Edible Coatings for Strawberry Based on Extracellular Compounds of *Humphreya coffeata*

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Edible coatings (EC) are a biodegradable alternative for food preservation. In this work EC based on extracellular compounds of *Humphreya coffeata* and pectin (PHC) were elaborated and characterized through testing on strawberries. Two PHC types were obtained (PHC1, which settled, and PHC2, which floated during the first separation). The conservation period of the strawberry and the phenol content and antioxidant activity in strawberries with the EC were determined. Four EC were prepared (EC1, EC2, EC3, and EC4), in which EC1 did not contain any PHC, EC2 contained PHC1, EC3 contained PHC2, and EC4 contained both PHCs. Three EC types (EC2, EC3, and EC4) delayed the decay of strawberries approximately 90% at 20 °C during the first 10 days of evaluation, and at 4 °C the effect was between 75 and 90% after 25 days of experimentation. However, EC3 and EC4, both of which contained PHC2, presented the best results. The content of total phenols (approximately 55 mg (gallic acid equivalent (GAE)/mL) and the free radical scavenging activity were 35% with ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and 75% with DPPH (2,2-diphenyl-1-picrylhydrazyl) in strawberries with EC. Thus, EC are an alternative for preserving strawberries without losing their antioxidant capacity.

Keywords: Edible coating; Strawberry; Shelf-life; Extracellular compounds

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INTRODUCTION

The postharvest technology of fruits and vegetables has advanced a lot in conservation issues, seeking to promote the fresh consumption of these products. Fruits are an important source of nutrients; however, it has been reported that approximately 45% of fruits and vegetables are wasted due to deterioration caused by physical-chemical and microbiological damage (Snyder and Worobo 2018). In order to extend the shelf life of fruits, plastic packaging made from polymers has been used widely and been successful, due to its versatility, performance, and cost (Valdés *et al*. 2015). However, these polymers are not biodegradable, and they represent a major pollution problem. For this reason, packaging alternatives are being sought to reduce the amount of plastic waste. During the last decade, interest has increased in the development of edible coatings (EC) derived from biopolymers, which are raw materials that are environmentally friendly, available, and

easily degradable, in addition they are metabolized by the human body together with food (Valdés *et al*. 2015).

It is important to mention that the strawberry is one of the most important soft fruits in the world. The main strawberry producing countries are: China (38.6%), USA (16.9%), Mexico (5.6%), Turkey (4.6%), Spain (3.6%), and Egypt (3.5%) (Sharma *et al*. 2019). Strawberries have an active metabolism, are perishable fruits, and have high postharvest physiological activity. Thus, they have short periods of shelf life and senescence; they can remain viable for seven to eight days under refrigeration conditions and only one to two days in ambient conditions, due to high-water content (90%), texture, and the high level of respiration, in addition to the ease of microbial contamination (Samadi *et al*. 2016). Due to this, several strategies have been developed to prolong the postharvest life of strawberries, such as: refrigeration, chemical fungicides, modified atmosphere packaging, osmotic treatments, hypobaric treatments, heat treatments, and EC (Petriccione *et al*. 2015). Kumar *et al.* (2020) reported the use of an EC of chitosan-pullulan (50:50) and pomegranate peel extract in litchi (*Litchi chinensis* Sonn.). The browning (color) and the loss of physiological weight, of total soluble solids, of acidity were reduced. The content of phenols, flavonoids and antioxidant activity were controlled by the presence of EC during storage for nine days (4 °C and 23 °C) compared to the control batch (litchi without EC). The EC in tomato (*Solanum lycopersicum*) was also evaluated and postharvest quality was improved (lower weight loss, maintained total soluble solids, titratable acidity, color, pH and greater retention of phenolic content, flavonoid content, and antioxidant activity were observed) and sensory characteristics during storage (18 days) at 4 and 23 °C (Kumar *et al*. 2021).

It has been reported that semipermeable edible coatings are functional in strawberry, due to the reduction of humidity, gas exchange, respiration, oxidative reaction, and retention of firmness, most of the EC that present these characteristics have been elaborated with chitosan that has antibacterial and antifungal properties. It has been reported that chitosan-based EC have good mechanical strength and antimicrobial activity and that EC also controls the loss of nutritive compounds (Kumar 2019). Chitosan is a naturally occurring non-toxic biopolymer derived from the deacetylation of chitin that can be obtained from crustaceans and some fungi (Shiekh *et al*. 2013). In contrast, fungi are an important source of obtaining molecules that can be used in the food industry; such compounds can be low molecular weight (terpenoids, polyketides, and alkaloids) and/or complex high molecular weight compounds such as proteins, lipids, and polysaccharides (De Silva *et al.* 2013). Several studies reported that polysaccharides are one of the main bioactive compounds in different types of edible and medicinal mushrooms that have antiviral, antibacterial, and antifungal activity both *in vitro* and *in vivo*, which depending on their molecular weight, composition, branching, *etc.*, their effect can vary (Khan *et al*. 2014). However, until now, this type of molecules obtained from fungi have not been used in the preparation of EC. Therefore, it is important to explore sources for obtaining polysaccharides. *Humphreya coffeata* (Berk.) Steyaert is a medicinal mushroom (Porras-Arboleda *et al*. 2009) and is part of the Polyporaceae family, which includes several species of fungi that produce industrially important polysaccharides and are applied in different biotechnological areas, due to their biodegradable, non-toxic, and biocompatible nature (Ullah *et al*. 2019). This fungus can produce exopolysaccharides in liquid culture. The polysaccharides of *Humphreya coffeata* have been quantified but have not yet been identified; however, glucans are the polysaccharides most produced by fungi. Depending on biotic and abiotic factors, differences in their composition may occur including glucose, mannose, ribose, fucose, among other carbohydrates (De Silva *et al*. 2013; Khan *et al*.

