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Opuntia ficus-indica Mucilage and Edible Chitosan Biofilms Including *Brassica olearacea* Extract for Extending the Shell-life of *Capsicum annuum* var *Serrano*

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Authors' contributions

This work was carried out in collaboration between all authors. Author LGT coordinated the experimental part and wrote the first draft paper. Author CG carried out the experimental work. Author EB collaborated in the writing of the paper and review process. Author YGG is the Grant receiver and head of the research. She participated also in the writing and correction of the paper. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This work describes the manufacture of biofilms using a mixture of *Opuntia ficus-indica* (nopal) mucilage, chitosan and active ingredients extracted from plants. In this case, ethanolic extractions of white cabbage (*Brassica olearacea*) were performed, since it has been demonstrated in previous studies that these present antioxidant and antibiotic properties. *Chilies* (*Capsicum annuum*) were covered with only chitosan, chitosan+nopal mucilage, or the chitosan-mucilage mixture added with white cabbage extract. Triplicates or quintuplicates of the covered *chilies* and a control group were administered without any application.

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Place and Duration of Study: Assessments were carried out at Unidad Profesional Interdisciplinaria de Biotecnologia-IPN (Mexico) facilities during the end of 2016-2017.
Methodology: Covered and no-covered fruits were stored under two conditions: a) ambient temperature (24 to 28°C) and b) refrigeration at 4°C. They were observed day by day, photographed, and maturation was evaluated in all groups. Also, the weight loss, hardness and content of some metabolites (flavonoids and phenols), acidity and °Brix were determined every third day and the respiration rate of the chilies every five days.
Results: Results indicated that the addition of edible mucilage and chitosan films, added with the white cabbage extract, lengthened the shelf life of the chilies, reducing weight loss and improving the hardness of the fruits. There was also a delay in the maturation process measured as the colour changes from green to mixed green-orange, to yellow and orange and a decrease in the respiration rate of covered chilies compared to those that were not covered. Acidity, °Brix, phenol and flavonoids analyze also provided evidence of the improvement in the final quality of the mucilage-covered chilies, and even more those covered with mucilage + white cabbage extract.
Conclusion: The use of *Opuntia ficus*/chitosan biofilms amended with white cabbage extract resulted very useful in extending the shelf life of *Capsicum annuum* var Serrano fruits under refrigeration or environmental conditions, prolonging their shelf life with a better quality.

Keywords: Biofilms; Capsicum annuum; chitosan; with cabbage extract; Opuntia ficus.

1. INTRODUCTION

Mexico is the second largest producer of chile (*Capsicum annuum*), just behind China. In the post-harvest and transportation process for distribution, large amounts of product are lost. The major obstacle in the food industry is the limited shelf life of foodstuffs resulting from oxidation reactions such as degradation, enzymatic browning and oxidative rancidity.

Bayoumi [1] studied the way to keep the quality of white pepper fruits (*Capsicum annuum* L.) by hydrogen peroxide treatment. This treatment significantly reduced weight loss, rot rate index and nitrate content of fruits, especially with 15 mM H_2O_2 as compared with control treatment (no H_2O_2). Nevertheless, this method is not more than a disinfection technique similar to the application of sodium hypochlorite to fruits and vegetables. It does not protect for a long time the fruits because hydrogen peroxide decomposes in the environment, producing water and O_2.

Another method to reduce spoilage of foods is to use films and edible coatings. Applying edible coatings on fruits from renewable sources, such as lipids, polysaccharides and proteins, help improve quality and prolong the shelf life of fresh and processed foods [2]. Authors such as Lin and Zhao [3] have reviewed the benefits of the application of edible coating for fresh and minimally processed fruits and vegetables. In particular, Edirisinghe et al. [4] have studied the use of edible chitosan biofilms over *Capsicum annuum* fruits, ant they have demonstrated that chitosan controls the Anthracnose, caused by *Colleothrcicum capsici*. It seems that the antifungal properties of chitosan (at 2% concentration) diminished the growth of *Colleothrcicum capsici* by inducing defence-related enzymes.

