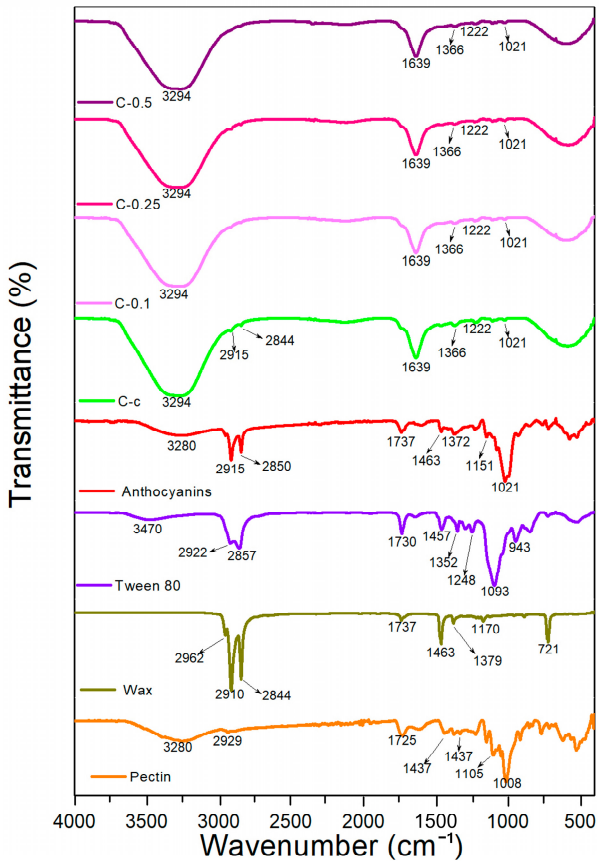

Figure 5. FT-IR spectra by ATR of the control and active coatings and their components. C-c: coating control; C-0.1: coating 0.10% (*w*/*v*) anthocyanins, C-0.25: coating 0.25% (*w*/*v*) anthocyanins, C-0.5: coating 0.50% (*w*/*v*) anthocyanins.

For the coatings, the most pronounced band at 3294 cm^{-1} corresponds to the oxygen–hydrogen (O-H) stretching. The band at 2915 cm^{-1} is related to the carbon–hydrogen (C-H)

stretching, while the band at 1639 cm^{-1} is due to the stretching of the carbon–oxygen (C=O) double bond. The bands at 1366 and 1021 cm^{-1} correspond to oxygen–hydrogen (O-H) bending and carbon–oxygen (C-O) stretching, respectively [53,55].

3.1.5. Scanning Electron Microscopy (SEM)

In the micrographs obtained by SEM (Figure 6), the formation of wax crystals embedded in a continuous pectin phase is observed in all formulations, the surface of the material being divided into two well-defined zones: continuous pectin film and surface encrustations of beeswax crystals. An important aspect to note is that the distribution of wax crystals over the entire surface increased with the addition of anthocyanins to the formulation. For the control coating, there is an area with a predominance of crystals and another one without, while for the active filmogenic solutions, there is a greater dispersion of crystals. This is confirmed when analyzing the images at 1000× (Figure 6e–h), where for the control filmogenic solution, no crystals can be seen because when the image was zoomed in, a surface area was captured where there was none. At the image amplitude of 1000×, the wax crystals are better appreciated, and we were able to better define their amorphous structure.