2014). Therefore, the objective of this work was to elaborate an EC with extracellular compounds from the *Humphreya coffeata* fungus and pectin to evaluate its application in fresh strawberries.

EXPERIMENTAL

EC Films Preparation

Extracellular compounds of *Humphreya coffeata* (PHC) from *Humphreya coffeata* (Berk.) Steyaert were obtained by submerged fermentation. This strain (HEMIM-140) was obtained from the Mycological Herbarium, from the Autonomous University of the State of Morelos, Morelos, Mexico. *Humphreya coffeata* were separated in two fractions (PHC1 and PHC2). The culture medium contained (g/L): 0.5 $MgSO_4$; 1.0 KH_2PO_4-H_2O; 5.0 peptone; 50 lactose; and 10 yeast extract (Porras-Arboleda *et al*. 2009). *Humphreya coffeata* was grown in a fermentation process, which was carried out in 125-mL Erlenmeyer flasks with 50 mL of medium at pH 5.0 and incubated at 30 °C with orbital shaking at 130 rpm. The production of PHC was observed from 4 days of culture, but the highest production was at 14 days. The biomass was removed by filtration and to separate PHC1 and PHC2, 95% ethanol were added to the culture broth (1:4 v/v), and the mixture was kept at 4 °C for 48 h. After that, it was centrifuged at 7800 x g for 20 min (Supramani *et al*. 2019). The PHC1 settled and showed a gelatinous consistency and the PHC2 remained on the surface of the supernatant and was sticky. The PHC1 and PHC2 were used in the form in which they were obtained for the preparation of the EC. Firstly, solutions were made in water (w/v): PHC1 15 g/10 mL and PHC2 1.5 g/15 mL, which were sonicated (3 min at 20 kHz; Fisherbrand™ Q500 Sonicator with Probe; Thermo Fisher Scientific, Waltham, MA, USA) and stirred (20 min at 1600 rpm; Thermo Scientific™ LP Vortex Mixer, Thermo Fisher Scientific, Waltham, MA, USA) until a homogeneous mixture was obtained. Subsequently, the EC films were made with water (Table 1). Each of the mixtures were placed in Petri dishes (60 x 15 mm), dried in a forced convention oven at 60 °C for 24 h, and then removed from the Petri dish. Subsequent measurements were made in triplicate.

Table 1. Formulation of EC

EC	PHC1 Solution (%)	PHC2 Solution (%)	Pectin (%)	Glycerol (%)
EC1	-	-	0.7	0.5
EC2	21	-	0.7	0.5
EC3	-	49	0.7	0.5
EC4	21	49	0.7	0.5

Characterization of EC Films

Thickness measurement

Thickness was measured instrumentally with the help of a Mitutoyo digital micrometer IP65 (MDC-1 "MJ"; Mitutoyo Corporation, Takatsu-ku, Kawasaki, Kanagawa Prefecture, Japan), with a permissible error of ± 0.00005, and at least six measurements were made in different positions of the EC to verify that the average thickness did not exceed 1 mm thick, as marked in ASTM D882-02 (2002).

Evaluation of EC solubility

The evaluation of EC solubility was made according to the ASTM D570-98 (2010) standard. Samples of 2 x 2 cm in dimension were cut and their weight was recorded. Each one of them was deposited in 30 mL of water for 24 h. Subsequently, the water was removed, and they were placed in an oven at 100 °C for 24 h, and the EC pieces were weighed to determine the non-solubilized weight. The percentage of solubility by weight difference was calculated.

Water vapor permeability (WVP)

The water vapor permeability was carried out according to the ASTM E96-00 (2000) standard. A circular section of 8 mm in diameter was cut from each EC and the thickness was measured with the help of the MDC-1 "MJ". The samples were placed in 1 mL of supersaturated solution of potassium nitrate (KNO_3) to generate a constant relative humidity. The samples were placed in a desiccator with silica gel and moisture indicator. The variation in weight was recorded every hour on analytical balance PX224 (OHAUS, Latin America, Mexico) for 8 h.

Scanning electron microscopy (SEM)

Scanning electron micrographs were obtained with a JEOL scanning electron microscope (JSM-6010; Nanosurf AG, Liestal, Switzerland), located at the Zacatepec Institute Technological (Morelos, Mexico) at an acceleration voltage of 3 kV. The film surfaces were examined without the metallic coating and observed at different magnifications.

Characterization of EC in Strawberry

Strawberry preparation and storage conditions

Fresh strawberries (*Fragaria ananassa*) were obtained from the "Hortalizas finas" orchard located in Cuernavaca, Morelos, Mexico. The strawberries were used in the experiment on the same day to avoid any deterioration, and they were selected based on their uniform size, weight, color, and absence of physical or pathological damage before being placed on a grid. The strawberries were disinfected by immersing them in a sodium hypochlorite solution (2%) for 2 min, then they were washed and kept in a clean area until dry. The strawberries were dipped in each of the EC (1 through 4, prepared as indicated earlier) for 1 min to ensure a uniform coating, and then they were dried in the open air. All treatments were kept at 20 ± 2 °C and 4 °C, including the uncoated one (negative control; UEC). Its shelf life was analyzed for 25 days, and some parameters were analyzed every 5 days. The experiment was carried out in triplicate.

Strawberry decay percentage

The number of rotten fruits (loss of firmness and weight) due to an infection by fungi or any microorganism was recorded in all treatments at intervals of 5 days and was reported in percentage, calculated as the number of rotten fruits divided by the initial number of all fruits multiplied by 100 (Gol *et al*. 2013).

Weight loss percentage

Strawberries were weighed at the beginning of the experiment (0 days) and every 5 days until the 25^{th} day of treatment. Total weight loss was expressed as a percentage

according to the standard method of Association of Official Analytical Chemists (AOAC 1994).