Opuntia ficus-indica mucilage has been studied widely, in regards to their many biochemical characteristics, which derive from interesting pharmaceutical potential. To mention some, these are antioxidant, antimicrobial, photoprotective, [5], cytoprotective [6], anti-inflammatory, anti-ulcer, neuroprotective, anticancer, anti-viral, antidiabetic, hepatoprotective and alcohol hangover activities [7]. Many of these activities are related to the chemical composition. This mucilage is composed of single sugars such as D-glucose, D-galactose, L-arabinose, D-xylose, L-rhamnose, as well as D-galacturonic, and glucuronic acids [7]. Other activities, such as the antioxidant and antibacterial ones are related with the flavonoids and coumarins contents (including quercetin, isorhamnetin, luteolin, kaempferol and rutin) and dihydroflavonols, flavonones and flavonols and betalain pigments [7]. A nice discussion of the *Opuntia-ficus* antioxidants properties and potential pharmacological use in chronic diseases is presented by Osuna-Martinez *et al.* [8].

Some authors have studied the gelling properties of *Opuntia-ficus* mucilage [9] and other have suggested the use of this mucilage for the preparation of edible biofilms for fruits and vegetables [10]. In a previous work [11], our

research group proposed the use of alginate biofilms including white and purple cabbage extracts in order to protect *Capsicum annuum* fruits from oxidation process and microbial contamination. Biofilms were prepared using alginate, peanuts oil, glycerol and one of two natural extracts: purple cabbage or white cabbage. Results showed that at room temperature, the weight loss values for the alginate, the alginate+purple cabbage extract and the alginate+white cabbage extract showed weight losses of 63, 52 and 73%, respectively in comparison with the loss weight found in the blank experiment (82%). At day 19, the last day of the experiment (room temperature), the firmness of the blank, alginate, alginate+purple cabbage and alginate+white cabbage were as follows: 1.1, 25.7, 21.8 and 17.5 N. Besides, at room temperature films containing alginate+purple cabbage extract augmented the shelf life (quality value >3) from 6 (blank) days to 14 days (alginate+purple cabbage). At 6°C, the shelf life of *C. annum* fruits was augmented from 8 (blank) to 19 days (alginate+purple cabbage). Finally, at 28°C, the shelf life of *Capsicum annuum* was augmented from 1 (blank) to 8 days (alginate+white cabbage).

This work describes the manufacture of these biofilms using a new formulation: a mixture of *nopal* mucilage, chitosan and an active ingredient extracted from a plant, i.e., white cabbage. Some assessments (weight loss, hardness, and maturation state delay, contents of phenols and flavonoids, as well as respiration of covered and uncovered fruits) are employed to demonstrate the capabilities of these edible biofilms to enlarge the shelf life of *Capsicum annuum* var Serrano fruits.

2. MATERIALS AND METHODS

2.1 Extracts and Biopolymer Solutions

In the manufacture of these biofilms was used a mixture of *nopal* mucilage, chitosan and active ingredients extracted from plants. In this research, ethanolic extractions of white cabbage (*Brassica olearacea*) were performed, since it has been demonstrated in previous studies that these have antioxidant and antibiotic properties. *Chilies* were selected with similar sizes and washed with 1% sodium hypochlorite.

The white cabbage ethanolic extract was prepared as follows. 10 g of dry white cabbage was extracted with ten mL of ethanol; the solution was filtered. A new ten mL portion of solvent was used to extract more material from the dry white cabbage, and the two extraction solutions were mixed after filtration in filter paper. A third extraction was carried out. The 30 mL of solution was heated in a commercial oven at 60°C maximum, until total dryness. Then the white cabbage residue was suspended in the nopal mucilage+chitosan solution and in order to speed the dissolution process, the mixture was sonicated by 10-20 min in a commercial water bath sonicator [12]. Phytochemical analysis were carried out as described in Gomez-y-Gomez 2018 [11].

2.2 Experimental Design

At random, four groups of *chilies* were formed to be covered: only chitosan (group 1), chitosan + *nopal* mucilage (group 2), and the chitosan-mucilage mixture added with white cabbage extract (group 3). In each group, triplicates or quintuplicates of the covered chilies were managed and a control group without any application (group 4). They were stored under two conditions: a) room temperature (24 to 28°C) and b) refrigeration at 4°C. The exact formulation of the biofilms were in percentage %: 1 (only chitosan): chitosan 2, sorbitol 0.6, TW80 0.4; 2 (chitosan + nopal mucilage): chitosan 1, nopal mucilage 1, sorbitol 0.6, TW80 0.4; 3 (chitosan+nopal mucilage and white cabbage extract): chitosan 1, nopal mucilage 1, sorbitol 0.6, TW80 0.4, white cabbage ethanolic extract 2. Sorbitol was employed as the plasticizer in accord to recommendations of other authors [13], and the TW80 (sorbitan monooleate polyethoxylated) helps to incorporate the polar components (ethanolic white cabbage extract) into the aqueous solution. Fruits were characterized for 13 days. Experiments were carried out at room temperature (with variable values during the day, around 24-28°C).

