Evaluation of a biodegradable edible coating on the quality of the habanero chili

María de Lourdes Vargas y Vargas ^{1,§}

María de los Ángeles Mayorga-Patrón ¹ Jorge Tamayo-Cortez ¹ Víctor Toledo-López ₁

1 Tecnológico Nacional de México-Instituto Tecnológico de Mérida-Departamento de Ingeniería Química-Bioquímica. Carretera Mérida Progreso km 5, s/n, Mérida, Yucatán, México. CP. 97118. (LE16081464@merida.tecnm.mx; jorge.tc@merida.tecnm.mx; victor.tl@merida.tecnm.mx).

Autora para correspondencia: maria.vy@merida.tecnm.mx

Abstract

This research aimed to maintain the quality and shelf life of *Capsicum chinense* Jacq. var habanero by developing and applying an innocuous, environmentally friendly, and economical edible coating based on agar and mango peel extract as a potential postharvest treatment alternative for habanero chili as it is a perishable fruit susceptible to physiological deterioration. The study was carried out during 2021, in the city of Mérida, Yucatan. The coating was tested for solubility, moisture, opacity, antioxidant capacity, and biodegradability, while the habanero chilies with coating were evaluated for texture, acidity, moisture, total polyphenol content, vitamin C, capsaicin, and antioxidant capacity. The results showed that coated chilies stored at 4 °C had a longer shelf life and a significant effect of coating was observed on the parameters of texture, capsaicin content and the percentage of free radical inhibition, which makes it an appropriate technology for the preservation of habanero chilies.

Palabras clave:

agar, cáscara de mango, chile habanero, vida útil.



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Introduction

It is estimated that postharvest losses in fruits and vegetables are 62%, which are generated mainly during activities related to transport, storage, packaging, and the marketing process, which is why the challenge arises to ensure that the product reaches the consumer with quality similar to that at the time of harvest (IICA, 2018). This makes it necessary to apply sustainable, economical, environmentally friendly, non-destructive, loss-reducing techniques based on food safety criteria.

Among the alternative technologies is the study of edible coatings to extend the shelf life of a fruit against the use of plastic materials. An edible coating (EC) is the product of the formation of three-dimensional networks derived from the denaturation of edible substances, such as proteins, polysaccharides, or lipids, and is defined as a thin layer of edible material formed directly on the surface of the food by immersion or spraying (Otoni *et al.*, 2017).

Among the functions of ECs are: prolonging the shelf life by controlling the transfer of gases, avoiding the loss of firmness and moisture, regulating ripening, reducing metabolic processes during storage, allowing the addition of other compounds; in addition, they can improve the appearance and quality of the coated product to make it more striking due to its brightness or color; bio-based ECs are gaining importance due to their biodegradability, sustainability, and respect for the environment (Mora-Palma *et al.*, 2021).

Research has been carried out applying EC to fruits: Kumar *et al.* (2021) managed to extend the shelf life of the green bell pepper since the quality of storage at 4 °C, the phenolic content, firmness, and antioxidant activity were maintained, this due to the application of an EC based on pomegranate peel extract and chitosan. Mthembu *et al.* (2021) studied the effect of an EC based on moringa leaf extracts and carboxymethylcellulose on tomato fruits, they reported decreases in the changes of texture, total soluble solids, and reduced spoilage compared to untreated fruit, so shelf life was extended by delaying ripening.

Coatings based on basil seed gum and oregano essential oil in apricots improved their quality, odor, and acceptability, while the essential oil decreased water vapor permeability and showed an increase in moisture in the coating (Hashemi *et al.*, 2017). Most of the matrices that make up ECs are intended to be from renewable sources or byproducts of agribusiness, in order to make them biodegradable and reduce the use of non-biodegradable plastic packaging.

One of the polymers used is agar, which is extracted from red algae, considered as Gras, it forms good quality, colorless, flexible, and low moisture content films, with excellent oxygen barrier properties (Mostafavi *et al.*, 2020). The mango peel represents 20% of the weight of the fruit, is considered an agro-industrial waste and has a high potential to be used to obtain molecules with antioxidant activity due to the presence of bioactive compounds such as polyphenols, flavonoids, and carotenoids.

The mango peel of the Ataulfo variety contains 68.13 mg of polyphenols per gram of dry weight (Lizárraga and Hernández., 2018) and can be used to incorporate it through powder, oils, or extracts into ECs. *Capsicum chinense* var. habanero is one of the most important crops in Mexico, it is a product with a high socioeconomic impact that is characterized by the presence of various secondary metabolites of great value for the pharmaceutical and food industries, such as capsaicinoids, polyphenols, vitamins, and carotenoids, which give it added value (Oney-Montalvo *et al.*, 2020).

