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Danielle Fabíola Pereira Silva[1], Luiz Carlos Chamhum Salomão[2], Laércio Zambolim[3], Aline Rocha[4]*

ABSTRACT

This present work aimed to assess the effect of different concentrations of cassava starch, associated or not with prochloraz fungicide, on the postharvest conservation of 'Pedro Sato' guavas. Physiologically mature fruits were immersed in a solution of prochloraz (49.5 g/100 liters of water) for 5 min. Fruit treated with distilled water and air dried were used as control. They were immersed in cassava starch suspension at concentrations of 0, 20, 30 and 40 g/L, plus 0.5 mL/L of mineral oil. The fruits were stored at $21.0 \pm 1.0\,°C$ and relative humidity of $85 \pm 5\%$, for 12 days, and were examined at every three days. The combination of prochloraz and cassava starch resulted in delayed loss of firmness and yellowness and inhibited the incidence of lesions caused by *Colletotrichum gloeosporioides* during the 12 days of storage. Fruits treated with 40 g/L of starch, whether containing prochloraz or not, had unpleasant taste and odor, which suggests the occurrence of fermentation. Control fruits, with and without prochloraz, and those treated with starch with no prochloraz, had nearly 100% lesion occurrence within the 12 days. Starch suspension of 30 g/L, containing prochloraz, was the most effective in maintaining fruit quality.

Key words: *Psidium guajava*, cassava starch, quality.

RESUMO

Uso de biofilme na conservação pós-colheita da goiaba Pedro Sato

O presente trabalho teve como objetivo avaliar o efeito de diferentes concentrações de fécula de mandioca, associada ou não ao fungicida prochloraz, na conservação pós-colheita da goiaba 'Pedro Sato'. Frutos fisiologicamente maduros foram imersos em solução de prochloraz (49,5 g/100 L de água) por 5 min. Frutos tratados com água destilada e secos ao ar foram utilizados como controle. Eles foram imersos em suspensão de fécula de mandioca nas concentrações de 0, 20, 30 e 40 g/L, acrescida de 0,5 mL/L de óleo mineral. Os frutos foram armazenados a $21,0 \pm 1,0\,°C$ e umidade relativa de $85 \pm 5\%$, por 12 dias, sendo avaliados a cada três dias. A combinação de prochloraz e fécula de mandioca retardou a perda de firmeza e o amarelecimento e inibiu a incidência de podridão causada por *Colletotrichum gloeosporioides* durante os 12 dias de armazenamento. Frutos tratados com 40 g/L de fécula, com ou sem prochloraz, apresentaram sabor e odor desagradáveis, sugerindo a ocorrência de processo fermentativo. Nos frutos controle com e sem prochloraz e naqueles tratados com fécula sem prochloraz a ocorrência de podridão foi próxima de 100% aos 12 dias. A suspensão com 30 g/L de fécula, associada ao prochloraz, foi a mais eficiente na manutenção da qualidade dos frutos.

Palavras-chave: *Psidium guajava*, fécula de mandioca, qualidade.

INTRODUCTION

Guava is a very perishable fruit and it undergoes conditions during and after harvest that accelerate its physiological processes and increase their effects (Chitarra & Chitarra, 2005). In the postharvest period, the onset of fruit senescence occurs rapidly, hindering their storage for long periods of time (Basseto *et al.*, 2005; Soares *et al.*, 2011). This makes it difficult to export or send to more distant consumer centers, since there will be great losses during the journey.

Anthracnose, chocolate spot, is a major postharvest disease of guava (*Psidium guajava* L.) in Brazil (Piccinin *et al.*, 2005). *Colletotrichum gloeosporioides* (Penz) Penz and Sacc. was long considered the only causal agent of such disease (Soares *et al.*, 2008). However, more recently, based on molecular technique for identification of *Colletotrichum,* Peres *et al.* (2002) have found *Colletotrichum acutatum* J. H. Simmonds associated with anthracnose lesions on guava. Thus, guava anthracnose may be caused by single or multiple infections of these two fungal species (Soares *et al.*, 2008).

The fungus initiates infection in the fruit during its development in the field, stays inside in a quiescent state, and no symptoms manifest until the fruit start ripening (Araújo Filho, 1980). The fact that there is no fungicide registered for the control of postharvest guava diseases, makes it necessary to search for alternative products that are cheaper and less toxic to humans and the environment.

The use of edible coatings (films) or biofilms is latest technology, which has as raw material the derivatives of amylose, of cellulose or collagen, and they can be removed with water or ingested along with the product being protected and are inexpensive (Vicentini *et al.*, 1999). Edible biofilms, that have starch as biopolymer for their formation, are beginning to be studied in more detail (Coqueiro *et al.*, 2011), and cassava starch has been selected as the most suitable raw material for their preparation, because it forms resistant and transparent films (Queiróz *et al.*, 2010). Moreover, they are effective barriers to water loss, provide good appearance and brightness, making fruits and vegetables more attractive to the market (Vila *et al.,* 2007; Lemos *et al.*, 2007). However, it is important to be careful when using edible films or wax as surface protection for fruits and vegetables. The thickness of the layer, when too thin, does not affect moisture loss, and when too much, can lead to the development of unpleasant flavors (Damasceno *et al.*, 2003).