The covered and uncovered fruits were observed day by day, they were photographed, and the maturation was evaluated in all the groups. Also, weight loss, hardness and the value of some attributes (phenols, flavonoids, acidity and Brix degrees) were determined every third day and the respiration rate of the chilies every five days. °Brix is an indirect measure of the soluble solids in the sample as mg/L. Phenols and flavonoids were determined by the wet method as reported by Hurtado-Mariles [14]. The biofilm formulation of the following: 1% mucilage, 2% white cabbage extract, 0.6% sorbitol, 0.4% Tween 80 and 1%

chitosan. The procedures can be consulted in detail in Gómez-y-Gómez, et al. [11]. The acidity of the samples was determined as follows: one chile was ground, and ten mL of deionized water was added. To this solution, two drops of phenolphthalein were added and titrated with 0.1 N NaOH, in accord to the Mexican Norm NMX-F-102-S-1978 [15].

2.3 Respirometric Studies

The respirometry of the chilies with and without covers was determined based on the methodology proposed by Guerrero-Ortiz et al. [16]. Analyzes of the antioxidant capacity of mucilage are detailed in Gumecindo [12]. With these methodologies, we measured the percentage of inhibition of antioxidant activity, vitamin C content and Trolox, respectively.

3. RESULTS AND DISCUSSION

Table 1 shows the results of the phytochemical sieve performed on the *nopal* mucilage (*Opuntia ficus*). The results were interpreted as positive or negative, referring to the presence or absence respectively of the groups of secondary metabolites. It is important to emphasize the presence of phenols, reducing sugars, saponins, tannins, and submarines. The presence of some of these compounds may be responsible for the antioxidant and antibiotic activities of the *nopal* mucilage. Specifically, it is well known that phenols, flavonoids and submarines have an important antioxidant activity. This activity will be key to the preservation of chilies throughout the days of storage.

The quantification of phenols and flavonoids can be useful to measure the ripening process of fruits (in this case the chilies). It is well known that the products along the time of storage are diminishing their contents of phenols and flavonoids, responsible for the antioxidant capacity of the chilies. Table 2 shows the values of these two metabolites, in chilies stored at 4°C or at room temperature (28°C on average), with or without coating, at the end of the storage process.

As for the phenols, it can be observed that the chilies covered with mucilage and white cabbage extract at room temperature or in refrigeration maintained higher values of phenols (72.7 and 100% of the initial phenols, for *chilies* stored at 28 and 4°C, respectively) than uncoated chilies (55.4 and 77.4%, respectively) (See Table 3). A similar situation was observed for flavonoids, where the percentages of this metabolite in uncoated chilies stored at 28 and 4°C were 20.3 and 70%, respectively. The chilies covered with mucilage and white cabbage, presented final percentages of 56.7 and 100%, for storage at room temperature and 4°C, respectively.

Table 1. Results of the preliminary phytochemical assessments for white cabbage extract

Metabolites	Result	Metabolites	Result
Phenols	+	**Tanines:**	
Steroids:		1% potassium ferrocyanide reaction	-
Lieberman-Bouchard	+	Gelatinreaction	+
Alkaloids:		Iron chloride reaction	-
Dragendorff	+	**Quinones:**	
Mayer reaction	-	Ammonium hydroxide reaction	-
Wagner reaction	-	Sulfuric acid reaction	-
Flavonoids:		Börntraguer reaction	-
Shinoda reaction	-	**Coumarins:**	
10 % no reaction	-	Erlich reaction	+
Cyanogenic glycosides	-	Ammonium hydroxide reaction	-
Reducing sugars:		Hearth glucosides	
Fehling reaction	+	Legal reaction	-
Benedict reaction	+	Baljet reaction	-
Saponins:		**Sesquiterpenlactones:**	
Foam hight and stability	-	Ferrichidroximate reaction	-
Lieberman Bouchard reaction	+		
Rosenthaler reaction	-		

Table 2. White cabbage and *Opuntia ficus* mucilage metabolites profile and antioxidant capacity

Metabolites	White cabbage methanolic extract	*Opuntia ficus* mucilage aqueous extract
Phenols	✓	✓
Steroids		✓
Flavonoids		✓
Reducing sugars	✓	✓
Tannins	✓	✓
Heart glucosides	✓	✓
Alkaloids		✓
Quinones		✓
Saponins		✓
Camarines		✓
Antioxidant capacity (%)	68.78+/-0.771	55.8+/-0.542