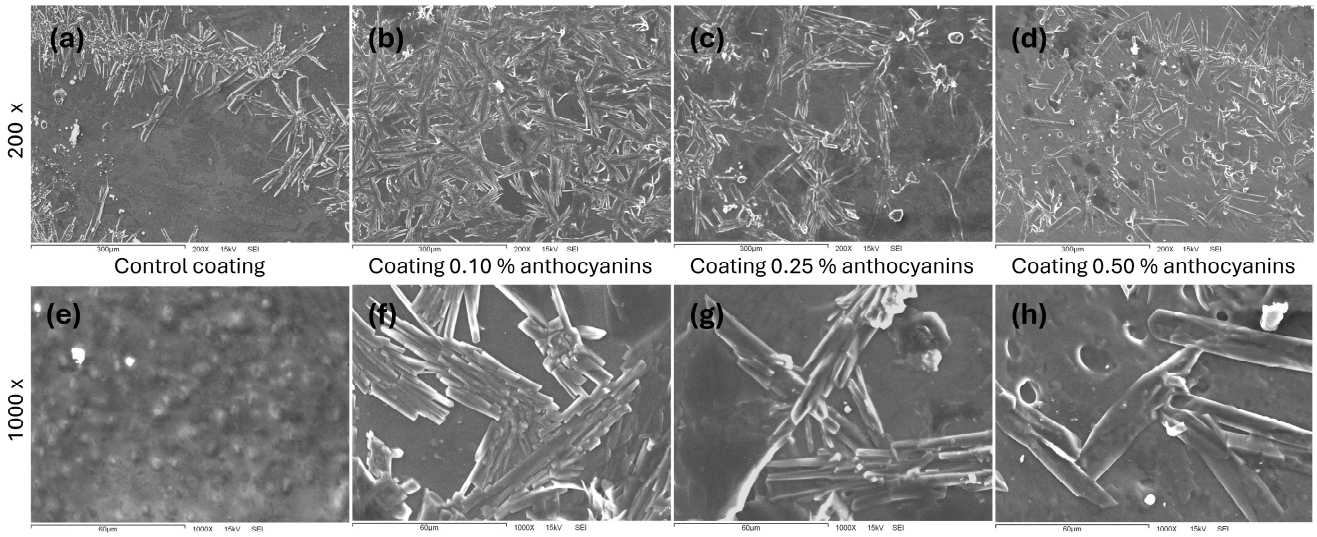

Figure 6. Micrographs of the control and active coatings transformed into films at 200× and 1000× amplitudes. (**a**) Control coating at 200×. (**b**) Coating with addition of 0.10% (*w*/*v*) anthocyanins at 200×. (**c**) Coating with addition of 0.25% (*w*/*v*) anthocyanins at 200×. (**d**) Coating with addition of 0.50% (*w*/*v*) anthocyanins at 200×. (**e**) Control coating at 1000×. (**f**) Coating with addition of 0.10% (*w*/*v*) anthocyanins at 1000×. (**g**) Coating with addition of 0.25% (*w*/*v*) anthocyanins at 1000×. (**h**) Coating with addition of 0.50% (*w*/*v*) anthocyanins at 1000×.

3.2. Antioxidant Activity

3.2.1. Antioxidant Activity of Commercial Anthocyanins

The results of the antioxidant activity are shown in Table 3 where the results of both the antiradical activity and the reducing power, as well as the content of total phenols and flavonoids, are shown.

The commercial anthocyanins presented a low IC50 for $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, which evidences their good antiradical capacity, while, for the reducing power by FRAP, a high value of 8.27 mmol TE/g sample was obtained. The content of the phenolic compounds was 386.11 mg GAE/g sample, a high value, since anthocyanins fall within the group of phenolic compounds, more specifically, within the group of flavonoids, which justifies the value of 1.26 mg QE/g for the flavonoid content.

Table 3. Antioxidant activity and total phenol and flavonoid content of commercial anthocyanins.

Antioxidant Activity	Results
Antirradical DPPH$^{\bullet}$	IC50 → 79.52 µg/mL
Antirradical ABTS$^{\bullet+}$	IC50 → 56.14 µg/mL
Reducing power (FRAP)	(8.27 ± 0.29) mmol TE/g
Total phenol	(386.11 ± 19.98) mg GAE/g
Total flavonoid	(1.26 ± 0.14) mg QE/g

All determinations were performed in quintuplicate. The concentration that inhibited the 50% radicals (IC50) of DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ was determined by linear regression. The reduction power, phenols, and total flavonoids are expressed as mean ± standard deviation.

3.2.2. Antioxidant Activity of Control and Active Coatings

The results of the antioxidant activity of the control and active coatings are shown in Figure 7. The lowest results were obtained for the control coating (without the addition of anthocyanins), with values close to zero and statistically lower than the results for the active coatings. The highest antioxidant result for the control coating was obtained against the radical DPPH$^{\bullet}$. When analyzing the results of the active coatings, they were significantly higher than those of the control coating, and the higher the anthocyanin addition, the more the results for DPPH and FRAP increased significantly, while for ABTS, there were no significant differences between the 0.25 and 0.5% anthocyanin additions. The results of the reducing power were considerably superior with respect to the antiradical techniques. When comparing the reducing power of the coatings with commercial anthocyanins, the result is significantly lower, recovering 9.08% of the reducing power of the anthocyanins by adding them to the coating formulation at 0.5%.

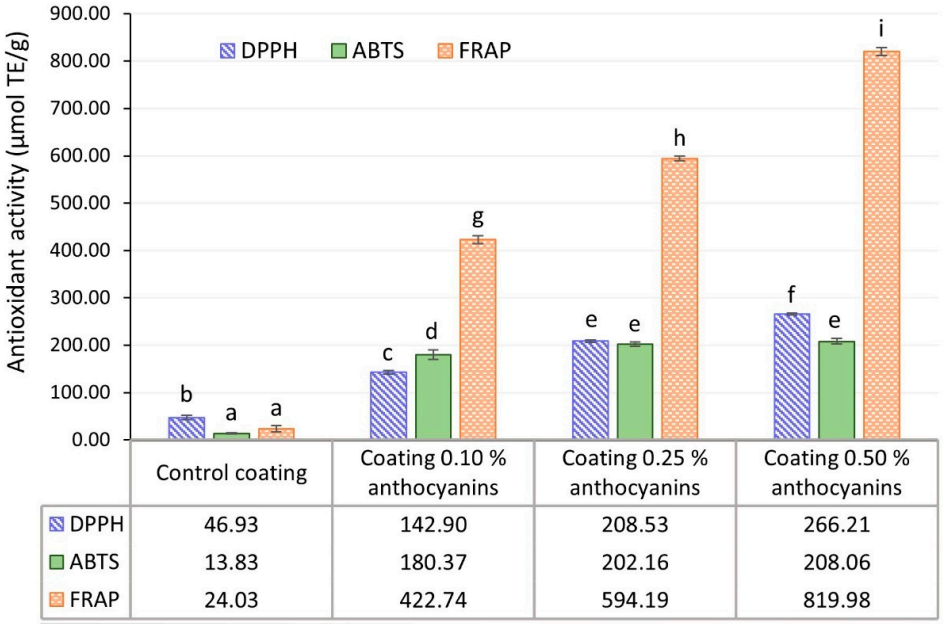

Figure 7. Antioxidant activity results for control and active coatings by DPPH, ABTS, and FRAP methods. Different letters indicate significant differences by Tukey's test ($p < 0.05$). All determinations were performed in triplicate.