Obtaining Strawberry Extracts After Treatment With EC

To conduct the subsequent tests, strawberries were obtained every 5 days of each one of the treatments stored at both temperatures tested, the strawberries were weighed and water was added to macerate them in proportion (w/v, 1:1), and the strawberry extracts (SE) obtained were filtered and stored in Eppendorf tubes for later analysis.

Measurement of pH, soluble solids of SE, and maturity index

The pH of each of the samples was measured with a potentiometer (Sartorius PP-50, Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany) previously calibrated with buffer solutions of pH 4, 7, and 10. Soluble solids (SS) were measured with a Milwaukee MA871 refractometer, Milwaukee Instruments, Rocky Mount, NC, USA). The maturity index was calculated as the quotient of soluble solids and acidity.

Content of total phenols

To determine the content of total phenolic compounds, the Folin-Ciocalteau reagent was used (Singleton and Rossi 1965). Two mg of each fungus extract were dissolved in 1 mL of 4 % methanol and mixed with 1 mL of Folin-Ciocalteu reagent. After 3 min, 1 mL of sodium carbonate solution (20%) was added, and the mixture was adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min and then the absorbance was read at 725 nm. Gallic acid was used as standard for the calibration curve (0.02 to 0.14 mg/mL). The result of the concentration of total phenols was reported in mg GAE (gallic acid equivalents) per mL sample.

The ABTS and DPPH assays for the assessment of the antioxidant activity

The ABTS radical scavenging activity was evaluated according to the methodology previously reported (Re *et al.* 1999; González-Palma *et al*. 2016). The ABTS radical cation ($ABTS^{\bullet+}$) (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) was obtained after the reaction of ABTS (7 mM) with potassium persulfate (2.6 mM final concentration), the mixture was kept in the dark at room temperature for 12 to 16 h, and after that, the $ABTS^{\bullet+}$ solution was diluted until obtaining an absorbance of 0.700 ± 0.2 (734 nm). The reaction mixture (0.07 mL of each extract and 3 mL of the ABTS radical) was kept in the dark for 6 min. The decrease in absorbance (734 nm) was expressed as the inhibition percentage of $ABTS^{\bullet+}$.

The DPPH radical scavenging activity was determined by measuring the decrease in absorbance (517 nm) of the reaction mixture after it had been incubated for 45 min in darkness and was expressed as the inhibition percentage of DPPH radical. The reaction mixture contained 0.5 mL of extract, 3 mL of methanol, and 0.3 mL of 0.5 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution in methanol (Moraes-de-Souza *et al*. 2008; González-Palma *et al*. 2016).

Statistical analysis

Each experiment was performed three times, and the data were expressed as mean ± standard deviation (SD). Statistical analyses were performed with Sigma Plot software (version 10.0; Systat Software Inc., Chicago, IL, USA). The level of statistical significance was established at 0.05.

RESULTS AND DISCUSSION

Characterization of EC

The EC were easily separated from the casting container. They were visually transparent, homogeneous, thin, flexible, easy to handle, and no fractures were observed. The visual properties of the EC are important for the acceptance of the product by consumers; the EC1 did not present coloration (Fig. 1a), the other three presented a slightly yellow appearance (Figs. 1b, 1c, and 1d), but there was no modification of the appearance of the strawberries.

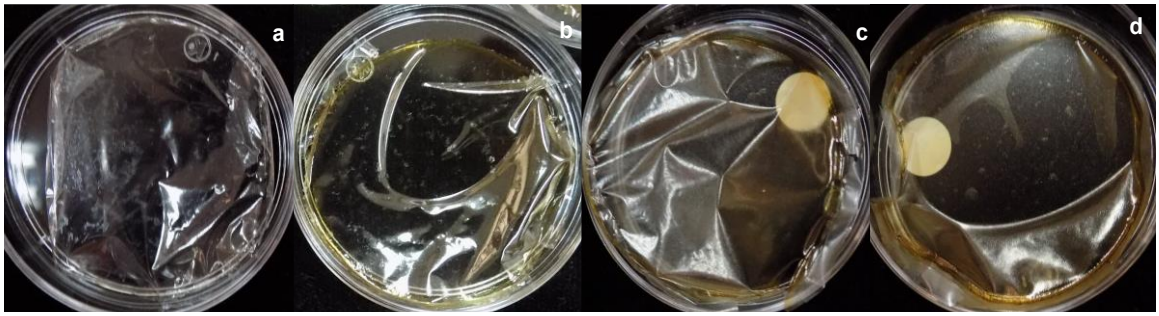

Fig. 1. Appearance of the EC: EC1 (a), EC2 (b), EC3 (c), and EC4 (d)

Pectin is vitreous at room temperature, so after evaporation of the water or drying, defects such as cracks or curling can occur in the EC. These are usually brittle and rigid due to the extensive interactions between the polymer molecules; therefore the addition of plasticizers is required, which reduce the intermolecular forces between the polymer chains, increasing their flexibility and extensibility (Šešlija *et al*. 2018). It is worth mentioning that the EC of this work did not show any apparent cracks.

