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Application of Biofilms on Fruits of Avocado (Persea Americana Miller) in Postharvest

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Abstract: Avocado (*Persea americana* Miller) is one of the fruit trees that have been gaining interest among producers and consumers. There is a wide variety of avocados, among which we can cite: 'Prince', 'Hass', 'Ouro Verde', 'Fuerte', 'Choquette', 'Manteiga', 'Breda' and 'Margarida'. Avocado is a very energetic fruit, rich in vitamins A, B, C and E and phenolic compounds, therefore, being provided with high antioxidant activity. Since it is a climacteric fruit, its shelf-life is short, thus, there is a high production loss in postharvest. In order to decrease this loss, some alternative techniques are used, such as, wax, irradiation, biofilms and refrigeration. In this work, we evaluated characteristics of fruits of cultivars 'Breda', 'Choquette' and 'Ouro Verde', covered with Chitosan 2% and manioc starch 2%, stored under refrigeration at 13ºC (± 1ºC, RH 80%). The fruits were submitted to analysis of color, pH, total titratable acidity, soluble solids, antioxidant activity, determination of lipids and phenolic compounds. From the results, we could observe that the use of biofilms was efficient for all cultivars, being that the best response for manioc starch 2% was cultivar 'Ouro Verde' and for Chitosan 2%, were cultivars 'Breda' and 'Choquette'. Under these conditions, the fruits maintained their quality for a period of 15 days.
Keywords: Avocado cultivars, Storage, Coverings, Conservation.

1. INTRODUCTION

Avocado (*Persea Americana* Miller) has a considerable nutritional quality, since it is provided with high contents of fiber, proteins, minerals (such as potassium) and vitamins (especially vitamin E). It is a climacteric fruit, which ripens only a few days after harvest, and its postharvest behavior can be influenced by temperature and storage time (Teixeira et al., 1991). The most used cultivars in Brazil are: 'Simmonds', 'Barbieri', 'Collison', 'Quintal', 'Fortuna', 'Reis', 'Solano', 'Imperador', 'Ouro Verde', 'Choquette' and 'Campinas', while the cultivars that are usually exported are: 'Prince', 'Breda' and'Geada', since these fruits are more resistant (Francisco and Baptistella, 2005).

The fruit's postharvest quality is not only related to the evaluated physical and chemical parameters, but also with the nutritional value that this fruit can offer. Nowadays, for consumers, the quality is also related to benefits that the fruits may bring to their health. The growing number of people interested in consuming more fresh and healthy foods causes the increase in consumption of fresh fruits, thus being necessary to reinforce production and postharvest conservation.

Among the techniques for loss decrease, the use of biofilms as coverage has been widely used lately. It is a low cost technique which aims at maintaining the fruit's quality of life and increasing its shelf life. Among the functional properties of biodegradable biofilms, we can mention transportation of gases (oxygen and carbonic gas) and solutes, retention of aromatic compounds and incorporation of alimentary additives, such as: nutrients, scents, pigments or antioxidant and antimicrobial agents (Palmu et al., 2005). For several applications on food, resistance to moisture is the most important characteristic of the film or edible coverage. Not only does water loss in stored products result in weight loss, but also in quality loss.

In this work, we evaluated the characteristics of fruits of cultivars 'Breda', 'Choquette' and 'Ouro Verde' covered with Chitosan 2% and manioc starch 2%, stored under refrigeration at 13ºC (± 1ºC, RH 80%).

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2. MATERIAL AND METHODS

2.1. Fruit preparation

The fruits were harvested from an orchard in State University of Maringá (Campus Diamante do Norte, Paraná, Brazil) and were transported to the Laboratory of Biochemistry at State University of Maringá. The fruits were selected, washed and sanitized with a solution of Sodium Hypochlorite at 1%, and dried in a porous surface.

2.2. Application of biofilms

The fruits were divided into three groups: 1) Control, without treatment; 2) Chitosan 2% biofilm (2,000 g of Chitosan, diluted in 1 L of distilled water with 0.6% of ascorbic acid and 10mL of glycerol) and 3) Solution of manioc starch 2% (2,000g of manioc starch diluted in 1 L of distilled water, under constant agitation, at 70ºC).