Table 3. Percent concentrations of phenols and flavonoids present in the *Capsicum annum* fruits with and without biofilm after treatment

Treatment	Phenols (%)		Flavonoids (%)	
	28°C	4°C	28°C	4°C
Blank	55.4	77.4	20.3	70.0
Mucilage + white cabbage	72.7	100	56.7	100

Fig. 1 shows the results of experimentation with chile Serrano at 4°C. The groups of chilies were the control, chilies covered with chitosan, chilies covered with chitosan, chilies covered with chitosan and mucilage, and finally with chitosan and mucilage, in addition to white cabbage extract. The mucilage was characterized as to its antioxidant activity, and it was found that presented percent of the antioxidant activity of 64.62%. That activity can be represented as the equivalent vitamin C content (0.1522 mg/mL) or as Trolox equivalents (0.6416 mg/mL). These data are probably related to the contents of phenols, submarines and flavonoids shown in Table 1 for mucilage. We assume that the mucilage's antioxidant activity and the extracts added to it will help preserve the physical integrity of mucilage-coated *chilies* and a specific plant extract.

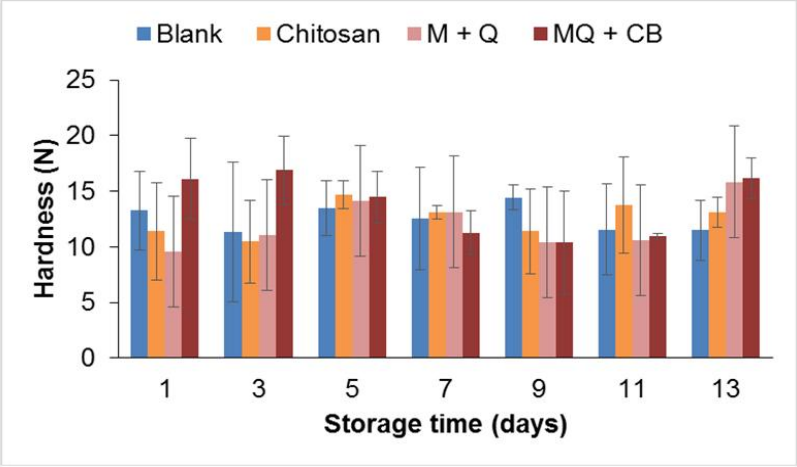

Fig. 1. Data represent an average of n = 3 ± SD of the *Capsicum annuum* fruits hardness at 4°C with three treatments: chitosan (Q), mucilage plus chitosan (M + Q), mucilage-chitosan plus white cabbage (MQ + CB) and the control group without treatment (blank)

Table 3 shows the percent concentrations of phenols and flavonoids present in the *Capsicum annum* fruits with and without biofilm after treatment. It is remarkable that covered fruits maintained the phenol and flavonoids concentrations (100%) in respect to the initial values at 4°C, while at room temperature the 73% of the phenols and the 57% of the flavonoids were present in the covered fruits, in comparison with the uncovered ones (55 and 20% of the initial values, respectively).

As for the storage studies of covered and uncovered chilies, chilies with chitosan/mucilage and vegetable extract presented at the end of the process (13 days) higher values of hardness. In second place are the *chilies* covered with the chitosan/mucilage mixture, followed by those with the only chitosan and in the end the chilies without any cover? In the case of experiments at room temperature, this did not happen, but on the contrary.

Fig. 2 shows the experiments with the shelled chilies stored at 4°C, regarding the weight loss they presented during the 13 days of storage. It can be observed that from the 3rd day of storage all the groups lost important quantities to reach day 13 with losses ranging from 15 to 18% by weight. In this case, the groups of chilies that lost more moisture were covered with chitosan and with chitosan/mucilage. The values of control loss and chitosan/mucilage covered chilies, and white cabbage extract did not present significant differences. Again, in the case of *chilies* stored at room temperature, the results were not good, as the covered chilies (all of them) lost more moisture compared to white (uncovered chilies).The final percentage concentrations of phenols and flavonoids are shown in the Table 3 for uncovered and covered chilies stored at room temperature and at 4°C.

In another type of tests, the acidity of the different groups of chilies was measured as a measure of the maturation level. In the case of chilies an attribute of the fruit is its acidity, so the greater the acidity of *chilies*, the more it will be accepted.