The thickness of the EC is important for maintaining the properties of the fruit to which it is applied. In general, the four EC of this study were thin; the one with the smallest thickness was the EC1 that contained pectin, and the one with the greatest thickness was EC4 that presents PHC1 and PHC2 from *Humphreya coffeata*. The insignificant ($p < 0.005$, Table 2) variation in thickness between the EC was because the same amount of solution forming each of the EC was added in Petri dishes, which coincides with the work of Fathi *et al*. (2018). However, the statistical differences were due to the presence of PHC in dry EC (Table 2). The EC containing PHC2 were the ones that presented lower permeability, compared to EC2 containing PHC1, which indicated that PHC2 is less soluble in water, which is why EC3 and EC4 presented lower solubility than EC1 and EC2 (Table 2). However, in general the solubility of all EC was high, which could be due to the presence of pectin, which is made up of molecules that have a great interaction with water and also by glycerol (plasticizer) that causes modification and reduction of molecular attractive forces in the EC matrix, decreasing rigidity and increasing the free volume of the system, which allowed a greater interaction with water molecules and increased solubilization (Jouki *et al*. 2013). However, it improves the flexibility of the EC by reducing the number of internal hydrogen bonds between the polymer chains and by increasing the free volume in the matrix to allow the diffusion of oxygen and water vapor through the coating film (Valdés *et al*. 2015). The solubility of the EC is not restrictive of functionality, because it depends on its application, and in some cases the high solubility of the EC is required when

having contact with water during the preparation of the packaged food (Gontard *et al*. 1992).

Table 2. Parameters of the EC

Treatments	Thickness (mm)	WVP (g m^{-1} s^{-1} Pa^{-1})	Solubility (%)
EC1	0.03 ± 0.0002^{c}	6.1 x 10^{-4} ± 6.2 x 10^{-05} ^{b}	100 ± 0^{a}
EC2	0.04 ± 0.0003^{b}	5.9 x 10^{-4} ± 6.1 x 10^{-05} ^{b}	100 ± 0^{a}
EC3	0.045 ± 0.0007^{a}	4.1 x 10^{-4} ± 8.6 x 10^{-05} ^{ab}	98.9 ± 0.2^{b}
EC4	0.05 ± 0.0003^{a}	3.8 x 10^{-4} ± 7.9 x 10^{-05} ^{a}	98.3 ± 0.1^{b}

Values are mean of three replicates ± SD; Means with different letters in the same line are significantly different ($p < 0.005$) by Tukey's multiple range test

SEM Morphology

The EC images are shown in Fig. 2. The EC1 exhibited a homogeneous surface without the presence of pores (Fig. 2a); however the other EC (Fig. 2b, 2c, and 2d) showed a rough surface with the presence of some particles (heterogeneous matrix); without evident presence of pores and/or bubbles, this is similar to that reported by Ye *et al*. (2019) in EC of pectin and cellulose. It has also been reported that glycerol helps in the organization of the crystalline structure of polymers in the film-forming matrix (Maniglia *et al*. 2017). Lorevice *et al*. (2016) observed through images some imperfections in the EC of pectin with a high degree of methyl esterification (HDM). The imperfections included pores and fissures that could confer weak mechanical properties, as well as present lower interactions between the pectin chains of HDM. In the EC with a low degree of methyl esterification, some cracks were observed, which may be due to stronger interactions (higher content of carboxylic groups). These imperfections were not detected when analyzing the surface of the EC of the current work. In EC1 a more homogeneous structure was observed. Compared to the other EC, probably the PHC were congregated during the drying process. It has been reported by several authors that during the drying of the EC there may be the presence of agglomerates of nanoparticles, which can result from solubility changes when working with ethanolic extracts or different particle sizes (Villasante *et al*. 2020), but despite presenting a heterogeneous matrix there may be antifungal activity (Pastor *et al*. 2010).

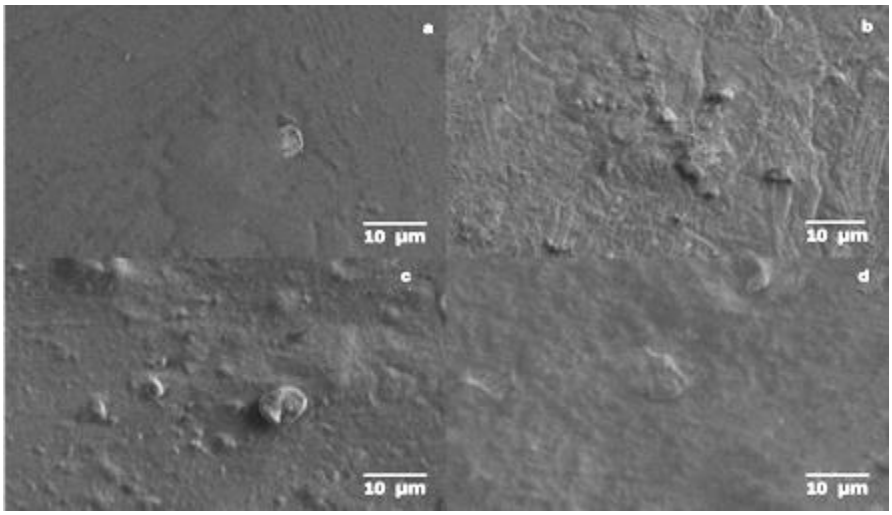

Fig. 2. SEM images of EC: EC1 (a), EC2 (b), EC3 (c), and EC4 (d)

Strawberry Decay Percentage

Strawberries are highly perishable; their maturity stage is rapid due to their high respiration and softening rate as well as the appearance of microorganisms (bacteria and fungi) (Nadim *et al*. 2015). Figure 3 shows that the percentage of decomposition of the strawberry increased with storage time. In general the presence of EC reduced the decomposition rate during storage at both temperatures evaluated. The EC containing PHC remained longer without the presence of microbial contamination, 20% of the strawberries stored at 20 °C showed decay at 10 days, while the strawberries stored at 4 °C showed the same percentage of decay at 20 days. For this reason, the PHC were shown to have a protective effect, delaying surface microbial growth and making them a great alternative for the conservation of strawberries.