3.3. Antifungal Activity

3.3.1. Antifungal Activity of Commercial Anthocyanins

Radial Mycelial Growth

The effect of different concentrations of commercial anthocyanins (0.06, 0.13, 0.25, 0.50, and 1.0% *w*/*v*) on the radial growth of *Colletotrichum siamense* were analyzed every

24 h until 168 h, as shown in Figure 8a. It was observed that the fungus had growth for all the anthocyanin concentrations studied, but it was lower than for the control sample (in culture medium without addition of other substances). For the concentration of 0.25% anthocyanins, the lowest radial growth was observed, while the treatment with the highest growth was that of 1.0% anthocyanins.

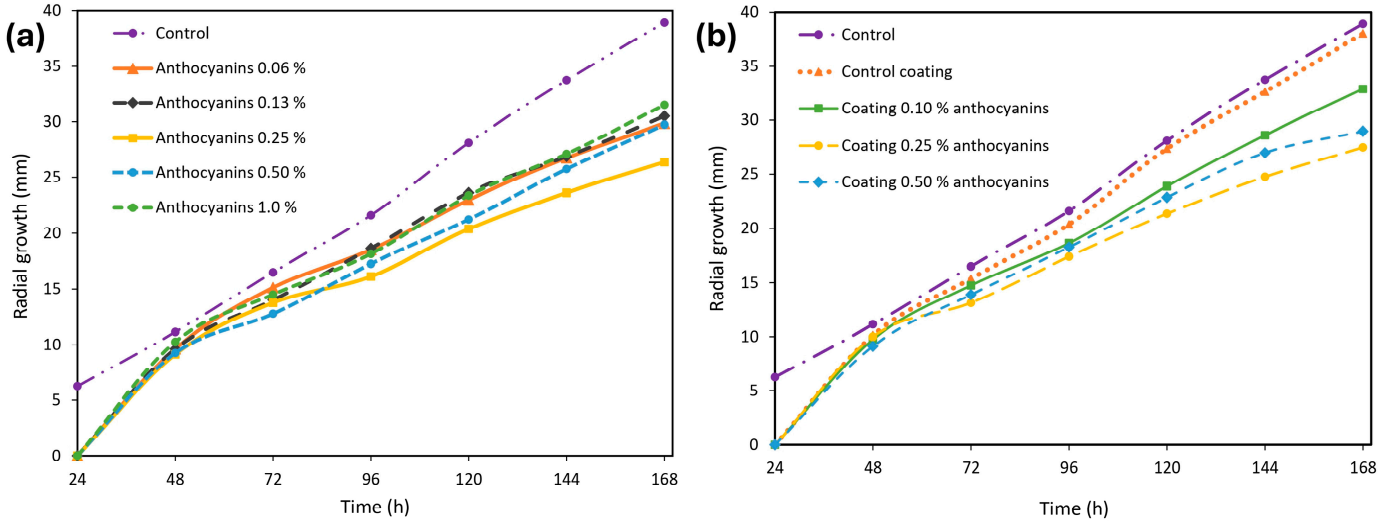

Figure 8. Radial mycelial growth of *Colletotrichum siamense*. (**a**) Against different concentrations of anthocyanins. (**b**) Against control and active coatings. All determinations were performed in triplicate.

The inhibition percentages of *C. siamense* are shown in Table 4 where it can be seen that during the first 24 h, there was no mycelial growth. A variation in these percentages is also observed, where the highest value obtained was 32.16% (anthocyanins at 0.25%).

Table 4. Mycelial growth inhibition (%) of *Colletotrichum siamense* against commercial anthocyanins and control and active coatings.

Time (h)	Anthocyanins Concentration (% *w*/*v*)					Coatings			
	0.06	0.13	0.25	0.50	1.00	Control	Ant. 0.10%	Ant. 0.25%	Ant. 0.5%
48	13.52 ± 2.25^{b}	13.52 ± 2.25^{b}	18.01 ± 2.25^{b}	16.89 ± 2.59^{b}	7.91 ± 2.59^{a}	9.03 ± 2.25^{a}	12.40 ± 2.59^{ab}	10.15 ± 3.67^{ab}	18.01 ± 2.25^{b}
72	8.33 ± 2.90^{a}	15.15 ± 2.47^{b}	16.67 ± 1.75^{bc}	22.73 ± 3.91^{c}	12.12 ± 2.47^{ab}	6.82 ± 2.90^{a}	10.61 ± 3.03^{ab}	20.45 ± 3.81^{c}	15.91 ± 1.52^{bc}
96	14.47 ± 2.67^{b}	13.89 ± 2.91^{b}	25.45 ± 2.21^{d}	20.25 ± 1.33^{c}	16.20 ± 1.16^{bc}	5.80 ± 3.47^{a}	13.89 ± 1.16^{b}	19.67 ± 2.21^{c}	15.63 ± 2.98^{bc}
120	18.24 ± 2.51^{b}	16.01 ± 2.67^{b}	27.57 ± 2.24^{d}	24.46 ± 1.03^{c}	16.90 ± 3.04^{b}	2.68 ± 1.70^{a}	15.13 ± 2.67^{b}	24.01 ± 1.70^{c}	18.68 ± 2.24^{b}
144	20.74 ± 1.48^{c}	20.37 ± 2.22^{c}	30.00 ± 2.22^{e}	23.70 ± 2.57^{cd}	19.63 ± 0.74^{c}	3.33 ± 1.42^{a}	15.19 ± 2.22^{b}	26.67 ± 0.86^{d}	20.00 ± 1.21^{c}
168	23.16 ± 1.23^{cd}	21.55 ± 3.79^{c}	32.16 ± 3.22^{e}	23.48 ± 1.66^{cd}	18.98 ± 1.82^{c}	2.26 ± 1.05^{a}	15.44 ± 1.23^{b}	29.27 ± 1.05^{e}	25.41 ± 1.05^{d}

All determinations were performed in triplicate. Different letters in the row indicate significant differences by Tukey's test ($p < 0.05$).