Nevertheless, it is a perishable fruit, has a shelf life of two weeks with storage at 22 °C, while at 7 °C in combination with modified atmospheres using perforated polyethylene bags, it can be kept for up to 20 days followed by 5 days at 22 °C (Pérez-Ambrocio *et al.*, 2018). Therefore, the objective is to evaluate the effect of an edible coating based on agar and mango peel extract on the quality and shelf life of *Capsicum chinense* Jacq. var. habanero.





Materials and methods

Raw material

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The green chilies were purchased in the local market in the city of Mérida, Yucatán, which were harvested on the same day in the town of Dzidzantún, Yucatán, and stored in plastic containers; they were selected according to the size, color of the peduncle and integrity, discarding those that presented physical damage. These fruits were transported to the Laboratory of Fruit and Vegetable Food Technology, located at the Technological Institute of Mérida, where they were washed with tap water and soaked in a solution of 100 ppm of sodium hypochlorite in order to reduce microbial load.

Deiman food grade agar (Mexico City) and J.T. Baker commercial glycerin (Mexico City) were used. The mango peel, a total of 3 kg, was obtained from the Ataulfo variety, which was in an organoleptic state for consumption, yellow in color, and free of black spots. The peels were weighed on an Alpha triple beam balance and dried at 45 °C in a Binder natural convection drying oven (model ED115-UL) and then ground in an Oster commercial blender to a fine powder.

Solid-liquid extraction was performed with the Soxhlet method and 96% ethanol as solvent (J. T. Baker, Mexico City) in a proportion of 5 g to 200 ml of solvent. Finally, the extract was obtained by concentrating 50 ml in an Ovan diagonal rotary evaporator (model RE30) at a temperature of 65 °C for 30 min and stored at 4 °C in a two-door refrigerator (Torrey) until use the next day.

Preparation of film-forming solutions

The total volume of water (100 ml) was divided into two parts of 50 ml each; in the first, the agar was dispersed (2% w/v) and the solution was heated in a magnetic stirrer with temperature (Eslab) at a temperature of 80 °C for 30 min with stirring at 250 rpm. In the second part, the dispersion of 2% glycerol (w/v) and the extract of the peel (2 and 3%, w/v) was prepared, which was slowly added to the agar solution and the whole mixture was kept for half an hour in heating and stirring until a bright hue was observed; it was left to cool at 60 °C, the solution was emptied into Petri dishes and they were dried at 50 °C in an oven for 24 h.

Formulation of edible coatings (ECs)

Two ECs were formulated: the first formed with 2% agar, 2% glycerol, and 2% mango extract (EC1); the second coating (EC2) contained the same concentrations of agar and glycerol, but 3% of mango peel extract. Both ECs were characterized by evaluating the parameters of moisture, solubility, opacity, and percentage of free radical inhibition, and from the results obtained from these variables, the appropriate EC to be applied to habanero chili fruits was determined.

The application of the coating was carried out by immersion of 200 chilies for 2 s in a liter of filmforming solution; they were left to drain and once dry, 20 chilies were placed in rigid polypropylene trays, making a total of 10 trays and they were stored at 3 °C \pm 1 °C for 35 days; this batch was considered as chilies with coating (W/C); a second batch called control was used, which consisted of uncoated chilies (U/C), 10 trays with 20 chilies each were used; two trays per batch were analyzed in each sampling and representative samples were taken every seven days; likewise, the characterization of the raw material was carried out before the treatment (day 0).

Response variables analyzed to determine the quality and shelf life of habanero chili

Acidity. It was performed according to the methodology described in the (AOAC, 2012), assessing an aliquot of the sample with 0.1 N NaOH. The result was expressed as a percentage of citric acid.

Firmness. It was evaluated with a GY-3 manual texture meter. The chilies were compressed in three different places with the compression tube until the skin of the fruit broke slightly. The results were expressed in kg cm⁻².



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Vitamin C. The official method of 2-6 dichloroindophenol of the (AOAC, 2012) was used. The results were expressed as mg of vitamin C in 100 g-1 of sample (mg of vitamin C 100 g-1).