Papaya fruit (Tainung 1), for instance, when coated with edible biofilm made with cassava starch at 1, 2 and 3%, had their shelf-life extended for four days without affecting their quality (Pereira *et al.*, 2006). These treatments have delayed fruit ripening, which changes in skin color, pulp firmness, soluble solids and titratable acidity were significantly slower than those of untreated fruits.

Since the cassava starch is an edible polymer and effective in delaying the ripening of certain fruits, this present work aimed to assess the effect of different concentrations of cassava starch, associated or not with prochloraz fungicide, on the postharvest conservation of 'Pedro Sato' guava.

MATERIALS AND METHODS

Physiologically ripe fruit, with 179.2 ± 34.3 g weight and pale green peel, were gathered from a commercial orchard in the city of Paula Cândido (20º52'29"S and 42º58'11"W), Minas Gerais, Brazil, in February 2007, at the rainfall season. After being washed, they were divided into two groups of 180. One of them was immersed into prochloraz solution (Sportak 450 CE) at a dosage of 49.5 g/100 L of water, for 5 min, as control. The other group of fruits was treated with distilled water only. After air drying, the fruits were dipped in aqueous suspensions of cassava starch at concentrations of 0, 20, 30 and 40g/L, added with 0.5 mL/L of Assist mineral oil (Bayer Cropscience). The fruits were stored at an average temperature of 21.0 ± 1.0 °C and relative humidity of $85 \pm 5\%$, for 12 days. Samples were collected on day zero (day of the assembly of the experiment), and thereafter every three days until 12 days.

The assessed characteristics were: fruit fresh weight loss, color of peel and pulp, incidence of decay caused by fungi, respiration rate (CO_2 production), firmness and soluble solids content of pulp. Fresh weight loss was determined gravimetrically and the results were expressed as percentage of weight loss, considering the difference between the initial weight of the fruit and that found in each sampling date.

Peel and pulp color development was determined with the aid of a Konica Minolta CR-10 colorimeter, that provided the values of L^*, a^* and b^*. The coefficient L^* (lightness) ranges from 0 (black) to 100 (white), a* ranges from green (negative) to red (positive), and b* from blue (negative) to yellow (positive). The results were expressed as $\triangle E = (\triangle L^{*2} + \triangle a^{*2} + \triangle b^{*2})^{1/2}$. The $\triangle E$ (color difference) defines the saturation and intensity of the color defined by L*, a^* e b^* (Minolta Corporation, 1994) and was determined by the color difference between the values recorded for the fruit at each storage period of time and the values obtained for the fruit at harvest. Two readings were conducted of coordinates L^*, a^* e b^* for the peel, one on each diametrically opposite side of the fruit and one in the placental region, representing the pulp.

The incidence of decay was expressed as the percentage of fruit naturally infected with *Colletotrichum* sp.

Pulp resistance was determined by subjecting each fruit to a force, using the digital penetrometer Shimpo model DFS 100 (Digital Force Gauge) with 12 mm circular flat head until the tissue no longer presented resistance. The results were expressed in kPa. The soluble solids content was determined by refractometry, by means of direct reading of pulp samples.

Fruit production of CO_2 was determined by gas chromatography. In order to do so, the fruits were packed in airtight seal glass bottles with a volume of 1680 mL. Sixty minutes after the closing of glass bottles, 1 mL aliquots of the solution are removed with a hypodermic syringe and injected into a GOW MAC gas chromatograph Series 550, with thermal conductivity detector, equipped with aluminum column filled with Porapak Q. Working conditions included: helium flow rate of 40 mL per minute, which was the carrier gas, electric current of 150 mA, column, detector and injector temperatures were 50, 70 and 80 °C respectively, and room temperature was 20-23 °C. The quantification of CO_2 was made by comparison of the peaks produced by the sample in the chromatogram and by the injection of a standard aliquot made up of 5.96% mol of CO_2 per mol of the $CO_2 + N_2$ mixture. The results were expressed as mg CO_2 kg^{-1} h^{-1}.

The experiment was conducted in a completely randomized split-plot design, with three replications, and three fruits per experimental unit. The plots consisted of different concentrations of cassava starch, the subplots of the presence or absence of prochloraz, and the splits were the time intervals of sampling.

Data were examined using analysis of variance and regression. The models adjusted by means of regression were chosen based on the significance of regression coefficients at 5% probability, using "t" test. Regardless whether the dose x sampling time interval interaction may or not be significant, we opted for its deployment, given the interest in study. The statistics analyses were made in the Software SAEG 9.1.