Morphology and Dimensions of Spores

As can be seen in the images in Figure 9, the morphology of *C. siamense* is shown under normal conditions and in anthocyanins-enriched agar. In all images, the hyphae are well defined, with normal growth observed for the control, whose hyphae are straight, practically without folds and with a slightly elevated production of inclusion bodies. Spores are visible in almost all the images and inclusion bodies are more abundant in the treatments of *C. siamense* subjected to different concentrations of anthocyanins. Cell lysis is not noticeable at any of the anthocyanin concentrations studied.

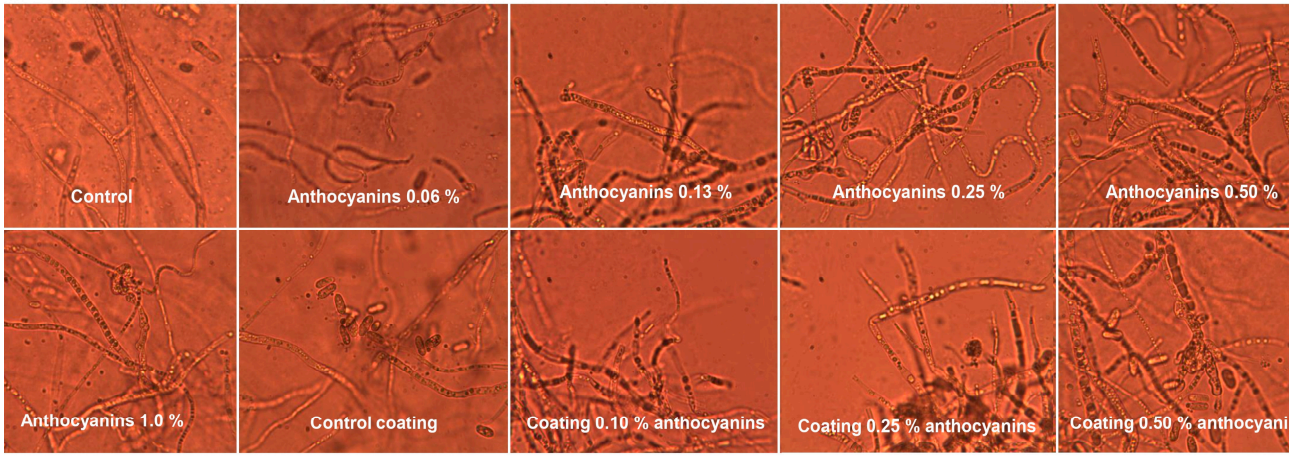

Figure 9. Morphological images of *Colletotrichum siamense* control at different concentrations of anthocyanins and for the control and active coatings.

Figure 10 shows the dimensions of the spores, the longest spores being those of the control fungus, significantly longer (Tukey, $p < 0.05$) than all the anthocyanin treatments, with the shortest spores being those of the treatments with anthocyanin concentrations of 0.25 and 1.0%, with results slightly lower than 12 µm; however, despite being the shortest, they did not show significant differences with respect to the other anthocyanin concentrations, all having equal influence on spore length. On the other hand, the behavior of the width was different from the behavior of the length. The anthocyanin concentrations of 0.06 and 1.0% did not show significant differences in the width of the spores with respect to the control, while the greatest width was obtained at an anthocyanin concentration of 0.25%, which is significantly different from the anthocyanin concentrations of 0.06 and 1.0%, but not from those of 0.13 and 0.50%.