Total polyphenols. For the determination of phenols, the Folin-Ciocalteu method was followed (Singleton et al., 1999); the absorbance was read at a wavelength of 765 nm using a UV-Visible spectrophotometer (Velab) with glass cells of 1 cm optical length. The concentration of polyphenols in the medium was calculated from a calibration curve with the use of gallic acid as a standard. Results were expressed as mg gallic acid equivalents 100 g-1 sample (mg GAE 100 g-1).

Antioxidant capacity. It was performed according to the technique described by Kuskoski et al. (2005), which consisted of the formation of the ABTS radical [2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] and reading at a wavelength of 754 nm. The results were expressed as a percentage of free radical inhibition.

Quantification of total capsaicinoids. They were measured by ultraviolet spectrophotometry reading at an absorbance of 280 nm, acetonitrile was used as an extractor medium, for this we worked with a UV-Visible spectrophotometer (Velab) and glass cells of 1 cm optical length were used. For quantification, a calibration curve was prepared with standard concentrations of capsaicin in a range between 0 and 100 ppm. Results were expressed as mg capsaicin per g-1 sample (mg capsaicin g-1).

For the characterization of the ECs, the following variables were performed

Moisture. It was determined by gravimetry according to Oregel-Zamudio et al. (2016). The result was expressed as a percentage of water to the total weight. For the solubility of the films, the method described by Oregel-Zamudio et al. (2016) was used, the dry weight films of the moisture test were used. These were immersed in 30 ml of distilled water at room temperature for 24 h, after which the films were recovered and dried in an oven at 80 °C for 24 h, and the percentage of solubility was calculated, which was expressed as solubilized dry matter content.

Thickness. Each film was measured at three different points at room temperature with a digital micrometer, taking the average of the measurements as the thickness in mm.

Opacity. It was determined according to the methodology of Nouraddini et al. (2018), determined at 600 nm and calculated using the ratio of absorbance at 600 nm/film thickness in mm.

Biodegradation. It was performed in natural soils where the films were buried in environmental conditions and watered every three days to simulate rainy conditions, after 3, 8, 15, 20 and 30 days they were recovered, cleaned, and dried at 60 °C in a natural convection drying oven (Binder) for 24 h to constant weight. The degree of degradation in the films was measured as the percentage of weight loss.

The soil was characterized in terms of the content of microorganisms present with the methodology for quantification of aerobic microorganisms through the rapid method of seeding in Petrifilm dishes and the total nitrogen content was determined according to the Kjeldahl method (AOAC, 2012).

Statistical analysis. The results were analyzed using an analysis of variance (Anova) and the statistical significance of the means by the least significant difference (LSD) test. Fisher's Least Significant Difference with a 95% confidence level. The statistical program used was Statgraphics Centurion 19. Each response variable was analyzed in triplicate.

Results and discussion

The results of the characterization of the coatings are shown in Table 1; it was observed that the EC1 with the lowest percentage of mango peel extract (2%) presented greater thickness compared to the one containing 3% of the extract, there is no significant difference between them with a $p \le 0.5$. Agar is a hydrocolloid that binds water within the film, by increasing the extract content and having less water content, it allows an easier exit of water during drying and consequently, a slight decrease in thickness.



Table 1. Characterization of edible coatings.					
EC	Thickness (mm)	Moisture (%)	Solubility (%)	Opacity	(%) free radical inhibition
EC1	0.13 ± 0.014^{a}	24.166 ±1.248 ^ª	48.748 ±0.668 ^ª	1.783 ±0.012 ^ª	99.319 ±0.313ª
EC2	0.12 ± 0.014^{a}	24.053 ±0.476 ^ª	51.204 ±0.226 ^b	0.783 ±0.066 ^b	99.244 ±0.212 ^ª
	, 2% glycerol and 2% m lard deviation of three r				

Most ECs are hydrophilic in nature and it has been found that the thicker the film, the greater the resistance to the transfer of water and gases through them, since the thickness determines the distance that the permeate (water vapor and gases) must travel to diffuse from one side of the film to the other (Park and Chinnan, 1995). It is mentioned that this parameter must be considered so that the edible coating in general forms a fine and thin layer on the food (AI-Hassan and Norziah, 2012).

Significant differences ($p \le 0.05$) were found in opacity, where it was higher in EC1 compared to EC2; this property is desirable since light catalyzes the oxidation and degradation processes of nutritional compounds, such as vitamin C content (Solano-Doblado *et al.*, 2018), in this case, mango peel extract gave the opacity by acting as a barrier to light.