The fruits remained immersed in the solutions for 3 minutes and were later placed in nylon nets, in order to remove the excess of solution and dry.

2.3. Refrigeration

The fruits were stored in B.O.D. (Tecnal) at a temperature of 13ºC (± 1ºC, RH 80%). They remained in storage for 15 days and we carried out physical and chemical analyses every three days.

2.4. Color and Mass Loss

On the first day, the fruits were sanitized, covered, weighed and identified. Afterwards, they were weighed every three days. Color parameter was analyzed by means of a colorimeter Konica Minolta Spectrophotometer, model CR410, with determination of parameters L, Hue, a* and b* (peel and pulp) (Castro et al., 2012).

2.5. pH and Total Titratable Acidity

We determined pH by means of a potentiometer and direct reading in pHmeter Hanna Instruments model pH300, according to method No. 981.12 of AOAC (1997).

Total titratable acidity was quantified by titration with NaOH 0.1M (IAL, 2008).

2.6. Soluble Solids and Lipid Determination

In order to determine soluble solids in avocado pulp, we used a portable digital refractometer, brand Atago, model Pocket pal-1, ranging from 0% to 35% ºBrix. The readings were made directly on the device, using concentrated juice and the results were expressed in ºBrix (AOAC, 1997). Lipid quantification was made using Bligh and Dyer (1959) method.

2.7. Phenolic Compounds and Antioxidant Activity

In order to determine phenolic compounds, we prepared an extract with the sample dissolved in methanol. In essay tubes, we put 125µL of the extract, in addition to extra 125µL of solution Folin 50% and 2250µL of a solution of Sodium Carbonate 3.79M. Afterwards, the tubes remained in a dark room for 30 minutes. The reading was made in a spectrophotometer at 725nm (Singlenton and Rossi, 1965).

Antioxidant activity was determined by means of DPPH radical (1,1-diphenil-2-picrylhydrazyl), according to the method described by Mensor et al. (2001), with modifications. The reading was made in a spectrophotometer at 517nm.

2.8. Statistical Analysis

The results gathered in the experiment were submitted to Analysis of Variance (ANOVA) and, for media comparison, we used Tukey Test at the level of significance of 5% of probability, by means of software SISVAR, version 5.3 (Ferreira, 2008).

3. RESULTS AND DISCUSSION

3.1. Color and Mass Loss

We verified that during storage, mass loss was low, though crescent, being significant from the 9th day on, both for covered and control fruits (Figure 1). These low values are due to the summed effects of storage temperature and use of pellicle. This fact caused a decrease in respiratory activity and water loss in the fruits. Regarding percentage of weight loss, none of the cases differentiated from each other and the mass loss was not higher than 1%. Similar results were found by Joyce et al. (1995), who reported that avocados from cultivar 'Hass' treated with biofilms, showed 0.51% of mass loss and fruits without treatment, 0.99%.

As for parameter L (for peels) there was an increase in luminosity in all of the three cultivars. Cultivar 'Breda' had an average of 16.64, 17.38 and 18.01, for fruits without coverage, fruits covered with chitosan and manioc starch, respectively. On its hand, cultivar 'Ouro Verde' had averages of 17.64, 17.37 and 18.03, for fruits without coverage, fruits covered with chitosan and manioc starch, respectively. Last, but not least, cultivar 'Choquette' had averages of 14.79, 16.63 and 15.46 for fruits without coverage, fruits covered with chitosan and manioc starch, respectively.

It was possible to observe that color yellow appeared on the peels and values for Hue (h) remained between 90º and 174º, being that 90º represents yellow and 180º represents green, as displayed on Table 1. The values for luminosity of pulp were 30.95, 34.84 and 33.21 for fruits without coverage, fruits covered with chitosan and manioc starch, respectively, in cultivar 'Breda'. Cultivar 'Ouro Verde' had averages equal to

28.68, 32.54 and 30.55, while 'Choquette' had 33.90, 34.27 and 32.74, respectively.