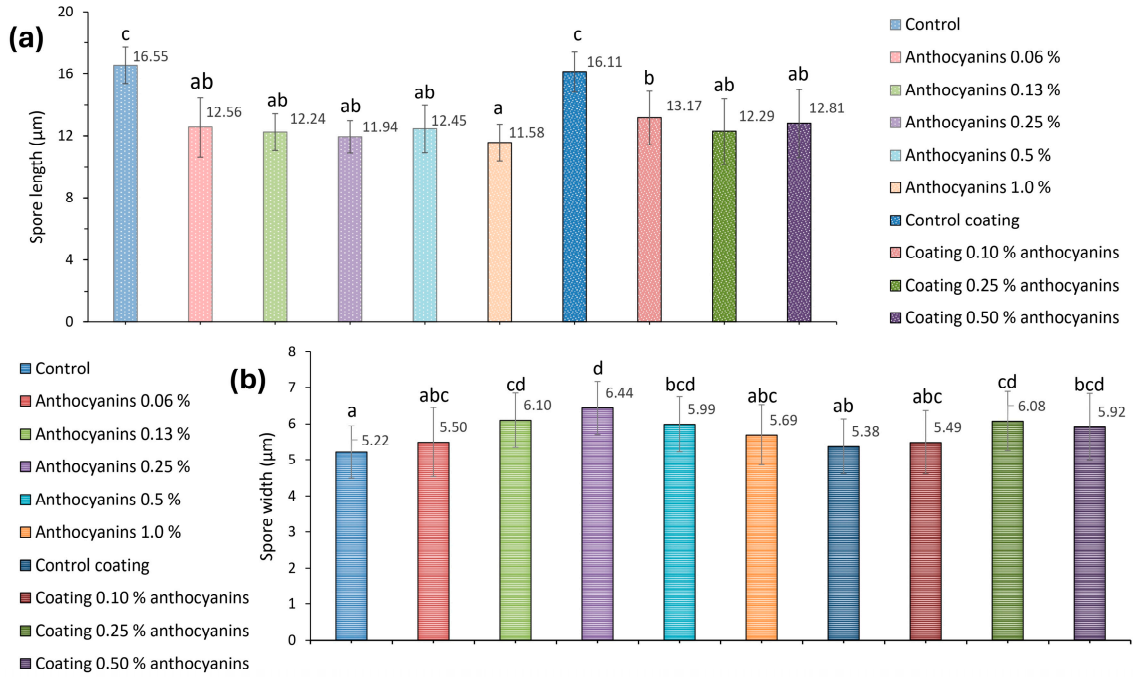

Figure 10. Spore dimensions of control *Colletotrichum siamense* spores at different anthocyanin concentrations and for the control and active coatings (**a**) spore length and (**b**) spore width. Different letters indicate significant differences by Tukey's test ($p < 0.05$). One hundred spores were measured per treatment.

3.3.2. Antifungal Activity of Control and Active Coatings

Radial Mycelial Growth

Figure 8b shows the development of *C. siamense* against the coatings where it can be seen that, for all the formulations, the fungus grew, but always less than for the control sample. Likewise, for the different concentrations of anthocyanins in the coatings, no growth was observed at 24 h; however, after 24 h, growth began and, for the control coating, growth was very similar to that of the control sample, the effect of the formulation without anthocyanins being minimal. On the other hand, for the fungus exposed to the active coatings, growth was slower, with the lowest growth at 168 h in the treatment with the active coating with 0.25% of anthocyanins. These results are contrasted with the inhibition percentages shown in Table 4, where it can be seen that the control coating achieved only 2.26% inhibition.

Morphology and Dimensions of Spores

In the image of Figure 9, it can be seen that for the fungus exposed to the control coating, there are few affectations with respect to the control sample, with a straight development of the hyphae, denoting little or no stress of the medium on them. However, when analyzing the images of *C. siamense* exposed to the active coatings (Figure 9), a greater cross-linking of the hyphae can be distinguished, tending to fold and return to the origin. As with the morphological images of *C. siamense* exposed to commercial anthocyanins, no cell lysis can be seen; however, the marked effect on the morphological development of the species can be appreciated. With respect to spore dimensions, it can be seen in Figure 10a that the spore length of the control coating does not differ from that of the control treatment but does differ from that of the active coatings. For the width of the spores (Figure 10b), there were no significant differences (Tukey, $p > 0.05$) between the control, the control coating, and the active coating with 0.10% anthocyanin, the spores being of lesser width.

4. Discussion

4.1. Physicochemical Characterization of Coatings

The physicochemical characterization of filmogenic solutions for coatings helps to predict the behavior of the material at the time of application and thus define its functionality. The rheological properties of the mixture of compounds to develop the coatings are crucial to identify the appropriate formulation to use. Analyzing the viscosity greatly helps to estimate how the solution will be distributed on the food when applied. It is desirable that the coatings are presented in the form of a thin, continuous layer over the entire surface of the food. However, if it is very fluid, it will not cover the entire surface, or if it is very dense, it will form a very thick layer with a possible formation of lumps on the product, characteristics that would result in the rejection of the coated products [59].

The rheological behavior of pectin solutions is due to the fact that pectin has a high viscosifying power, which correlates directly with the concentration of the solution [60]. Although it is desirable that the filmogenic solutions present a stable behavior in their viscosity, it can be seen in Figure 2b that the pectin solutions presented variations in their viscosity with respect to the shear rate. However, the variations are the result of a high centrifugal force, to which the coatings will not be subjected at the time of application. Their use would, in subsequent studies, be by immersion, and this process would involve a normal force of considerably lesser magnitude than that applied, so that the number and nature of chemical interactions present in the solutions would not be affected so considerably. This decrease in viscosity with increasing shear rate is known as reofluidization and is due to the fact that the applied stress causes the matrix molecules to become altered and disorganized [43]. The pseudoplastic flow behavior for all pectin concentrations studied

agrees with that reported by Lozada [61] for aqueous solutions of 2% (*w*/*v*) prickly pear peel pectin.

Based on the results of the rheological properties of the pectin solutions, it was determined that the 3% concentration solution for emulsification with wax and Tween 80 would be used because the viscosity of 0.1 Pa·s could favor the formation of the coating layer without being a very high value that hinders the emulsification with the oily phase.

For the pectin–beeswax–Tween 80 formulations, very similar behavior was observed among the three formulations, both for shear stress and viscosity, which evidences that pectin is the main component responsible for the rheological behavior. Based on the rheological stability results (Figure 3a,b), the formulation 3P-0.5W-1T was discarded for the addition of anthocyanins, and the formulations 3P-1W-1T and 3P-2W-1T can be used. Formulation 3P-1W-1T was chosen because it has a lower wax content and this factor could favor the stability of the emulsion, since the proportion of the dispersed phase is lower. This formulation constitutes the control coating.