Regarding the percentage of moisture, no significant differences were observed between the coatings, so the content of mango extract did not have an effect on this parameter but it did have an effect on the solubility, this has been an important factor that determines the integrity of the coatings in an aqueous medium, and on the biodegradability, when used as a means for food protection, this parameter involves the penetration of water molecules into the polymer matrix, followed by the breakdown of Van de Waals forces (Archundia *et al.*, 2016).

ECs had a moderate solubility, with EC1 having the lowest percentage, which is desirable during storage as it limits the exudate of fresh products, so the use of low solubility films is important in foods that require protection against moisture (Montalvo *et al.*, 2012).

Both coatings presented high values in the percentage of free radical inhibition, but they were not statistically significant between them. This antioxidant capacity was provided by mango peel extract, which provides antioxidant substances due to the presence of polyphenols (Lizárraga and Hernández, 2018).

According to the previous results, the EC1 formed by 2% agar, 2% glycerol, and 2% mango peel extract was used due to solubility, opacity, and percentage of free radical inhibition to be used in fresh habanero chili as a coating.

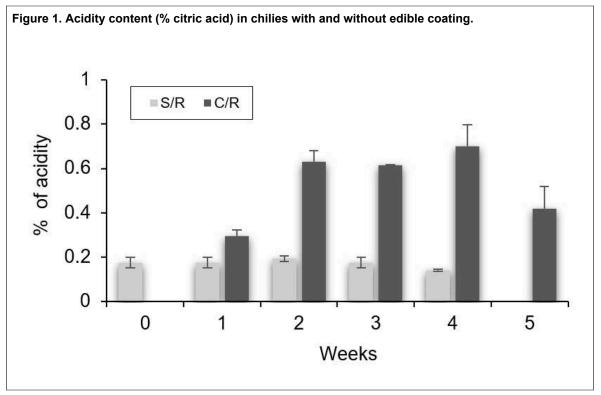
Regarding the biodegradation of EC1, in the first three days, its weight decreased drastically (56%) and at the end of the 30 days, there was a total weight loss of 60%, which is related to the loss of water-soluble materials or low molecular weight compounds (glycerol) and microbial activity, especially due to enzymatic degradation.

The microorganisms, represented by CFU g⁻¹ soil, were higher by 278.57% after 30 days of weight loss; likewise, the nitrogen content of the soil also increased (0.324 ± 0.011 at the beginning and 0.46 ± 0.009 at the end of 30 days), which may indicate the presence of nitrogen-fixing bacteria, therefore, the biodegradation process does generate significant changes in the soil.

It has been mentioned that this process depends on several factors such as temperature, moisture, the type of soil used, the microbial load present, among others (Emadian *et al.*, 2016). In habanero chilies, the decrease in moisture content is one of the main causes of spoilage leading to loss of weight, appearance, softening and nutritional value and is partly due to the limited waterholding capacity of the fruits due to their hollow nature, but differs between species, varieties, and maturation stages (Calvo *et al.*, 2018).

During refrigerated storage, there was an increase in moisture content, from $60.89\% \pm 1$ on day zero to $63.7\% \pm 0.5$ and $64.43\% \pm 0.487$ in uncoated and coated chilies, respectively, at the end of the fifth week. As it is a non-climacteric fruit, the low storage temperature, 3 °C, and the relative humidity conditions (> 95%) resulted in a decrease in the transpiration of the fruit and less permeability of the membranes, which caused a positive effect in the delay of its senescence and in the loss of moisture; this was reflected in the shelf life of the chilies, 35 days for the coated ones and 25 to 30 days for those in the control batch. Chenlo *et al.* (2005) reported an increase in moisture content in bell peppers and showed that storage with an external film affects the kinetics of moisture loss.

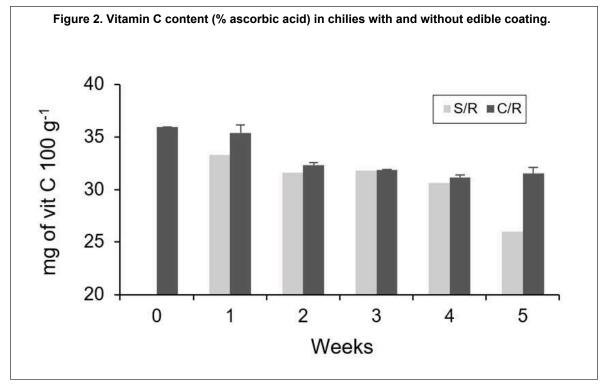
The acidity content of habanero chilies (Figure 1), was significantly different between W/C (coated) and U/C (uncoated) chilies during storage, where W/C chilies showed an increase in acidity content, this behavior is associated with the edible coating that acts as a modified atmosphere that slows down the metabolic process in the fruits, which results in an accumulation of organic acids that, together with temperature, delays the degradation of this acid, this causes the chilies to remain green and acidic (Figueroa *et al.*, 2013). On the other hand, in control chilies, this variable tends to remain constant throughout storage, with a decreasing dynamic due to an increase in the volatilization of organic acids as there is no coating.