According to Vieites (2012), negative a* values represent predominance of color green in the pulp. For the three cultivars, we observed a reduction in levels of green in the pulps, throughout the storage period, which is a result of ripening. This result was more intense in pulps of fruits covered with manioc starch in cultivars 'Breda' and 'Ouro Verde'. However, regarding cultivar 'Choquette', we could observe this result in fruits covered with chitosan (Figure 2). In studies carried out by Daiuto et al. (2012) with cultivar Hass, it was also possible to observe a color reduction (a*).

Parameter b* represents intensity blue/yellow (-b = blue; +b = yellow). The three cultivars had positive results, which indicate the presence of color yellow in their pulps. For the three cultivars and both types of biofilms, we observed an increase in levels of yellow and a decrease in levels of green.

3.2. pH and Total Titratable Acidity

Results for pH (Table 2) show that there were variations in accordance with the type of biofilm. The use of manioc starch in cultivars 'Ouro Verde' and 'Choquette' had value of 7.6 and 7.4, respectively. The fruits of cultivar 'Breda' covered with chitosan had a pH of 7.5.

The results for pH found in this work are in accordance with those cited in literature, in which cases, the values range between 6.0 and 8.0, as reported by Oliveira et al. (2000), in their study with cultivar 'Fuerte'.

Titratable acidity had a gradual decrease from the beginning to the end of storage period (Figure 3). This reduction in acidity is a natural consequence of fruit maturation, in which organic acids are metabolized in respiration and converted into non-acid molecules (Pech, 2002).

The percentage of titratable acidity for fruits of cultivar 'Choquette' was the highest one among fruits covered with chitosan (1.43% of organic acid), when compared with fruits covered in manioc starch (0.96%).

The fruits of cultivars 'Breda' and 'Ouro Verde' covered with starch showed a higher percentage of organic acid (1.44% and 1.48%, respectively), when compared with fruits covered with chitosan (0.95% for 'Breda' and 1.45% for 'Ouro Verde'). This fact could also be observed by Daiuto et al. (2012) and Oliveira et al. (2000).

3.3. Soluble Solids and Determination of Lipids

In Table 3, we display the results obtained for soluble solids. It was possible to observe that there was no statistical difference during the storage period or between the types of biofilm, for the three cultivars. The values for soluble solids remained around 6.46 to 10.46ºBrix.

From the three cultivars we tested, 'Breda' showed the highest quantity of soluble solids both for covered fruits and for control fruits. From the results, we could observe a decrease in soluble solids, as reported by Daiuto et al. (2012) and Oliveira et al. (2003). This decrease in total soluble solids, during conservation, can be explained by the fact that sugars and acids are used as respiratory substrates, therefore, having their reservations diminished.

With respect to lipid quantification in this study, there was no significant difference between the biofilms used during storage time. Despite this fact, fruits of cultivars 'Ouro Verde' and 'Choquette', covered in starch, had higher lipid amounts (14.94% and 11.13%, respectively). Nevertheless, cultivar 'Breda' covered in chitosan had a result equal to 9.95%.

Oliveira et al. (2008) determined the lipid content in avocados of cultivar 'Quintal' and found a yield of 4.2% in fresh pulp. Tango et al. (2004), while studying cultivar 'Ouro Verde', found 19.9%.

3.4. Phenolic Compounds and Antioxidant Activity

Figure 4 illustrates the results found for phenolic compounds. When we consider time and coverage, we observed that there were no variations in the levels of phenolic compounds for cultivar 'Choquette'. The values found for the control fruits, chitosan and starch were: 76.40, 83.23 and 70.55µg GAE/100g, respectively.

The averages for cultivar 'Breda' were 123.18, 130.46 and 123.51µg GAE/100g for control fruits, chitosan and starch, respectively.

The fruits of cultivar 'Ouro Verde' that were covered in manioc starch showed a highest amount of phenolic compounds (133.87µg GAE/100g) when compared with fruits without coverage or those covered with chitosan (114.71 and 79.30µg GAE/100g, respectively).