For the active coatings, the three formulations presented acceptable behavior and stability for their application, considering that the stability study was performed for a time of 180 s (3 min) and that the application of coatings by immersion is not usually performed for a time longer than 3 min. Authors such as Ramirez et al. [62] and Perez et al. [63] applied coatings by immersion to blackberry and mango fruits, respectively, and the application time was 30 s.

Comparing the viscosity values obtained with coatings applied on different products, Rojas et al. [64] developed an edible coating for fruit pieces and used a viscosity formulation of 0.14 Pa·s, a viscosity slightly higher than that of this study, while Castro-Parra [65] reported values of 0.015 and 0.025 Pa·s in the coating of hydroxypropylmethylcellulose-lipids and commercial wax, which are considerably lower than those obtained in the present study. Muñoz [66] developed a coating containing pectin, to which different treatments were applied with a magnetic plate and rod stirrer, and the viscosity ranged between 0.0738 and 0.1641 Pa·s, with the values identified in this study being in that range. Although there is no viscosity value established as the ideal for the development of coatings, the results obtained in this research coincide with the results of formulations that have been previously applied and have shown favorable results, so the formulations developed suggest a positive potential for their application.

In the case of pH analysis, it is important to take this factor into account because it can have a considerable influence on the application of the coating on the food. The main monomer of pectin is galacturonic acid, which is a weak acid due to the presence of the carboxyl group (COOH) in its structure, a group that, when in solution, releases H^+ ions to the medium and lowers the pH. If the functional group is bonded to a methyl ($COOCH_3$) it cannot donate H^+ atoms, thus decreasing its effect on pH [67,68]. Nelson et al. [69] suggest that 1% pectin solutions can give pH values of 2.7 to 3; however, for the control coating, a pH value of 3.78 was presented, which could be an indication of the methylation of the molecule, in addition to the fact that the pectin solution is emulsified with the beeswax. Anthocyanins, although differing considerably in structure from pectin, can also cause a decrease in pH in solution. These molecules have hydroxyl (OH) groups attached to aromatic rings, the conjugated double bonds of the ring can bring their electrons close to the oxygen atom of the hydroxyl, a product of the high electronegativity of oxygen, so that oxygen can release the hydrogen atom to the medium in the form of H^+, which would result in an increase in the hydrogen concentration of the medium and, therefore, a decrease in pH [70].

The low pH of coatings can affect the food at the time of application and can cause alterations in flavor and damage tissues, causing softening, discoloration, or lesions [71].

However, a positive factor to note is that the low pH helps to maintain the stability of anthocyanins and not lose their biological activity because at pH values in the range of 4 to 5, anthocyanin molecules are susceptible to a nucleophilic attack on carbon 2 (C2) by water [72]. However, there are multiple reports on the implementation in food of coatings or pectin-based coatings with pH values similar to that of this research and satisfactory results have been obtained. For example, Quilez-Molina et al. [73] studied the antibrowning effect on cut apples of filmogenic solutions of orange peel pectin at pH 2 and 4.5 and obtained promising results for those at pH 4.5, with the coated side exhibiting reduced browning compared to the uncoated side. In their research, this effect was due to the antioxidant activity of the extracted pectin, so with the addition of anthocyanins, the anti-browning effect would be expected to be greater. However, authors such as Ferrari et al. [74], Oms-Oliu et al. [75], Maftoonazad and Ramaswamy [76], and Moalemiyan et al. [77] have reported a positive effect on the shelf life of minimally processed fruits such as melon, avocado, and mango after the application of pectin-based coatings. However, they do not report the pH of the solutions, but pectin in solution has low pH values, and still the effect on the shelf life of the fruits was positive. It is important to note that it would be convenient to neutralize the pH of the coatings before application to avoid triggering degradative reactions in the coated foods, or another alternative would be their application in foods with acid pH values, similar to that of the active coatings.

With respect to color analysis, there are few reports on coatings with the incorporation of anthocyanins; however, several films have been developed with the addition of these compounds, as is the case of the films developed by Sohany et al. [78], using starch and sweet potato peel. In their study, for an addition of 1% of anthocyanins in the starch-based films, they obtained an L* value of 75.32, a very similar value to that obtained for the coating of 0.1% of the anthocyanins; however, the concentration of anthocyanins differs, but it should be noted that, since the films are a thin and solid material, their brightness should be higher for a coating with the same components. In addition, Sohany et al. [78] observed that the addition of anthocyanins decreased the luminosity of the material, and the same behavior was identified in this research. For the coordinate denoting red to green shades (a*), the effects of the addition and concentration of the anthocyanins increased the reddish shades due to the reddish coloration of this natural pigment, a characteristic behavior of this molecule, and the same behavior was reported by Sohany et al. [78] in their active films when anthocyanins were added. For the b* coordinate, a value very similar to that obtained for the films developed by Sohany et al. [78] (19.09) was obtained for the 0.5% anthocyanin coating whose value was 19.88. It should be taken into account that the values of the CIEL*a*b* scale coordinates obtained will vary if the pH is varied, since anthocyanins are characterized by varying their color as a function of pH, a product of chemical alterations in their chromophores, which is known as the bathochromic effect [72].