Vitamin C showed a tendency to decrease throughout storage and although the values found were slightly higher in the coated fruits, statistically there is no significant difference between them and the control batch ($p \ge 0.05$) during weeks 0, 2, 3 and 4; significant differences were observed at weeks 1 and 5, where the uncoated fruits of the latter showed a more pronounced reduction (Figure 2).





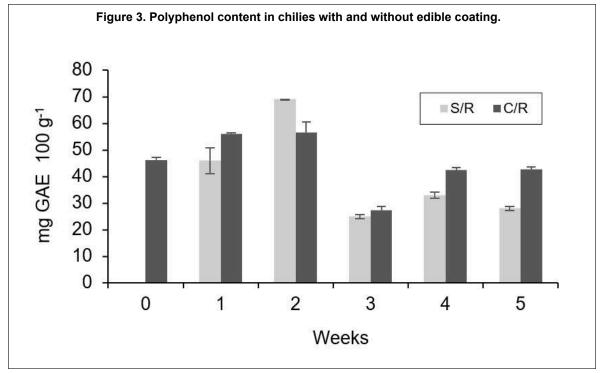


W/C chilies managed to retain 86.11% of the vitamin C at the end of storage, this decrease in the loss of its content can be attributed to the low permeability to O_2 induced by the coatings, which delays the oxidation reactions that degrade this nutrient. EC had a positive effect on the preservation of vitamin C, since Espinosa-Torres *et al.* (2010) reported losses of up to 40% concentration in manzano chilies coated with pliofilm and stored at 5 °C, so it is advisable to consume it freshly harvested.

Polyphenols are one of the main metabolites found in habanero chilies, which contribute to the antioxidant effect of this type of fruit. The results obtained revealed that the polyphenol content in the control fruits and in the coated fruits showed a tendency to increase until the second week, and then decrease and increase again in the 4th and 5th week for W/C chilies (Figure 3).



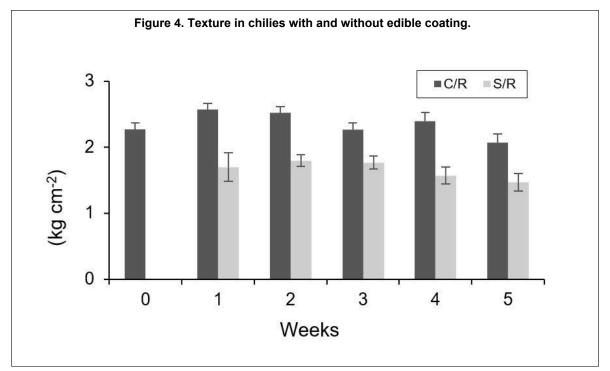
REMEXC



This increase in the content of polyphenols in chilies is related to the activity of the phenylalanine ammonium lyase (PAL) enzyme, as this is increased by the stress of the treatments (temperature control, coating + temperature), since it causes deterioration and generates the decomposition of the cell structure with the presence of a higher content of polyphenols (Oms-Oliu *et al.*, 2008), the values obtained are within the range reported by Oney-Montalvo *et al.* (2018), for green chilies grown in red, brown and black soils (64.9, 49.8 and 53.3 mg GAE 100 g⁻¹, respectively).

The loss of firmness caused by changes in the structure of the cell wall and by the decrease in moisture as a result of the decrease in cell turgor is one of the parameters that has the greatest impact on the quality of the habanero chili. According to the results obtained, the coating had a positive effect on the texture of the chilies, since the W/C fruits showed a significantly higher texture in terms of firmness with respect to the U/C fruits, the maintenance of this characteristic is due to the fact that the coating acts as a modified microatmosphere by creating a partial barrier to water and decreasing the concentration of oxygen, the enzymatic activity causing cell wall degradation is reduced, allowing the retention of firmness in chilies (Figure 4).