Fig. 3 shows the results of storage of the different groups of chilies at 4°C, although the results of the *chilies* at room temperature were very similar but not shown. It is noteworthy that there were changes in the acidity of all groups from day 3, when the acidity began to increase until day 5, since at day seven there was a sudden decrease of all groups of chilies and again it was observed an increase on days 9, 11 and 13.

At the end of the storage process, the group of chilies with the highest acidity was that of those covered with mucilage/chitosan and including white cabbage extract. After this group was the one, in which the chilies were covered with only chitosan/mucilage, with an acidity very similar to a blank group (uncovered chilies). In the end, the lowest acidity was found for the group where it was only covered with chitosan.

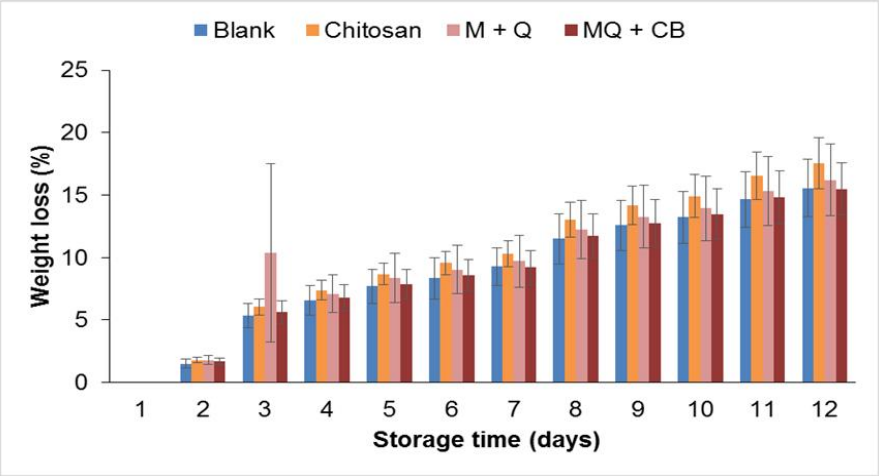

Fig. 2. Data represent an average of n = 3 ± SD of the weight loss of *Capsicum annuum* fruits at 4°C with 3 treatments: chitosan (Q), mucilage plus chitosan (M + Q), mucilage / chitosan plus White cabbage (MQ + CB) and the control group without treatment (white)

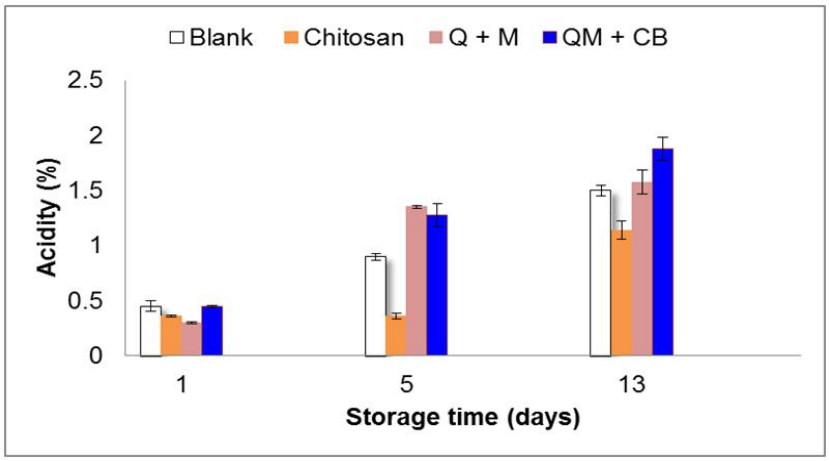

Fig. 3. Percentage of acidity in the *Capsicum annuum* fruits at 4 of with three treatments: chitosan (Q), mucilage plus chitosan (M + Q), mucilage-chitosan plus white cabbage (MQ + CB) and control group without treatment)

The data represent an average of n = 3 ± SD. T student p≥ 0.005

As for the experiments at 4°C (Fig. 4) where the °Brix grades of chilies were measured along the storage, and the following happened. There were no very radical changes, in the beginning, the unprocessed chilies had the highest Brix grades, and so it was until day 7. By day nine the uncovered chilies had lower Brix values than those that received a cover.

At day 13, chilies covered with chitosan/mucilage with or without the plant extract showed the highest values of degrees Brix. It is well known that when they lose moisture, the *chilies* increase their solids content, because they have the highest Brix degree and so it was until day 7. By day nine uncovered chilies had lower Brix values than those that received one coverage. At day 13, the results are congruent with those shown in the figure of weight loss.