RESULTS AND DISCUSSION

The peel $\triangle E$ levels increased over time for all treatments (Figure 1A), including the uncoated control, indicating that the yellowing of the fruit occurred during storage, regardless of the treatment used. According to Chitarra & Chitarra (2005), changes in fruit color during ripening are due to degradative or synthetic processes, one of the main criteria for judging the maturity of fruits and vegetables. Fruits treated or not with prochloraz turned yellow (Figure 1), but the yellowing was delayed by the increased concentration of cassava starch.

Fruits of the treatments that did not receive prochloraz and starch differed from those treated with prochloraz and starch (Figure 1A and 1B). Fruit from the control treatment (Figure 1A) showed higher $\triangle E$ levels throughout the entire experimental period, indicating their abrupt change of color in relation to the day of harvest. Regarding the fruits of concentrations 30 and 40 g/L, treated or not with prochloraz (Figure 1A and 1B), increased $\triangle E$ over the storage period was slower than the other treatments, indicating the potential of cassava starch to maintain the color of guava fruit for 12 days, providing improved appearance and longevity to the fruits of such treatments. Scanavaca Júnior et al. (2007), when working with mango cv Surpresa, found that fruit that was not treated with cassava starch turned yellow in 12 days, and that treated with 20 and 30g/L (maximum concentration) of starch changed from green to yellow-green in that same period. Similar results for peel color were found by Hernández-Muñoz et al. (2006) and Raybaudi-Massilia et al. (2007), who, when working with apple and strawberry, respectively, report that in fruit treated with chitosan coating, peel color was kept during storage.

The difference in pulp color in fruits not treated with prochloraz (Figure 2A) was lower for concentrations 0 to 20 g/L from the sixth day of storage on as compared with treated fruit (Figure 2B), indicating that fruit not treated with prochloraz had their ripening anticipated. At concentrations of 30 and 40 g/L of starch with prochloraz (Figure 2B), $\triangle E$ levels were lower compared to the other treatments, indicating that cassava starch in these doses has delayed the ripening of fruits.

Fresh weight loss during the postharvest period is primarily associated with water loss, since that of dry mass caused by respiration (respiratory intake of substrate) is low compared to the loss of water (Silva, 1995).

The use of prochloraz was effective in reducing weight loss compared to untreated fruit (Figure 3). Fruit with no cassava starch and without prochloraz (Figure 3A) showed the greatest loss of fresh weight, reaching 12 days of storage with loss of more than 8%. However, according to Oliveira & Cereda (1999), this level of fresh weight loss is still within the acceptable limits, which is between 10 and 15%.

Fruit weight loss was only 5.4% on the 12th day of storage at a concentration of 40 g/L of cassava starch with prochloraz (Figure 3B), while for fruits that did not receive any treatment, it was 7.8%. Fruit treated with cassava starch, with or without prochloraz, showed less fresh weight loss, which can be explained by the formation of a film around the fruit, after applying the coating, which acts as a barrier to gas exchange and loss of water vapor, changing the atmosphere and slowing the ripening of the fruit (Pereira et al., 2006). Oliveira (1996), studying white pulp guava fruit cv Kumagai, at cassava starch concentrations of 30 and 50 g/L, stored from 21 to 29 °C

and with relative humidity of 65-83%, also reported reduced weight loss compared to those treatments with no starch film and, the fruit remained in optimum conditions for consumption up to nine days of storage.

Fruit respiratory rate of all treatments was reduced on the third day of storage compared to day zero, due to field heat in fruits assessed after harvesting (Figure 4). Fruit treated with prochloraz had lower rates of CO_2 production compared to those not treated with prochloraz (Figure 4). Increased production of CO_2 was noted from the third day of assessment on at doses 0, 30 and 40 g/L of cassava starch, both for treated and not treated with prochloraz fruit, showing no regular climacteric pattern. The same was observed by Azzolini *et al*. (2005) while examining 'Pedro Sato' guava stored at 23 ± 1 ºC, reporting no typical climacteric behavior.

On the treatment with 20 g/L of cassava starch with or without prochloraz, CO_2 production reached its maximum at the ninth day and then decreased, showing climacteric fruit behavior. According to Oliveira & Cereda (1999), the climacteric or non climacteric respiratory behavior of guava is not yet defined, which is evidenced in this work, since on the other treatments (0, 30, and 40 g/L) no climatic pattern was found.