In FT-IR, the spectrum of the control coating presents high agreement to the results obtained by Mehraj et al. [54] for a pectin film with xylitol and *Terminalia catappa* Linn leaf wax incorporation. The band corresponding to oxygen–hydrogen (O-H) stretching manifested as a very broad band in the coatings because, in addition to being a functional group of the components, they contain water as the major component of the dispersant phase [79]. The stretching band of the carbon–oxygen double bond (C=O) is seen shifting towards lower energy zones, which may cause the polarization of the bond, causing the electrons to move closer to the oxygen atom so that it may have a higher dipole moment, and the displacement of this band may lead to the appearance of new intermolecular interactions [80]. The null influence on the spectra of the anthocyanin addition is evidenced by the absence of significant differences between the spectra of the control coating and the spectra of the active coatings. This is attributed to the low concentration of this compound

in the formulations, together with the presence of functional groups in the molecule that are also present in other molecules of the formulation, such as pectin, or to the overlapping of bands.

When studying the coatings by SEM, which were transformed into films by casting, it is important to highlight that it was necessary to place the samples in the equipment; however, the heat applied to volatilize the water affects the structure of the emulsion components. When the water evaporates, the dispersing phase is lost, leaving the pectin as the main structure of the material, since it is the polymer that is found in greater proportion. In addition, it can affect the beeswax, melting it again and allowing it to regroup through hydrophobic interactions. Once the drying process was completed, the films lowered their temperature to room temperature, this process being responsible for the wax crystals being visible in the material, resulting in the formation of a cream layer of the oily phase (beeswax), which affected the internal structure and surface of the film [81]. While Tween 80 as a plasticizer can be distributed both in the pectin phase as well as in the wax crystals, it is expected that it would be found in a greater proportion at the interface between them, binding the crystals to the pectin surface. The morphological results confirm that these formulations are best applied as coatings. The appearance of crystalline structures for a similar matrix was reported by Mehraj et al. [54], increasing the heterogeneity as the concentration of vegetable wax increases.

4.2. Antioxidant Activity of Commercial Anthocyanins and Coatings

Commercial anthocyanins presented significant results for their antiradical activity, managing to inhibit 50% of the radicals at low concentrations, which is attributed to the conjugated double bonds and hydroxyl groups present in their structure, which can donate electrons (antioxidant mechanism SET) or hydrogen atoms (antioxidant mechanism HAT) and stabilize the radicals. As for the reducing power, the result obtained is also due to the chemical structure and its ability to donate electrons; this technique only measures the antioxidant activity by the electron transfer mechanism (SET) [82]. The content of phenolic compounds was 386.11 mg GAE/g of sample, a high value, because anthocyanins fall within the group of phenolic compounds, more specifically within the flavonoid group, which justifies the value of 1.26 mg QE/g for flavonoid content. The results of antioxidant activity are attributed to the series of conjugated double bonds and benzene rings of anthocyanins, which can donate an electron and keep the molecule self-stable by resonance (SET antioxidant mechanism). In addition, anthocyanins have several hydroxyl (OH) groups, which are located near double bonds, so that the oxygen atom can donate the hydrogen atom and attract the electrons of the double bond to it, thus also being able to develop HAT mechanism [83].

Shen et al. [84] studied anthocyanin extracts and purified anthocyanins from *Lycium ruthenicum* Murr. and identified DPPH$^{\bullet}$ IC50 values of 724.7 and 458. 6 µg/mL for the anthocyanin extract and purified anthocyanins, respectively, with the IC50 of the commercial anthocyanins in the present investigation being nine times lower than for the extract and almost six times lower than for the purified *L. ruthenicum* Murr. anthocyanins. While comparing the antiradical activity for ABTS$^{\bullet+}$ with the results of Shen et al. [84], the behavior is similar to that of DPPH$^{\bullet}$, only that it was thirteen times lower than the IC50 of the extract of *L. ruthenicum* Murr. and nine times lower than the IC50 of the purified anthocyanins. In spite of attributing the results of both studies to anthocyanins, there is a great difference in the results, which could be due to structural differences; for example, in anthocyanins, the presence of hydroxyl groups in positions 3′ and 4′ of ring B confer greater antioxidant activity, an effect also caused by the free hydroxyl groups in position 3 of ring C and 5 of ring A, on a par with the carbonyl group in position 4 [85].

The reducing power for commercial anthocyanins was higher than that obtained by Li et al. [86] for zein nanoparticles loaded with blueberry anthocyanins, ranging in values obtained for nanoparticles with a zein–anthocyanin ratio of 1:0.3 between 300 and 400 μmol TE/g. While comparing the phenol and flavonoid content with existing reports, Park et al. [87] optimized the extraction process of the bioactive compounds from purple carrot by replicating the optimal conditions obtained phenolic and flavonoid compound values of 15.86 mg GAE/g and 0.91 mg QE/g, values lower than those found in the present study, even though the commercial anthocyanins come from the same plant source.