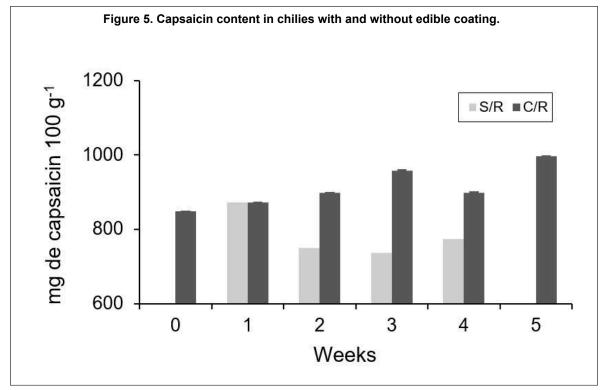
Similar results were found by Ordoñez-Bolaños *et al.* (2014) during the conservation of pepper (*Capsicum annuum* L.), who demonstrated that the application of an edible coating based on modified cassava starch and thyme essential oil presented significant changes in the barrier properties of the samples, delay in the loss of firmness, and decrease in deterioration compared to uncoated fruits.

The capsaicinoids responsible for the spiciness in chili fruits showed significant variation between those with the coating and the control batch, where the former were the ones with a higher content of this variable throughout refrigerated storage.

This increase in W/C fruits is due to an increase in the rate of synthesis and a decrease in degradation levels due to a decrease in the activity of the isoenzymes involved in oxidation, due to the oxygen-poor atmosphere created by the coating by also reducing the activity of peroxidases, which are involved in the degradation of capsaicin and dihydrocapsaicin (Barbero *et al.*, 2016), other authors mention that capsaicin tends to increase under conditions of greater stress, in the case of habanero chilies due to the coating plus temperature (Pedraza-Chaverri *et al.*, 2017) (Figure 5).





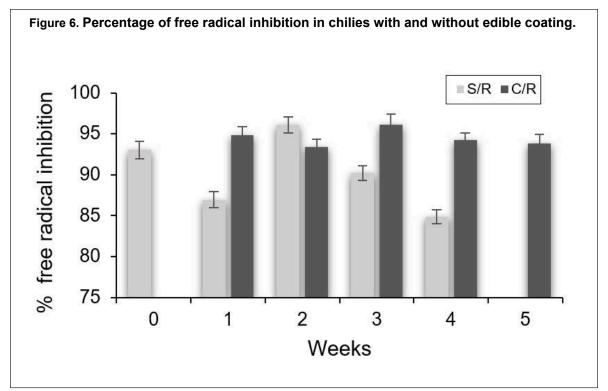


The values found for this metabolite are higher than those reported by Oney-Montalvo *et al.* (2018) for habanero chilies, of 247.5 to 537.6 mg 100 g^{-1} , this variation in capsaicin content is attributed to botanical variety, plant maturation, geographic origin of plants, soil type, and extraction and analytical methods.

Since the total antioxidant capacity of a fruit is the sum of its antioxidant capacity in the hydrophilic phase and lipophilic phase (Repo and Encina., 2008), the percentage of free radical inhibition was determined by the ABTS radical assay, this solution presents a decrease in absorbance when it is reduced by the hydrogen or electron donor species (antioxidant substances contained in the habanero chili).

The percentage of free radical inhibition for W/C chilies was significantly higher compared to U/C chilies, with values in a range of 96 to 93% inhibition, these percentages are higher than those reported by Oney-Montalvo *et al.* (2018) for habanero chilies, whose inhibition values range from 88 to 91% depending on the color of the soil in which they were sown (Figure 6).





The antioxidant activity of a food is the expression of the different polyphenolic components, which use different mechanisms of action to neutralize reactive oxygen species (Zapata *et al.*, 2013); in this case, a moderately strong correlation was observed between the content of polyphenols and the percentage of inhibition of free radicals (r= -0.5798), this indicates that the inhibition of these radicals depends mainly on polyphenolic compounds.

Conclusions

According to the results found in this work, the edible coating of 2% agar, 2% glycerol, and 2% mango peel extract presented good characteristics in solubility, antioxidant activity and opacity; it is a biodegradable coating friendly to the environment and easy to prepare, its application in *Capsicum chinense* var. habanero helped to maintain quality characteristics with a shelf life of up to 35 days at 3 °C compared to uncoated fruits, which reached only 30 days.

The coated chilies managed to maintain acidity, firmness, polyphenol, and capsaicin content, reached high values of free radical inhibition, so this coating is considered as an alternative method to extend the shelf life of the habanero chili, without altering its quality characteristics.

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