Cassava starch has reduced the respiratory rate during the storage period, and the treatment with 40 g/L resulted in the lowest production of CO_2 on fruit treated or not with prochloraz (Figure 4). This can be explained by the fact that the film formed by the starch around the fruit provides the effect of modified atmosphere, which causes reduced gas exchange and thus reduces the metabolism of the fruit (Kader, 1995).
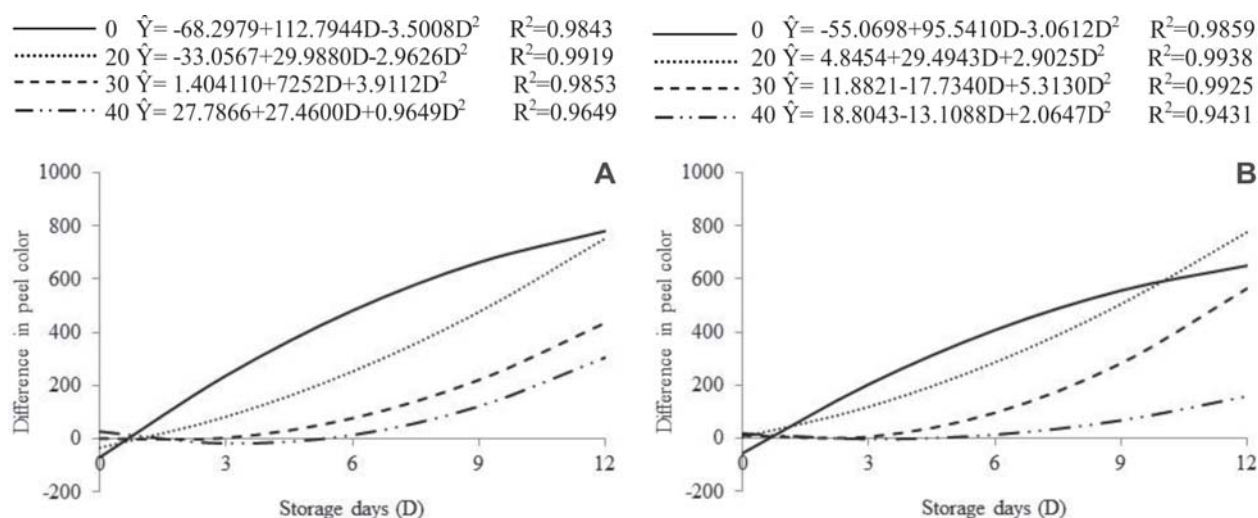
0	$\hat{Y}= -68.2979+112.7944D-3.5008D^2$	$R^2=0.9843$
20	$\hat{Y}= -33.0567+29.9880D-2.9626D^2$	$R^2=0.9919$
30	$\hat{Y}= 1.404110+7252D+3.9112D^2$	$R^2=0.9853$
40	$\hat{Y}= 27.7866+27.4600D+0.9649D^2$	$R^2=0.9649$

0	$\hat{Y}= -55.0698+95.5410D-3.0612D^2$	$R^2=0.9859$
20	$\hat{Y}= 4.8454+29.4943D+2.9025D^2$	$R^2=0.9938$
30	$\hat{Y}= 11.8821-17.7340D+5.3130D^2$	$R^2=0.9925$
40	$\hat{Y}= 18.8043-13.1088D+2.0647D^2$	$R^2=0.9431$

Figure 1. Peel color difference ("E) of 'Pedro Sato' guava treated with different concentrations of cassava starch (g/L) without (A) and (B) with prochloraz during storage at 21.0 ± 1.0 °C.

0	$\hat{Y}= -0.4547+45.6696D-2.6979D^2$	$R^2=0.9897$
20	$\hat{Y}= -15.1484+36.4146D-0.9364D^2$	$R^2=0.9943$
30	$\hat{Y}= -4.3937+21.8797D-0.6270D^2$	$R^2=0.9866$
40	$\hat{Y}= -16.0798+36.5245D-1.7703D^2$	$R^2=0.9717$

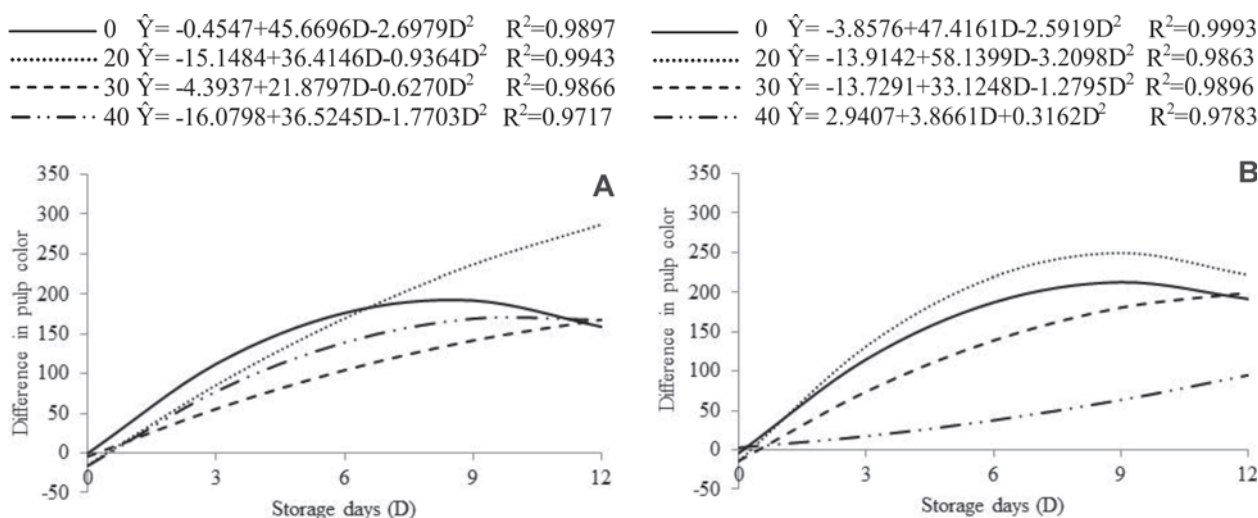|		
0	$\hat{Y}= -3.8576+47.4161D-2.5919D^2$	$R^2=0.9993$
20	$\hat{Y}= -13.9142+58.1399D-3.2098D^2$	$R^2=0.9863$
30	$\hat{Y}= -13.7291+33.1248D-1.2795D^2$	$R^2=0.9896$
40	$\hat{Y}= 2.9407+3.8661D+0.3162D^2$	$R^2=0.9783$