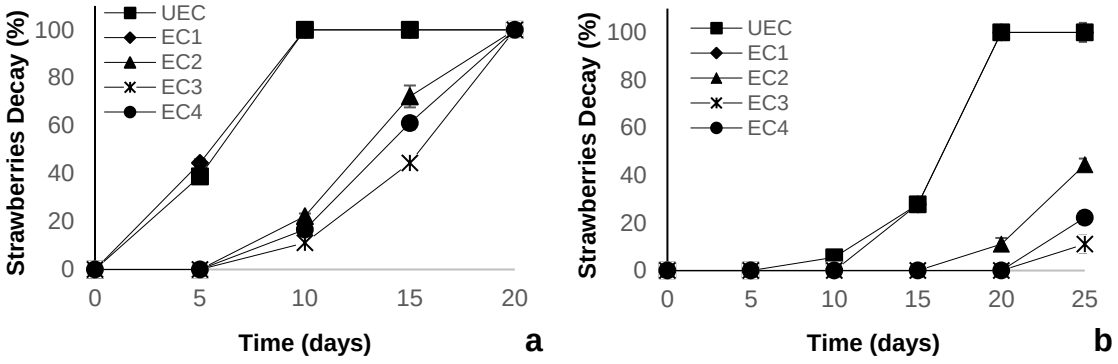

Fig. 3. Percentage of decay of strawberries as a function of storage time at 20 °C (a) and 4 °C (b)

The shelf life of strawberries without EC is usually around 2 weeks in cold storage (4 °C) and additionally 3 to 4 more days at room temperature (approximately 20 °C) (Romanazzi 2009). Edible coatings are a great alternative to extend the shelf life of strawberries. The difference in the shelf life of the fruits due to storage temperatures is influenced by the presence of microorganisms, among which are the genus *Rhizopus*, which grows rapidly in ripe or almost ripe fruits and their growth stops at low temperatures (below 5 °C) (Sommer *et al*. 1992) and the genus *Cladosporium* (a pathogen of senescent fruits), which can grow at fridge temperature (Jay 2000). Therefore, in this study at 4 °C the microbial contamination of strawberries was slower.

Bermúdez-Oria *et al*. (2017) reported that strawberries with EC based on pectin and fish skin protein presented less mold growth compared to strawberries without EC after 157 h of storage, which showed that these EC can protect strawberries from microbial attack and prolong shelf life; the combination of pectin with other polymers could improve the performance of the materials used in different fruits and vegetables. Ventura-Aguilar *et al*. (2018) reported that the application of a chitosan-based EC showed effect on the growth of *Colletotrichum fragariae*, because the fungus delayed its growth until the third day in strawberries stored at 20 °C and after 10 days at 5 °C symptoms of the pathogen were observed. It is known that the conidia of this fungus germinate between 12 and 24 h after inoculation (Curry *et al*. 2002). Wang and Gao (2013) observed that there was a reduction in the decomposition of strawberries during 12 days of storage at 5 °C. The fruit of the control group reached 80.1% decay and the fruits coated with chitosan (0.5, 1.0, and 1.5 mg/100 mL) presented 55.1%, 21.7%, and 0.0% of decay, respectively. Martínez *et al*. (2018) reported that EC of chitosan and thyme essential oil reduced the decomposition of

strawberries by up to 39% compared to those without EC. Chitosan has been shown to retard microbial growth, and the effect increased with the presence of the essential oil. It is suggested that sporulation and the germination of the few fungal spores were inhibited. In this work, the percentage of decay was also reduced 40% at 20 °C and 30% at 4 °C with the EC with PHC, so that the extracellular compounds of *Humphreya coffeata* could have some effect on the growth, sporulation, and germination of fungi. In this work, EC had a protective effect that can be an alternative for the preservation of fresh fruits.

Weight Loss Percentage

Weight loss is a key sensory characteristic to assess quality and is related to the transpiration and respiration of fruits (Lufu *et al*. 2019). Strawberries are susceptible to water loss, which causes contraction and weakening of the fruit tissue due to their very thin skin (Velickova *et al*. 2013). In this study, weight loss increased during storage at both temperatures (Table 3); however, it was higher at 20 °C, because several strawberries presented microbial contamination. When comparing the treatments, strawberries with the EC3 and EC4 lost approximately 50% of their weight at 25 days at 20 °C, and at a temperature of 4 °C the strawberries lost approximately 32% of their weight (no significant difference between the two EC in the two temperatures evaluated, $p < 0.005$) at 25 days. EC1 and EC2 lost weight much earlier than EC2 and EC3 (Table 3).

Table 3. Weight Loss Percentage of Strawberries

20 °C					
Treatments	5 Days	10 Days	15 Days	20 Days	25 Days
UEC	96.1 ± 6.7^{a}	-	-	-	-
EC1	35.93 ± 2.3^{b}	-	-	-	-
EC2	4.72 ± 0.4^{c}	9.21 ± 0.9^{a}	44.73 ± 8.3^{a}	-	-
EC3	4.56 ± 0^{c}	9.32 ± 0.75^{a}	14.21 ± 0.95^{b}	36.48 ± 1.1^{a}	50 ± 5.8^{a}
EC4	4.47 ± 0.22^{c}	9.17 ± 1.19^{a}	13.23 ± 0.83^{b}	33.18 ± 2.2^{b}	49 ± 4.13^{a}
4 ºC					
Treatments	5 Days	10 Days	15 Days	20 Days	25 Days
UEC	12.56 ± 1.5^{a}	22.3 ± 1.53^{a}	45.1 ± 1.64^{a}	-	-
EC1	4.94 ± 1.45^{b}	9.4 ± 1.62^{bc}	38.9 ± 6.62^{b}	-	-
EC2	2.74 ± 0.81^{bc}	7.97 ± 0.8^{c}	12.32 ± 0.7^{c}	15.88 ± 0.08^{a}	58.18 ± 2.87^{a}
EC3	1.81 ± 1.71^{c}	7.74 ± 1.51^{c}	11.28 ± 2.06^{c}	14.98 ± 3.72^{a}	31.01 ± 2.47^{b}
EC4	2.42 ± 1.41^{c}	11.26 ± 2.59^{b}	14.21 ± 3.4^{c}	17.12 ± 5.6^{a}	31.12 ± 5.48^{b}

Values are mean of three replicates ± SD; Means with different letters in the same line are significantly different ($p < 0.005$) by Tukey's multiple range test.