Fig. 5 shows the results of respirometry tests for chilies without biofilm, with chitosan/mucilage and with chitosan/mucilage + white cabbage

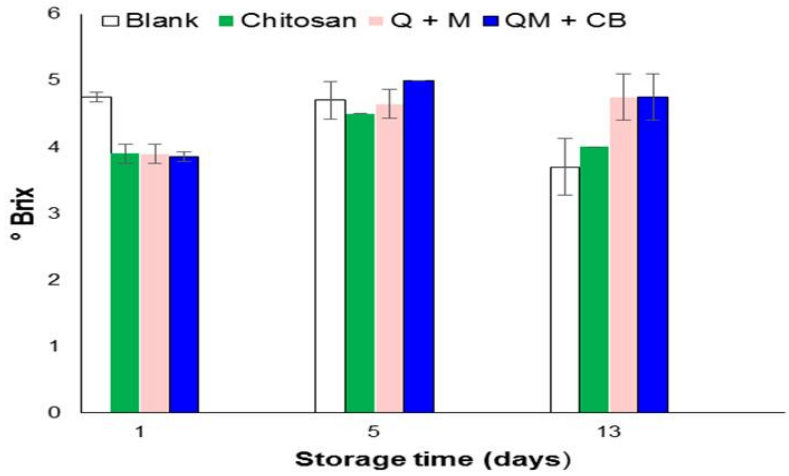

Fig. 4. Determination of °Brix degrees in the *Capsicum annuum* fruits at 4°C with three different treatments: chitosan (Q), mucilage plus chitosan (M + Q), mucilage-chitosan plus white cabbage (MQ + CB) Treatment (white).

The data represent an average of n = 3 ± SD. T student p≥ 0.005

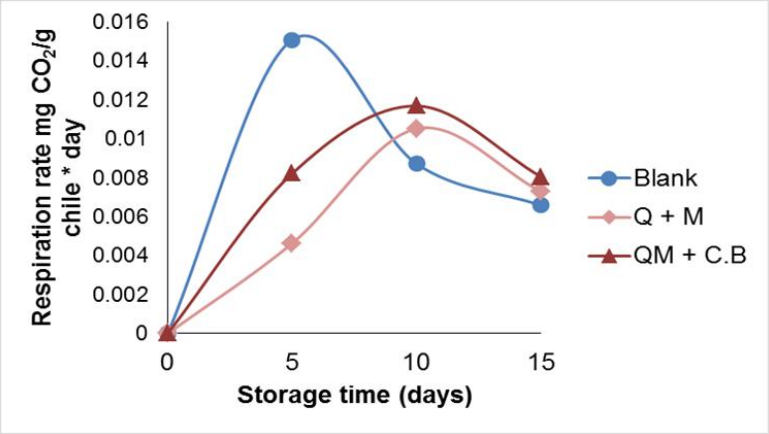

Fig. 5. Respiration rate for *Capsicum annuum* fruits with and without biofilm application

extract. As can be observed, the first days of the test, untreated chilies had respiration rates higher than those observed for the covered chilies (i.e., 0.015 mg CO_2/g chile.day. In the case of chilies covered with chitosan/mucilage + plant extract, the maximum velocities were about 0.012 mgCO_2/g chile.day, whereas at day 15, respiration rates of all treatments decreased to values of about 0.07 mg CO_2/g *chile*.day. The effect of the biofilm on the fruits, regarding the moderation of its respiration rate.

4. CONCLUSION

Results indicated that the application of edible mucilage and chitosan films, added with the extract of white cabbage, extended the shelf life of the chilies, reducing weight loss and improving the hardness of the fruits. Acidity, ^{o}Brix, phenol and flavonoids analyze also provided evidence of the improvement in the final quality of the mucilage-covered peppers, and even more those covered with mucilage + white cabbage extract. There was also a decrease in the respiration rate of the covered chilies compared to those that were not covered. Acidity, ^{o}Brix, phenol and flavonoids analyze also provided evidence of the improvement in the final quality of the mucilage-covered peppers, and even more those covered with mucilage + white cabbage extract.

The sum of the effect as a physical protector and respiration modulator of the mucilage/chitosan biofilm, plus the antioxidant activity of the mucilage and finally the antibiotic activity of the extract of white cabbage resulted in an improvement in the quality of the chilies after storage, In terms of weight loss, hardness, content of phenols and flavonoids, acidity, ^{o}Brix and general appearance of fruits.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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