Figure 2. Pulp color difference ("E) of Pedro Sato guava treated with different concentrations of cassava starch (g/L) without (A) and with (B) prochloraz during storage at 21.0 ± 1.0 °C.

Cassava starch was effective in keeping fruit firmness, both for fruit treated and not treated with prochloraz (Figure 5). The higher the dose of starch used, the greater retention of pulp firmness, indicating that cassava starch is associated with delayed ripening of fruit. Fruit treated with prochloraz retained greater firmness than those not treated with prochloraz, which indicates that the presence of pathogenic fungi can accelerate the maturation process (Figure 5). In this study we found a high incidence of anthracnose on postharvest fruit. Probably, *C. gloeosporioides* has been responsible for the accelerated ripening and fruit decay. Soares *et al*. (2008) have assessed the influence of environmental factors on the germination and appressorium formation of *C. gloesoporioides* e *C. acutatum in vitro* on Kumagai guava, employing temperatures of 10, 15, 20, 25, 30, 35 and 40 ºC, and leaf wetness duration (LWD) of 6, 12 and 24 hours. Guava infection occurred at temperatures of 15, 20, 25 and 30 ºC and leaf wetness duration of 24 hours. Infection of guava by two fungal species was increased with temperature, unlike conidium germination and appressorium formation. Incidence of 100% sick fruit occurred at 30 ºC for both species after 10 days of inoculation.

Significant decrease in fruit firmness due to the days of storage was also observed, associated with ripening and senescence. Carvalho (1999), using sealed and drilled package to store guavas at room temperature and 70% relative humidity for 10 days, found that unpackaged fruit and those packaged in perforated film showed no significant differences for firmness, while those wrapped with sealed film were firmer from the sixth day on than those from other treatments. On the other hand, when Oliveira & Cereda (1999) used guavas coated with cassava starch at room temperature, and reported no difference in firmness of fruits of all treatments.

For the soluble solids content, there were differences between fruit treated and fruit not treated with prochloraz. In fruit without prochloraz except the concentration of 40 g/L cassava starch film, a significant reduction in soluble solids content was noted from the ninth day of storage on (Figure 6A), which can be explained by the high percentage of fruit decay. Fruit disease accelerates ripening and senescence, by increasing the concentration of ethylene (Zambolim *et al*., 2002) and, thus, it may lead to the consumption of fruit reserves.
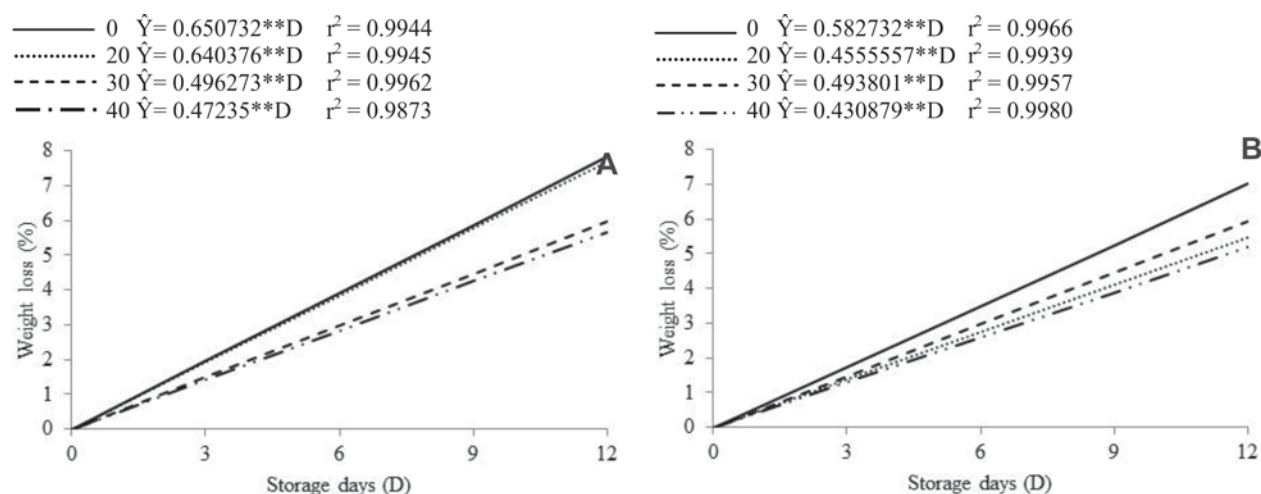