It is known that migration from water to the environment is the main cause of fruit weight loss during storage (Duan *et al*. 2011). The EC act as an external layer that covers the stomata, causing a decrease in perspiration. In addition, this also depends on the resistance of the fruit skin to the diffusion of steam and the steam pressure between the fruit tissues and the surrounding air, which is influenced by temperature and relative humidity (Kader 1992). Low temperatures help decrease weight loss. In this work, a reduction in weight loss was observed due to the presence and composition of EC, which serve as a semipermeable barrier against oxygen, carbon dioxide, and humidity, thus reducing respiration, water loss, and oxidation reactions. Such an effect has been reported in other studies (Maqbool *et al*. 2011; Gol *et al*. 2013; Guerreiro *et al*. 2015). Martínez-González *et al*. (2020) reported the effect in strawberries of two EC, one based on chitosan

and the other based on chitosan and propolis extract. The strawberries with the EC based on chitosan lost weight during the eight days of storage at 4 °C, but the strawberries with less weight loss were those with 10, 30, and 20% of propolis added to the EC (9.7, 10.2, and 11.0%, respectively). Additionally, the strawberries with the greatest weight loss (14.9%) were those that did not have EC, which coincides with the results of this study being 10 days in strawberries with EC based on PHC stored at 4 °C and 20 °C.

Soluble Solids (SS), pH, and Maturity Index

Among the main quality parameters of the fruits are the SS, pH, and maturity index. In general, the pH of strawberries increased from 3.4 to 4.0 during the storage period (Fig. 4), at both storage temperatures. Strawberry is classified as an acidic fruit because it has pH values that vary between 3.2 and 3.4 (Charalambous 1986). It has been reported by several authors that the increase in pH can be attributed to the decrease in available organic acids that are used as energy during the ripening process of the fruit, so that some components of the EC have an effect on metabolic activity and the senescence of the fruit (Perdones *et al*. 2012; Martínez *et al*. 2018).

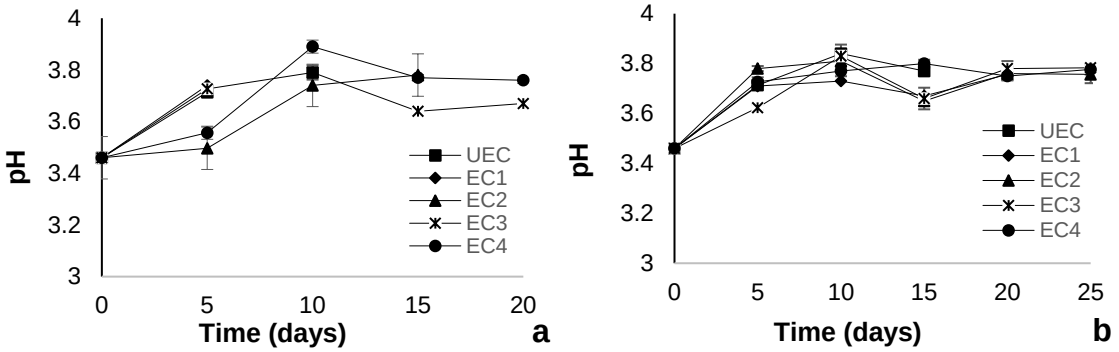

Fig. 4. Evolution of pH in strawberries with EC at 20 °C (a) and 4 °C (b)

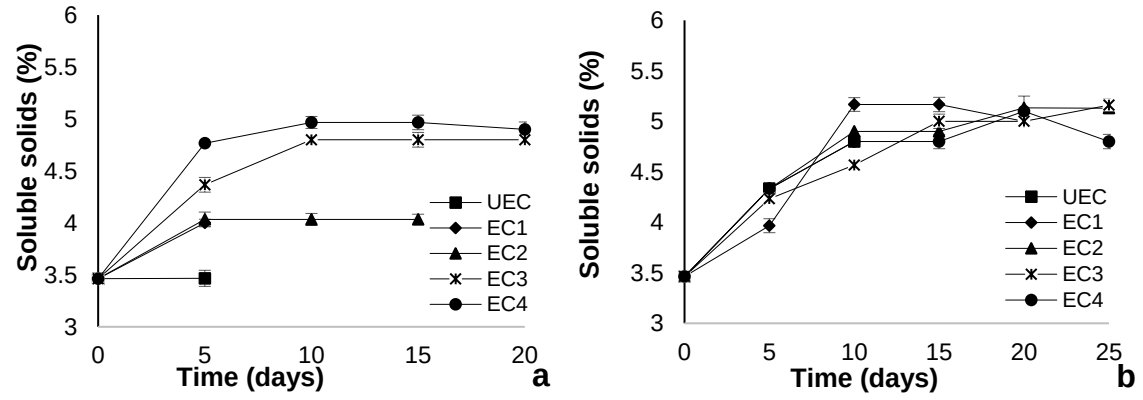

Fig. 5. Evolution of SS in strawberries with EC at 20 °C (a) and 4 °C (b)

The SS have an effect on the flavor and nutrients; the decomposition of these compounds leads to a loss of postharvest strawberry quality in storage (Zhang and Quantick 1997). The SS content of strawberries with EC1 and EC2 stored at 20 °C increased and stopped increasing after five days, while in strawberries with EC3 and EC4 they reached higher SS values at 10 days and subsequently remained stable (Fig. 5a). In contrast, in the strawberries stored at 4 °C, the increase in SS content was up to 10 days and in strawberries

with EC with the addition of PHC it was maintained until 25 days (Fig. 5b). The decrease in SS in strawberries during storage is due to the decrease in respiration and metabolic activity, thus delaying the ripening process, as it has been reported in several works that by using EC the SS content is maintained for more days of storage (Gol and Rao 2011; Velickova *et al*. 2013; Ali *et al*. 2015).