The cultivars we studied showed very oscillating values, regarding phenolic compounds, as it can be seen in Figure 4, though there was a constant increase in content. As it has already been confirmed by Antunes (2006), this increase in concentration of phenolic compounds can be associated to mass loss.

It is important to highlight that it has also been observed by Daiuto et al. (2012) with an increase of about 20µg GAE/100g for fruits under refrigeration and 30µg GAE/100 for fruits stored under room temperature.

In Figure 5, we displayed the behavior of antioxidant activity, which showed great variation during storage. It ranged from 13.23 to 93.56% for cultivar 'Choquette', from 17.46 to 55.05% for 'Breda' and from 29.38 to 90.05% for 'Ouro Verde'.

Fruits of cultivars 'Breda' and 'Ouro Verde' covered in manioc starch showed more intense antioxidant activity than fruits covered in chitosan. However, in case of cultivar 'Choquette', chitosan had better results than the others.

The values for antioxidant activity found in this work are higher than those reported by Daiuto et al. (2012), while studying cultivar 'Hass', with an average of 23% for refrigerated fruits and 30% for fruits under room conditions.

4. CONCLUSION

From the biofilms we used, the best response for fruit conservation was manioc starch 2% for cultivar 'Ouro Verde' and chitosan 2% for 'Breda' and 'Choquette'. They were able to maintain the fruits' good quality and appearance for 15 days in storage.

5. REFERENCES

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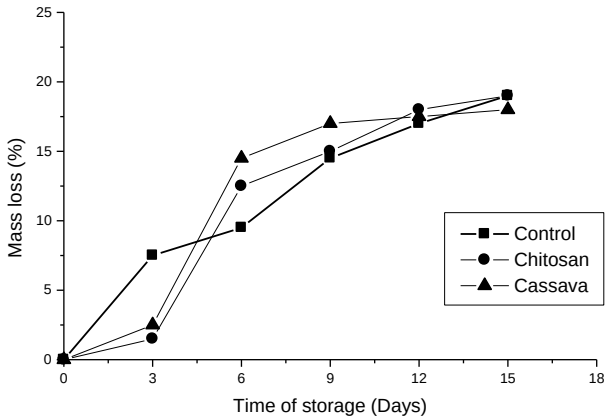
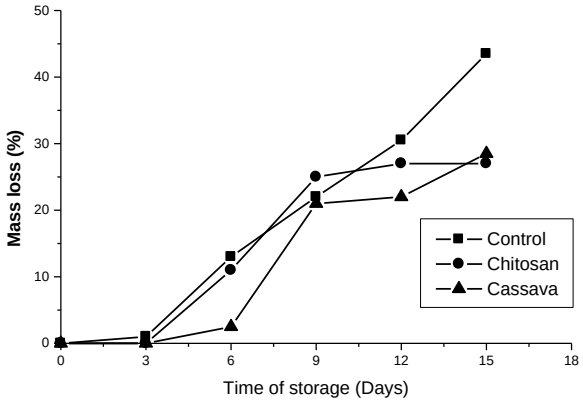
(A)



(B)



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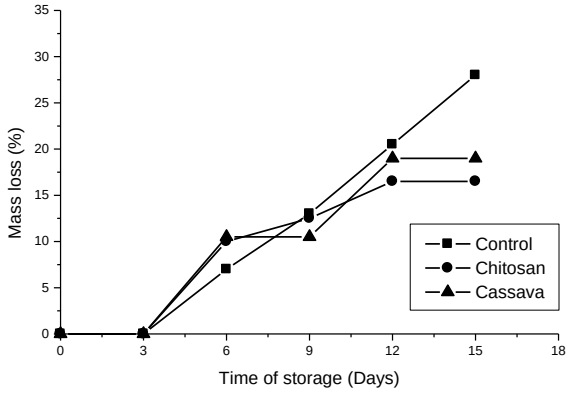
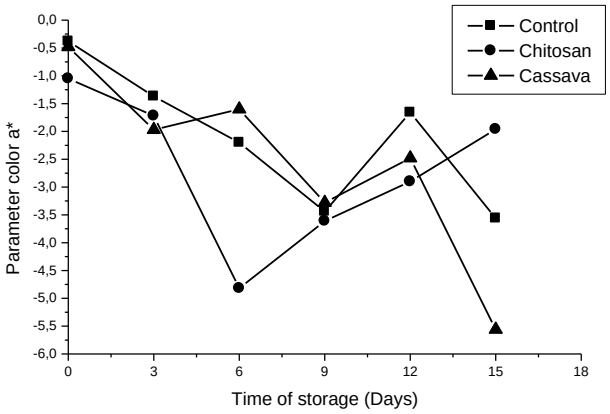