Figure 3. Weight loss (%) of 'Pedro Sato' guava treated with different concentrations of cassava starch without (A) and with (B) prochloraz during storage at 21.0 ± 1.0 °C.

Figure 4. Production of CO_2 (mg of $CO_2 \, kg^{-1}h^{-1}$) of Pedro Sato guava treated with different concentrations of cassava starch without (A) and with (B) prochloraz during storage at 21.0 ± 1.0 °C.
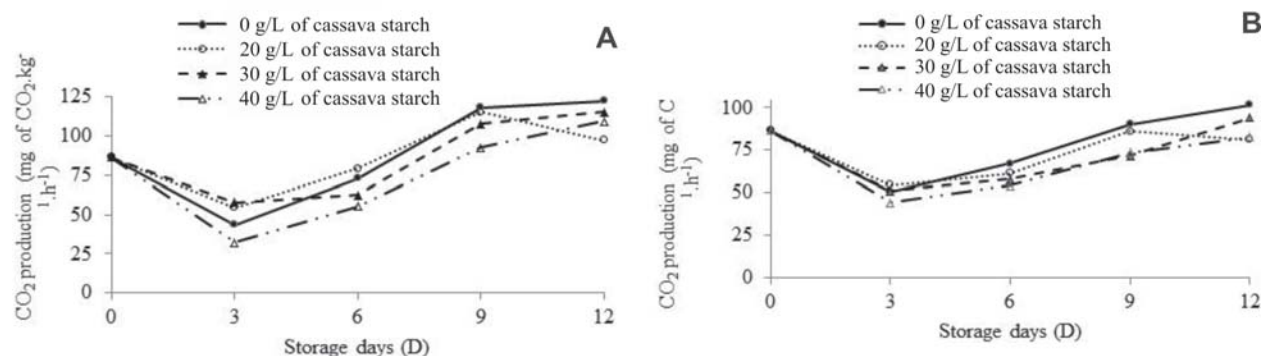
Fruit treated with prochloraz and cassava starch film at concentrations of 20 and 40 g/L showed soluble solids content increased throughout the storage period. However fruit with no cassava starch film showed significant reduction soluble solids content from the ninth day on, indicating the consumption of reserves. Fruit treated with prochloraz and cassava starch film at concentrations of 0 and 30 g/L showed slight increase in soluble solids content until the sixth day of storage and then decreased (Figure 6B), this reduction may be due to the consumption of reserves by breathing since this period there was an increase in respiratory rate (Figure 4B). Moreover, it was found that the higher the concentration of starch, the lower the soluble solids content, which proves the effectiveness of this product in delaying 'Pedro Sato' guava ripening.

The incidence of fruit rot treatments without prochloraz was adjusted to the quadratic model (Figure 7A) and prochloraz treatments with no adjustment being given the means observed (Figure 7B).

In fruits that were not treated with prochloraz (Figure 7A), the incidence of decay in the sixth storage day, was 60.32, 47.30, 30.47, and 31.43% for treatments of 0, 20, 30 and 40 g/L, respectively. The high incidence of decay since the middle of the trial shows the high level of inoculum to see the fruits of the field. Because *C. gloeosporioides* infecting flowers and fruit formation, the fungus remains quiescent in the stadium inside manifesting their symptoms with maturation (Zambolim *et al.*, 2002).