In the case of the coatings, the presence of anthocyanins causes satisfactory results for the active coatings, while, for the control coating, the highest value was obtained against the $DPPH^{\bullet}$ radical due to the fact that pectin can donate hydrogen atoms as a result of the high composition of D-galacturonic acid in its structure, stabilizing the free radicals through the antioxidant mechanism of hydrogen atom transfer (HAT). The DPPH and ABTS techniques are each capable of measuring the HAT antioxidant mechanism, as is SET; however, the DPPH technique has a preference for the HAT mechanism [83], justifying the better result by this technique. Zhang et al. [88] demonstrated that several pectin fractions evidenced antioxidant activity for the elimination of $DPPH^{\bullet}$ radicals, while Wang et al. [89] evaluated the antioxidant activity of crude citrus pectin and obtained a greater inhibition of the $DPPH^{\bullet}$ radical compared to the $ABTS^{\bullet+}$ radical. The results of this research are in agreement with the results of this investigation because the main activity is attributed to pectin, although beeswax may contain antioxidant compounds such as flavonoids, phenols, and terpenes [90], which are capable of scavenging radicals by both HAT and SET mechanisms. The antioxidant capacity of the coatings is mainly affected by the weak interactions between the components, mainly hydrogen bridges and electrostatic forces, altering the charges of the molecules and influencing their ability to donate or accept electrons.

4.3. Antifungal Activity of Commercial Anthocyanins and Coatings

The selection of the fungal genus used, *Colletotrichum siamense*, was due to the fact that this fungus is prevalent in multiple fruits such as mango, banana, and papaya, and is responsible for the disease known as anthracnose, a deteriorative and phytopathogenic species [91]. For commercial anthocyanins, the lowest radial growth corresponded to the concentration of 0.25% anthocyanins and the highest for the concentration of 1.0% anthocyanins, which could be the result of the adaptation of the fungus to higher concentrations, the same behavior identified by Márquez et al. [92] for *Fusarium oxysporum*. Above a certain concentration value, the fungus adapts more easily to environmental factors and can grow more rapidly [92]. For the inhibition percentages, the result of 100% inhibition during the first 24 h is attributed to the fact that during this period, the fungus was adapting to the conditions of the medium. The variability in inhibition for each concentration is mainly attributed to the phenotypic plasticity of adaptation to the factors that cause stress and to the intrinsic defense mechanisms resulting from the adaptation process itself [93].

In morphology, the increase in inclusion bodies in anthocyanin treatments may be due to the expression of resistance genes of the species and, although cell lysis is not ob-served, it is observed that not all hyphae develop straight but fold as a result of the stress caused, with greater twinning and irregularities in the thickness of the hyphae [94,95]. The greatest spore length was observed for the control fungus because it was not subjected to stress-inducing factors, having a less marked influence on spore width.

Anthocyanins, as a flavonoid compound, manifest their antifungal effects by binding to proteins and precipitating them, which can associate with nucleophilic amino acids of other proteins and trigger a protein inactivation sequence, stopping or slowing growth [96,97].

Solís-Silva et al. [98] inhibited *Colletotrichum gloesporoides* by 19.52% with a xoconostle extract rich in flavonoid compounds and phenolic acids, results similar to those of this research.

By analyzing the results of the antifungal activity of the coatings, it can be affirmed that anthocyanins constitute the compound responsible for the antifungal activity of the coatings. The results of the active coatings are consistent with the results previously analyzed for the different concentrations of anthocyanins, where the 0.25% concentration solution showed the greatest effect on growth. However, at 168 h, the active solution with this same value of anthocyanin addition presented an inhibition 3% lower than that reported for the anthocyanin solution, attributing the difference to the fact that the fungus could be using pectin as a nutrient, as a source of carbon and energy, or that an antagonistic effect of the mixture of compounds has occurred. Variations in fungal morphology and spore dimensions between coatings are attributed to the addition of anthocyanins to the formulations, which evidenced a marked effect on fungal growth and maturation.

4.4. Coating Manufacturing Process: Advantages, Limitations and Potential Applications

The process of elaboration of the coatings in this study is a simple process comprising a few steps (Figure 1) in addition to being easily replicable and using accessible and biodegradable components. It is very important to consider the emulsification process of the beeswax as the most critical step for the development of the material, and that, as evidenced by the SEM results, its application form must be as a coating as a film-forming solution, due to the influence of temperature on the internal structure of the material, making it impossible for it to dry to be applied as a solid film. However, in spite of presenting good properties, color and pH are the main limitations of this study, as mentioned above. The study of the emulsion stability is another aspect that would provide relevant information about the material, helping to define how long the coatings can be kept before their application, although the ideal would be recently developed to avoid degradation of the anthocyanins.

The material developed is mainly aimed at fruit and vegetable products, being a viable alternative for its study in minimally processed products. Although it could be studied as a material for any type of food susceptible to fungal and oxidative deterioration, favorable results are expected in the quality, safety, and shelf life of these products. The ingestion of food with this material could even have a favorable impact on the consumer's health because it has components such as pectin and anthocyanins.

5. Conclusions

Active coatings based on pectin and beeswax were successfully formulated using Tween 80 as a plasticizer and the commercial anthocyanins as the active compounds, with the formulation of 3% pectin, 1% beeswax, and 1% Tween 80 being the one that presented better properties and was used to add the anthocyanins. It was possible to identify a stable rheological behavior and the appearance of new interactions resulting from the mixture of compounds. However, the reddish tones are intense, and the morphological images show that the components separate when the water evaporates; in addition, the pH is lower than the normal pH of most foods. Despite this, the antioxidant and antifungal activities give it the desired active character, and the pH, which is the most critical factor for its use, can be neutralized, thus preventing it from affecting the coated product. Therefore, the coating based on pectin/beeswax and added to anthocyanins can be applied in the food industry to maintain food safety and improve product quality for a longer time in the market.

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