Figure 1. Weight loss (%) of avocados, cultivars: (A) Choquette, (B) Breda, (C) Green Gold, coated with chitosan and cassava starch, stored under refrigeration.

(A)



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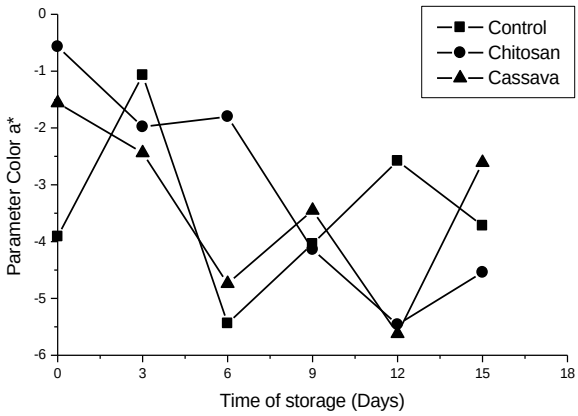

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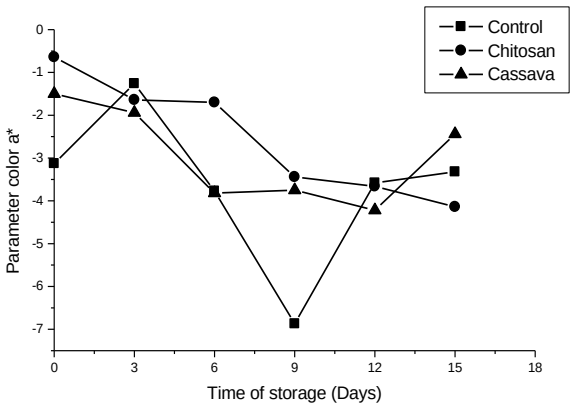
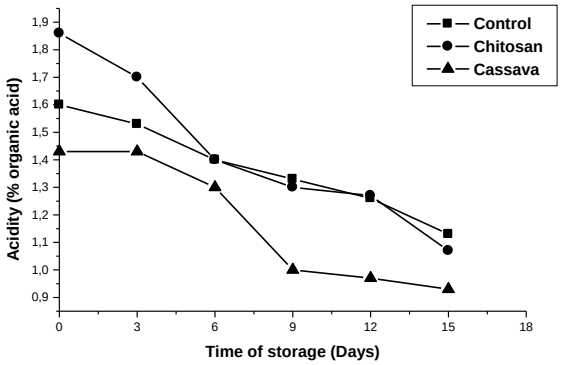


Figure 2. Parameter a* , squash cultivars(A) Choquette, (B) Breda, (C) Green gold coated with chitosan, cassava starch, stored under refrigeration

(A)



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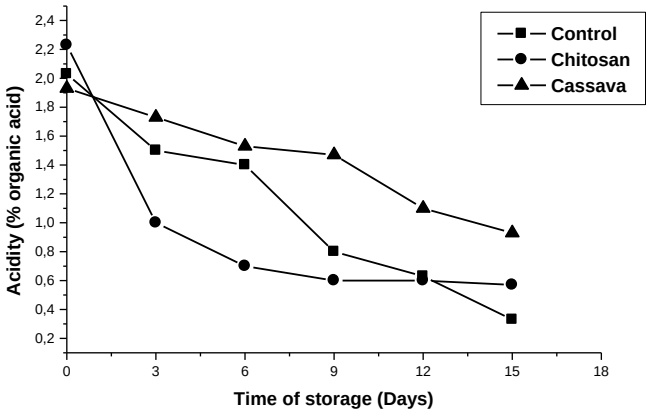