However, the fruits treated with prochloraz and cassava starch (Figure 7B), treatment 0 g/L prochloraz showed fruit rot incidence of 11.11% in the sixth and ninth days of storage. On the 12th day of storage for treatments 0 and 20 g/L prochloraz showed fruit rot incidence of 88.9 and 22.2% respectively and the other treatments did not show
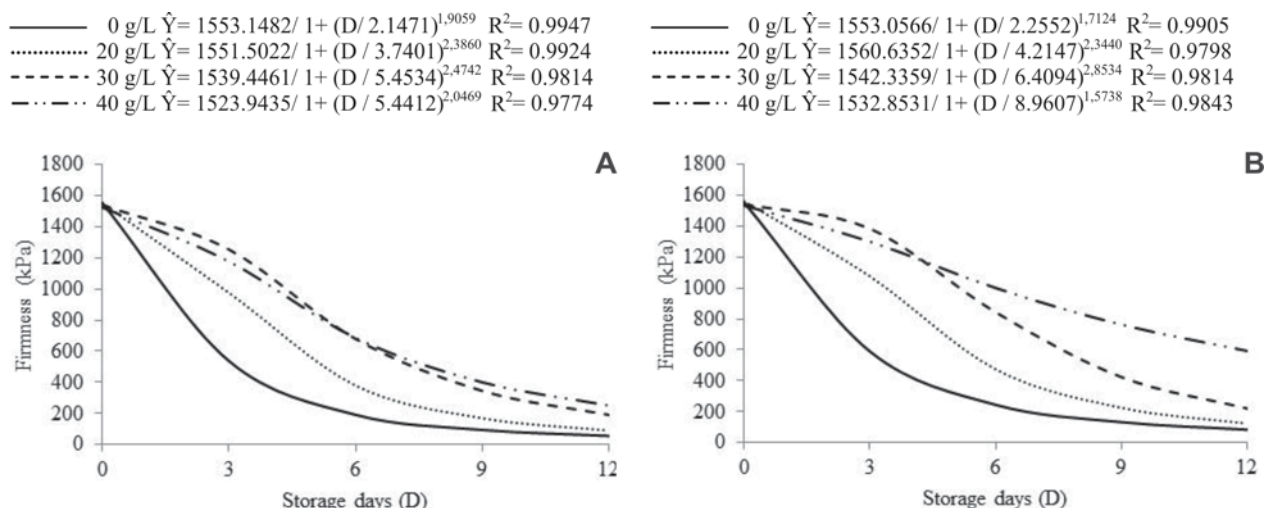
$$0\ g/L\ \hat{Y}= 1553.1482/\ 1+ (D/\ 2.1471)^{1.9059}\ R^2= 0.9947$$
$$20\ g/L\ \hat{Y}= 1551.5022/\ 1+ (D/\ 3.7401)^{2.3860}\ R^2= 0.9924$$
$$30\ g/L\ \hat{Y}= 1539.4461/\ 1+ (D/\ 5.4534)^{2.4742}\ R^2= 0.9814$$
$$40\ g/L\ \hat{Y}= 1523.9435/\ 1+ (D/\ 5.4412)^{2.0469}\ R^2= 0.9774$$

$$0\ g/L\ \hat{Y}= 1553.0566/\ 1+ (D/\ 2.2552)^{1.7124}\ R^2= 0.9905$$
$$20\ g/L\ \hat{Y}= 1560.6352/\ 1+ (D/\ 4.2147)^{2.3440}\ R^2= 0.9798$$
$$30\ g/L\ \hat{Y}= 1542.3359/\ 1+ (D/\ 6.4094)^{2.8534}\ R^2= 0.9814$$
$$40\ g/L\ \hat{Y}= 1532.8531/\ 1+ (D/\ 8.9607)^{1.5738}\ R^2= 0.9843$$

Figure 5. Pulp firmness (kPa) of 'Pedro Sato' guava treated with different concentrations of cassava starch without (A) and with (B) prochloraz during storage at 21.0 ± 1.0 °C.

$$0\ g/L\ \hat{Y}= 7.3267+0.4485*D-0.0463*D^2\ R^2=0.7785$$
$$20\ g/L\ \hat{Y}= 7.4683+0.3545*D-0.0376*D^2\ R^2=0.8548$$
$$30\ g/L\ \hat{Y}= 7.2905+0.5323*D-0.0524*D^2\ R^2=0.7917$$
$$40\ g/L\ \hat{Y}= 7.7270+0.2568*D-0.0139*D^2\ R^2=0.6756$$

$$0\ g/L\ \hat{Y}= 7.5635+0.5788*D-0.0526*D^2\ R^2=0.7661$$
$$20\ g/L\ \hat{Y}= 7.6971+0.2097*D-0.0073*D^2\ R^2=0.6733$$
$$30\ g/L\ \hat{Y}= 7.3914+0.3490*D-0,0318*D^2\ R^2=0.6664$$
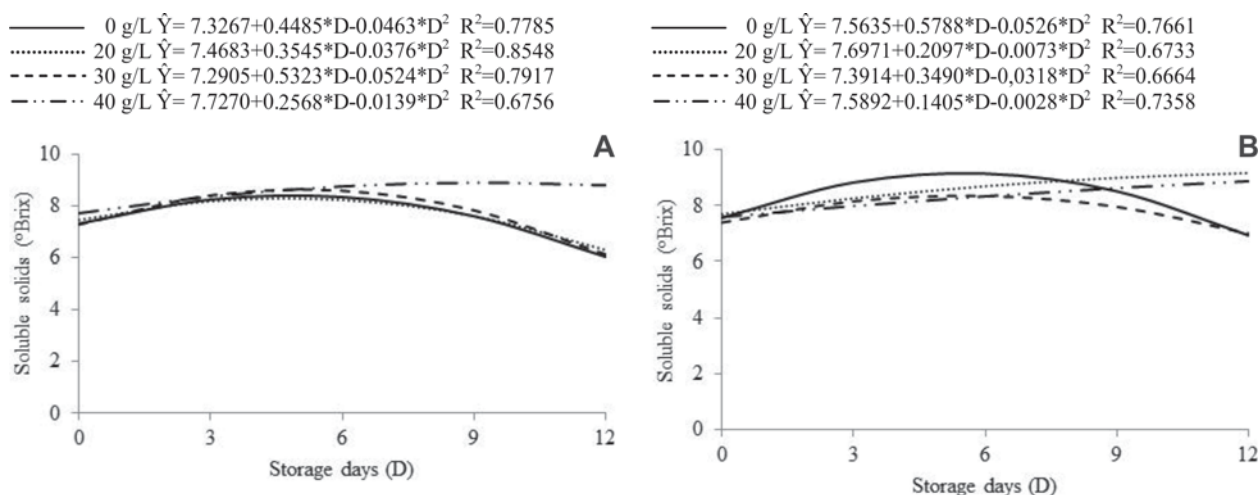$$40\ g/L\ \hat{Y}= 7.5892+0.1405*D-0.0028*D^2\ R^2=0.7358$$