The maturity index is used in the industry to determine the internal quality of fruits (Ménager *et al*. 2004). During the storage time, in the strawberries there was an increase in the maturity index in both temperatures (Fig. 6), with a maximum of 14.05% in strawberries with EC2 in 20 days. According to reports, the sugar content in strawberries increases during fruit ripening (Salamat *et al*. 2013) and depends on the variety of strawberry and abiotic factors. Through using EC, the aging process of the fruit is delayed. Martínez *et al*. (2018) reported a delay in maturity, because the index was around 11.6% in strawberries stored at 5 °C for 15 days with EC of chitosan and thyme oil. In another work EC of chitosan and lemon oil was used, where the increase in the maturity index was reported during the days of storage at 4 °C, with a maximum of approximately 12% (Perdones *et al*. 2012). Noting that essential oils can have an effect on the senescence of the fruit by helping in the conservation of fruits, it is worth mentioning that in this work this effect was presented by the PHC, since it has been reported antiviral, antibacterial and antifungal effect of some extracellular compounds such as polysaccharides of polyporales fungi (De Silva *et al.* 2013; Khan *et al.* 2014) including *Humphreya coffeata*.

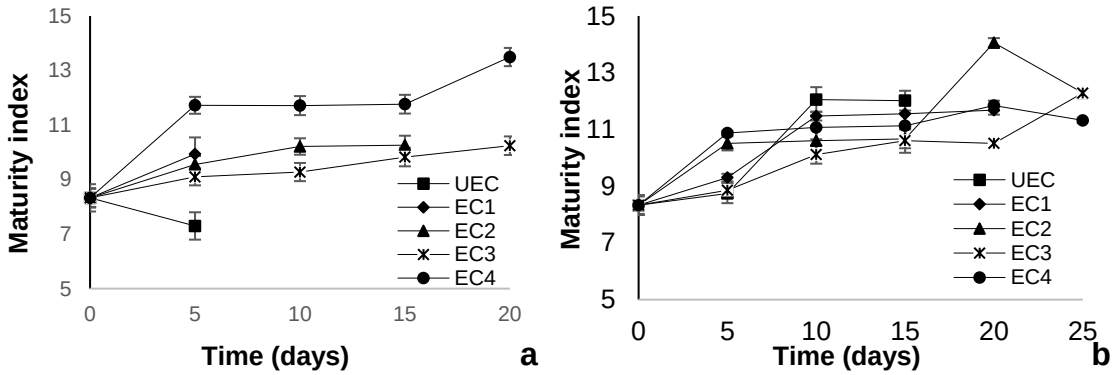

Fig. 6. Evolution of the maturity index in strawberries with EC at 20 °C (a) and 4 °C (b)

Phenol Content and Antioxidant Activity

The strawberry is a non-climacteric fruit that is highly appreciated due to its organoleptic characteristics, in addition to being a source of vitamin C, vitamin E, carotene, and phenolic compounds (Van De Velde *et al*. 2013). Anthocyanins are responsible for the red color of strawberries, and quantitatively they are the most important phenolic compounds (da Silva *et al*. 2007) because these compounds have a beneficial effect on consumer health (Van De Velde *et al*. 2013). The initial total phenol content of the strawberry was 87 mg GAE/mL and it decreased during storage (Fig. 7a and 7b). In general, the loss in strawberries with the EC with the addition of PHC was approximately 30% of the initial content phenols at both temperatures. The maximum DPPH free radical scavenging activity was 88%, and it decreased during storage; EC3 and EC4 at 20 days of storage presented approximately 69% (4 °C) and 74% (20 °C) (Fig. 7c and 7d, respectively). The ABTS free radical scavenging activity was lower for all samples. The

maximum capacity was 46%, and at 20 days at 4 °C for EC with PHC it was on average 37% (Fig. 7e), but at 20 °C for EC3 and EC4 it was 35% (Fig. 7f).