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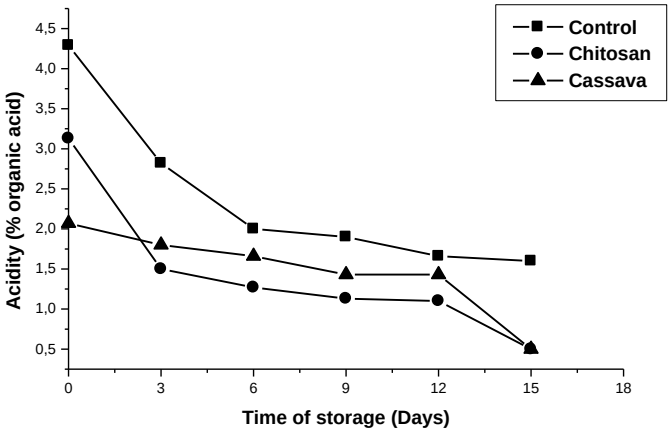
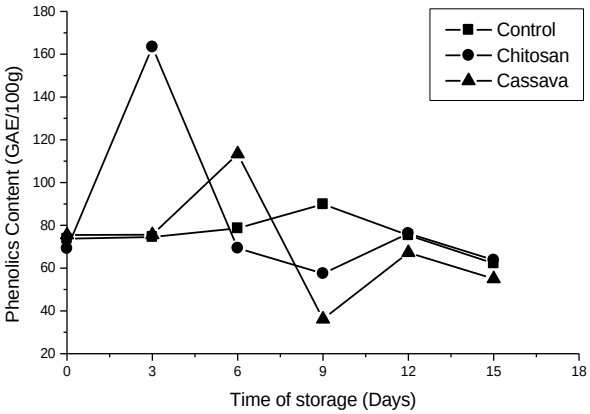


Figure 3. Content evolution of titratable acidity (% organic acid), cultivars (A) Choquette, (B) Breda, (C) Green gold coated with chitosan and cassava starch and stored under refrigeration

(A)



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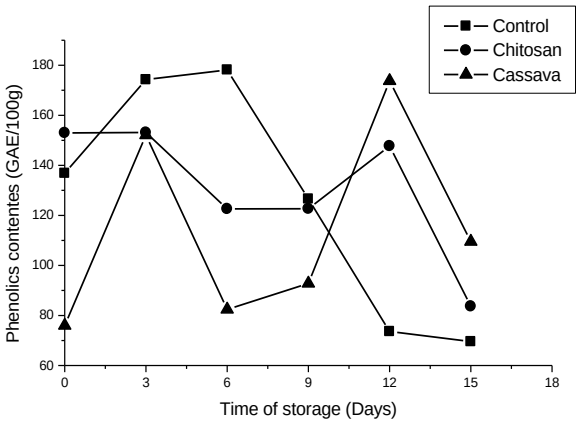

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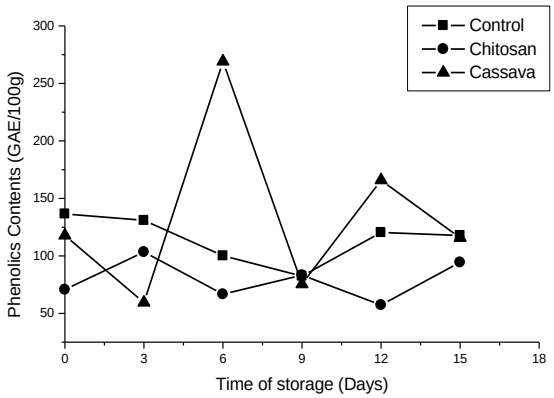
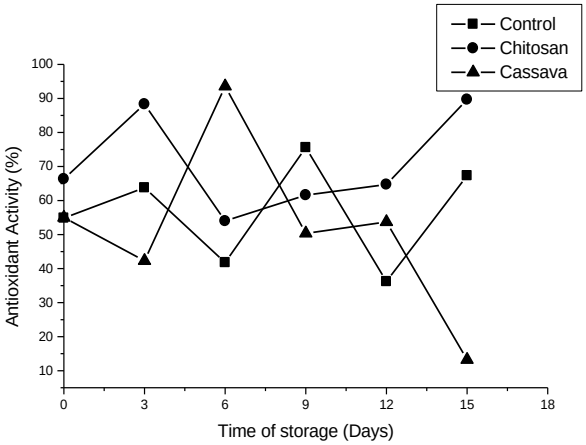