Figure 6. Soluble solids content (°Brix) in 'Pedro Sato' guava treated with different concentrations of cassava starch without (A) and with (B) prochloraz during storage at 21.0 ± 1.0 °C

— 0 g/L $\hat{Y}= 4.1270+10.9524*D-0.2646*D^2$ $R^2=0.9862$
········· 20 g/L $\hat{Y}= 3.8086+6.7190*D+0.0883*D^2$ $R^2=0.9886$
- - - - 30 g/L $\hat{Y}= 5.0797+1.0569*D+0.5292*D^2$ $R^2=0.9705$
—··—··· 40 g/L $\hat{Y}= 4.1274+2.4324*D+0.3529*D^2$ $R^2=0.9762$
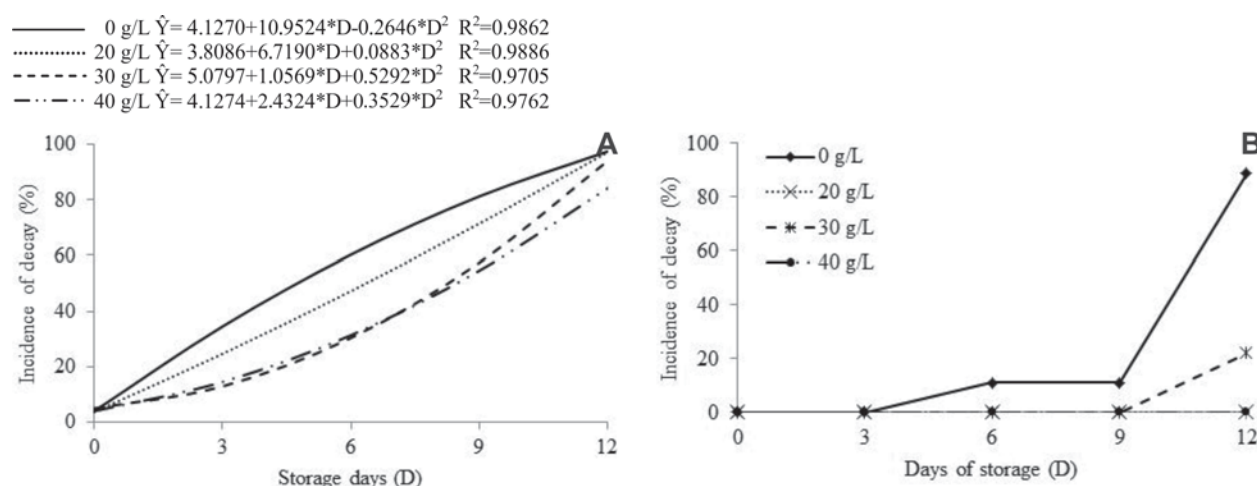
Figure 7. Incidence of decay (%) in 'Pedro Sato' guava treated with different concentrations of cassava starch without (A) and with (B) prochloraz during storage at 21.0 ± 1.0 °C

the incidence of fruit rot throughout the experimental period. The cassava starch has fungicidal and its benefit in rot control was indirect, the delay in ripening fruit (Botrel *et al.*, 2007).

Cassava starch film at a concentration of 40 g/L has provided similar results to those of 30 g/L; however, for fruit treated with 40 g/L of cassava starch, with or without prochloraz, unpleasant taste and odor can be noted, which suggest the existence of a fermentation process. Also for fruit treated with 40 g/L of cassava starch film, starch film peeling occurred in several fruits, which showed a need to add some affixing product.

CONCLUSION

The use of cassava starch film, with fungicide prochloraz, was effective in delaying the ripening of guava fruit, allowing them to extend their life for 12 days.

The suspension of 30 g/L of cassava starch was the most effective in maintaining the physicochemical characteristics of guava.

ACKNOWLEDGEMENTS

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