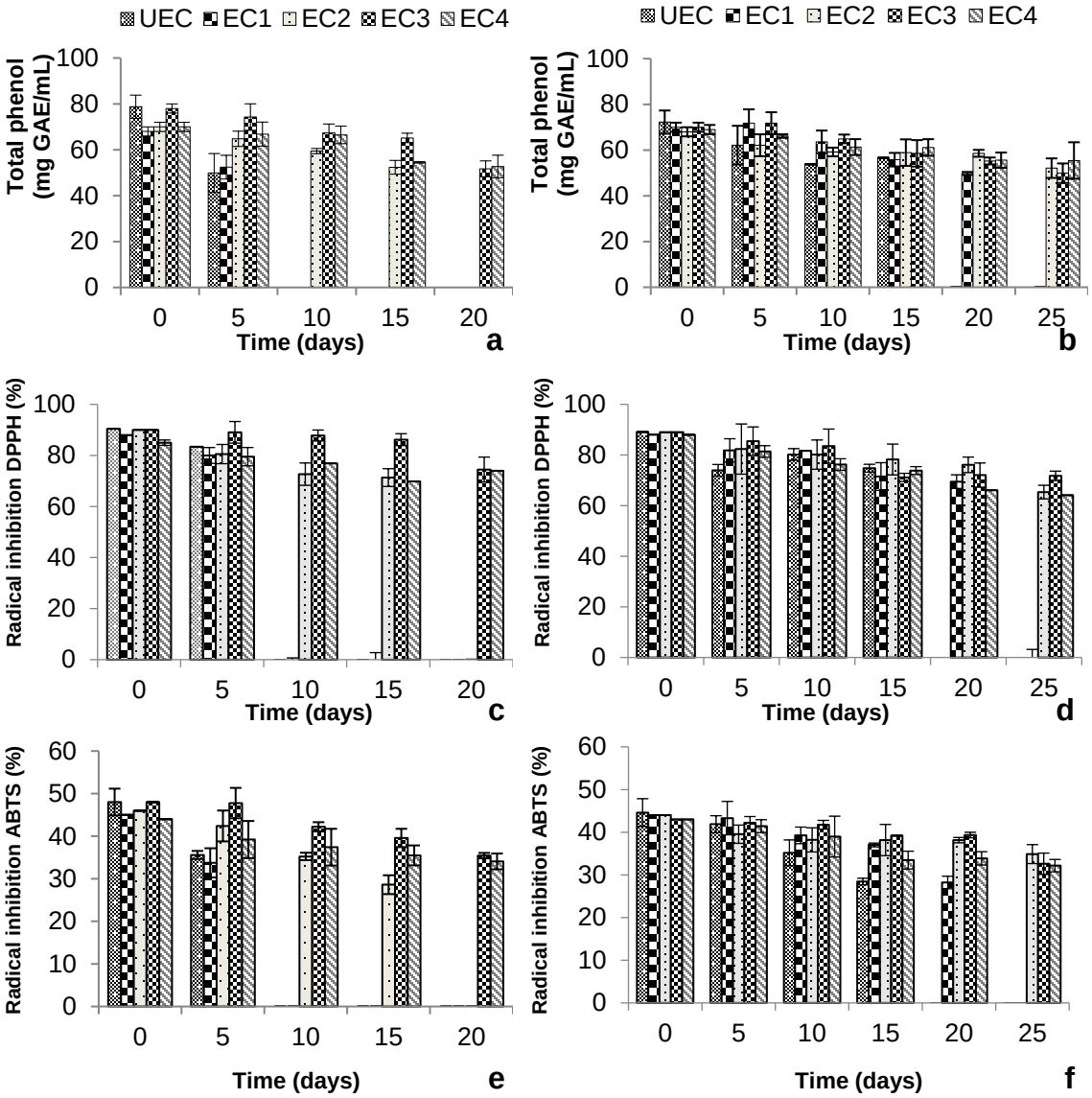

Fig. 7. Total phenol content and antioxidant in strawberries. Phenol with EC at 20 °C (a) and 4 °C (b); DPPH free radical scavenging activity with EC at 20 °C (c) and 4 °C (d); and ABTS free radical scavenging activity with EC at 20 °C (e) and 4 °C (f)

Zhang and Quantick (1997) reported that EC with chitosan can form a protective barrier on the surface of the fruit and reduce the oxygen supply for the enzymatic oxidation of phenolics, which allows for no considerable loss of said compounds. Martínez-González *et al*. (2020) reported that strawberries with EC of chitosan and propolis had a higher content of total phenols (69 µg GAE/g) and higher antioxidant capacity (between 92 and 79%) compared to strawberries without EC at 9 days at 5 °C, so they were effective in increasing antioxidant activity synergistically. Martínez *et al*. (2018) reported the antioxidant activity of strawberries with EC of chitosan and thyme essential oil, being between 23 and 13.9% with ABTS and 35 to 15% with DPPH at 15 days of storage. These

findings indicate that the retention capacity of antioxidants coincides with the presence of essential oil. It is worth mentioning that in this work a similar effect was observed, because the percentage of inhibition of radicals decreased during the days of storage, and the decrease in phenolic compounds and antioxidants during the storage could be due to the breakdown of the cell structure during fruit senescence (Macheix *et al*. 1990). Petriccione *et al*. (2015) reported the effect of a coating with 2% chitosan and managed to improve the nutraceutical properties of the strawberry, maintaining and in some cases increasing the content of phenols (average of 341.6 mg GAE/100 g), anthocyanins (50 mg CGE/100 g), and flavonoids (120 mg CE/100 g) and delaying the senescence of the fruit; however, there were differences in nutrients depending on the origin of the fruit. Therefore, the nutrient content of strawberries will depend on biotic and abiotic factors. Based on the above, the current authors consider that the strawberries used in this work had low phenol content.

The coating mechanism works as a permeable physical barrier between the fruit and the environment, which restricts contact with oxygen and pollutants. Therefore, there is a decrease in the oxidation and ripening process, so as storage time passes there is loss of phenolic compounds and antioxidant capacity. Most EC that exhibit antimicrobial activity (fungi or bacteria) use chitosan. It should be mentioned that chitosan was not used in this work, but the PHC obtained from the *Humphreya coffeata* culture broth could protect the strawberries for up to 20 days without microbial contamination and without the loss of phenolic compounds and antioxidants. However, EC formulation can be improved by increasing the amount of extracellular compounds of the fungus or by using chitosan instead of pectin.

CONCLUSIONS

1. The results of the present study showed that the incorporation of the complex of pectin and extracellular compounds produced by *Humphreya coffeata* (PHC) in the edible coatings (EC) based on pectin increased the useful life of the strawberry, because it presented less weight loss and less microbial contamination, delayed ripening, and increased the conservation of the phenol content and of the antioxidant activity.
2. With the addition there was no modification of the visual appearance of the strawberries. As the strawberries presented high solubility, they are an alternative to foods that require eliminating the EC before consumption.

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