Figure 4. Phenolics content (GAE/100g^{-1}mg) of cultivars (A) Choquette, (B) Breda, (C) Green gold coated with chitosan and cassava starch stored under refrigeration.

(A)



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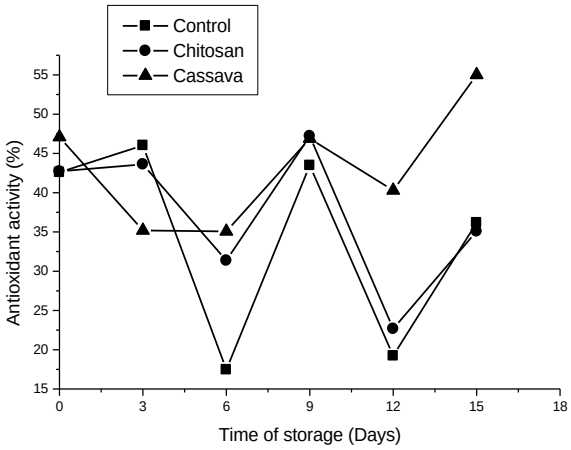

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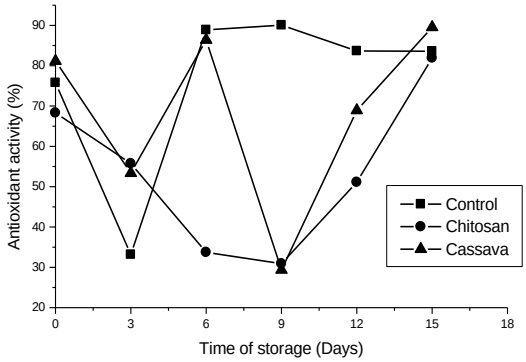

Figure 5.Antioxidant activity(%) of cultivars (A) Choquette, (B) Breda, (C) Green gold coated with chitosan and cassava starch, stored under refrigeration.

Table 1. Parameter– Coloring expressed "Hue" bark of avocados grow Choquette Breda Green gold coated with chitosan 2% and 2% cassava starch stored under refrigeration. Time for each biofilm within each genotype with respect to time.

Time	Biofilm	Cultivar		
		Choquette	Breda	Green gold
0	Control	174,01 Aa	97,43 Bb	94,43 Bb
	Chitosan	111,50 Aa	95,12 Bb	86,12 Aab
	Cassava starch	105,85 Aa	102,44 Aa	102,44 Aa
3	Control	202,71 Aa	97,79 Ab	96,79 Bb
	Chitosan	105,10 Aa	96,47 Bb	96,47 Ab
	Cassava starch	106,14 Aa	100,27 Aab	99,27 Ab
6	Control	108,43 Bb	115,33 Aab	168,33 Aa
	Chitosan	109,12 Ab	128,30 Aa	109,30 Ab
	Cassava starch	104,20 Aa	105,24 Aa	105,24 Aa
9	Control	106,21 Ba	105,73 Ab	103,73 Bab
	Chitosan	102,82 Ab	105,35 Ba	105,35 Aa
	Cassava starch	102,55 Ab	103,89 Aa	103,89 Aa
12	Control	104,68 Ba	100,26 Ab	100,26 Bb
	Chitosan	104,72 Aa	101,90 Bb	101,90 Ab
	Cassava starch	94,66 Ab	101,10 Aa	101,10 Aa
15	Control	114,42 Aa	102,42 Ab	102,42 Bb
	Chitosan	105,28 Aa	104,08 Ba	104,08 Aa
	Cassava starch	101,85 Aa	100,16 Aa	99,16 Aa

*Means followed by the same capital letter in the column do not differ by Tukey test ($p<0.05$), and averages followed by the same letter in the line do not differ by Tukey test ($p<0.05$).

Table 2. Values of the pH value of cultivars of avocado in relation to each biofilm to grow over time.
*Means followed by the same capital letter in the column do not differ by Tukey test ($p<0.05$), and averages followed by the same letter in the line do not differ by Tukey test ($p<0.05$).

Time	Biofilm	Cultivar		
		Choquete	Breda	Green gold
0	Control	5,39 Ba	6,01 Ba	5,74 Ba
	Chitosan	5,86 Ba	5,94 Ba	5,70 Ba
	Cassava starch	6,90 Aa	7,13 Aa	6,61 Aa
3	Control	6,53 Aa	6,46 Aa	6,47 Ba
	Chitosan	6,74 Aa	6,74 Aa	7,05 Aa
	Cassava starch	6,89 Aa	6,93 Aa	7,04 Aa
6	Control	6,65 Bab	7,26 Aa	6,47 Cb
	Chitosan	6,91 Ba	7,50 Aa	7,15 Ba
	Cassava starch	7,73 Aa	7,64 Aa	7,74 Aa
9	Control	6,69 Aa	6,56 Aa	6,24 Ca
	Chitosan	6,94 Aa	6,69 Aa	6,97 Ba

	Cassava starch	6,76 Ab	7,07 Aab	7,63 Aa
12	Control	6,80 Ab	7,73 Aa	6,70 Bb
	Chitosan	6,87 Aa	7,47 Aa	7,33 Aa
	Cassava starch	7,22 Aa	7,27 Aa	7,42 Aa
15	Control	6,58 Aa	7,15 Aa	7,07 Aa
	Chitosan	6,84 Aa	7,20 Aa	7,21 Aa
	Cassava starch	6,57Aa	6,96 Aa	6,42 Ba

Table 3. Values of soluble solids (°Brix) of avocado cultivars in relation to biofilm for each cultivar in relation to time.

Time	Biofilm	Cultivar		
		Choquete	Breda	Green gold
0	Control	6,73 Aa	7,53 Ba	9,00 Ba
	Chitosan	8,47 Ab	11,2 Aa	6,87 Ab
	Cassava starch	6,93 Ab	6,97 Bb	9,70 Aa
3	Control	7,07 Aa	7,6 Ba	7,20 Aa
	Chitosan	6,46 Ab	9,77 Aa	8,10 Aab
	Cassava starch	6,57 Aa	8,17 Aba	8,10 Aa
6	Control	6,40 Aa	7,97 Aa	8,57 Aa
	Chitosan	7,33 Aa	8,8 0 Aa	9,30 Aa
	Cassava starch	6,70 Aa	8,43 Aa	8,67 Aa
9	Control	8,37 Aa	7,03 Aa	8,43 Aa
	Chitosan	7,97 Aa	7,03 Aa	6,97 Aa
	Cassava starch	8,20 Aa	9,67 Ba	8,60 Aa
12	Control	7,06 Aa	9,37 Aa	7,83 Aa
	Chitosan	7,80 Aa	9,37 Aa	7,23 Aa
	Cassava starch	7,93 Aa	8,73 Aa	8,80 Aa
15	Control	7,77 Aa	9,60 Aa	10,06 Aa
	Chitosan	7,00 Aa	9,3 Aa	7,23 Ba
	Cassava starch	7,60 Aa	9,53 Aa	8,80 A Ba

* Means followed by the samecapital letter in the column do not differ by Tukey test ($p<0.05$), and averages followed by the same letter in the line do not differ by Tukey test ($p<0.05$).