Investigation note

# Application of hydrothermal, fungicide and wax treatments on the superficial darkening of soursop

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## Abstract

The soursop (*Annona muricata* L.) is a climacteric fruit that has a short shelf life (6 to 8 days). This characteristic limits their transport and commercialization. The objective of the present work was to know the effect of some pretreatments on the superficial darkening in soursop. The effect of the hydrothermal treatment at 50 °C for 20 min, the application of the fungicide Azoxystrobin® at 300 mg $L^{-1}$ and the Semperfresh® coating (1:20) on the response to maturation and superficial darkening of soursop fruits was evaluated. stored at 16 ±2 °C and 25 ±2 °C. A shelf life of 7 days was observed in the treatments stored at 25 ±2 °C and 9 days for those stored at 16 ±2 °C, compared with the control that was 6 days. The combination of fungicide and the coating was the condition that produced the best results, because the darkening of the shell was delayed. The best treatments that showed the greatest control against decay and anthracnose were the application of the fungicide and the hydrothermal-fungicide treatment with 46 and 38.5% rot severity, respectively. The temperature of 16 ±2 °C was the best in keeping fruits viable for longer. The hydrothermal treatment caused an accelerated darkening of the shell.

**Keywords:** *Annona muricata* L., anthracnose, Azoxystrobin®, post-harvest life, Semperfresh®.

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The soursop (*Annona muricata* L.) is native to the tropical regions of America (Worrell *et al*., 1994). The cultivation of soursop has gained importance nationally and internationally due to beneficial effects such as antitumor, cytotoxic and antiparasitic (Pinto *et al*., 2005) and for its sensory characteristics appreciated by consumers. In Mexico, according to the SIAP (2017), the soursop was cultivated in 2015, mostly in the states of Nayarit, Colima, Michoacán and Guerrero. The production of soursop in Mexico in that same year was 16 620 t, with a value higher than 102 000 000 pesos (SIAP, 2017).

The soursop is highly perishable, because it ripens quickly. It has a high respiration rate and ethylene production. The rapid softening, and the darkening of the husk encourage the attack of fungi and other pathogens shortly after harvest (Ploetz, 2003; Mayer, 2006; Sunil *et al*., 2011). All of the above limits its storage and commercialization. The fruits have a maximum shelf life of 6 to 8 days after being harvested, and losses in postharvest have been reported due to darkening of the skin of around 30% (Tovar-Gómez *et al*., 2011). The use of different treatments that help to conserve quality, to prolong the post-harvest life and to prevent rots in fruits and vegetables include the application of edible coatings, combined with hydrothermal treatments. In mango, this combination reduced breathing rates and ethylene production (Alache *et al*., 1998; Pérez *et al*., 2004).

In studies carried out in passion fruit, it was found that hydrothermal treatment combined with fungicides reduced the severity of decay. There are reports that the application of fungicides with hot water allows to use lower doses of the product, achieving that the residues in fruits are within the maximum permissible limits and with the consequent reduction of operating costs (Yang *et al*., 2011). So far there are few studies of post-harvest treatments that exist in soursop fruits, so the general objective of this work was to know the effect of hydrothermal treatment, the application of fungicide and an edible coating, on surface darkening in soursop during ripening at two storage temperatures (16 and 25 °C ±2 °C).

The fruits were harvested at the stage of physiological maturity, from the municipality of Actopan and were donated by the Experimental Field Cotaxtla, Veracruz of the INIFAP, Veracruz. Thus, those with mechanical damage, infestation or insect bites were selected and discarded. They were randomly divided into 16 treatments at two storage temperatures: 16 ±2 °C and 25 ±2 °C. These were divided into groups for the application of the respective treatments, according to Table 1. For the hydrothermal treatment, the fruits were submerged in water at 50 ºC for 20 min. Subsequently, the treatment with fungicide (300 mg $L^{-1}$ of Azoxystrobin®) was applied by spraying. Finally, the Semperfresh® edible coating was applied by immersion using a concentration of 1:20 (wax:water).

The fruits were stored in chambers at 16 ±2 °C or 25 ±2 °C. Samples were taken every two days in fruits stored at 25 ±2 °C and every third day in stored at 16 ±2 °C. The experimental unit was 1 fruit and 3 biological repetitions were made.

**Table 1. Treatments applied to soursop fruits.**

| | |
|---|---|
| Control | |
| Hydrothermal treatment | TH |
| Fungicide treatment | TF |
| Coating treatment | TR |
| Hydrothermal treatment + fungicide | TH+TF |
| Hydrothermal treatment + coating | TH+TR |
| Fungicide treatment + coating | TF+TR |
| Hydrothermal treatment + fungicide + coating | TH+TF+TR |

The response variables were: weight loss, firmness, total soluble solids (°Brix), acidity as % malic acid, pH and color. The enzymatic activity of pectinmethylesterase (PME) was determined by titration with the modified method of Ranganna (1979). The activity of PME was considered as the mg of methoxyl released by the enzyme per gram of sample. The enzymatic activity of the polyphenoloxidase (PFO) was determined in the shell, from which 4 g were weighed and 20 mL of extracting solution (0.8 g of polyvinylpyrrolidone and 40 mL of 0.1 M phosphate buffer at pH 7) were added, homogenized for three minutes, it was filtered and the extract was centrifuged at 10 000 rpm for 20 min at 4 °C. The absorbance of each sample was measured at 410 nm. Respiratory rate and ethylene production were determined by gas chromatography. The results obtained were analyzed by a completely randomized design. An analysis of variance and Tukey's mean comparison test ($p \leq 0.05$) were performed with the statistical package SAS (version 9.1, SAS®, Cary, NC).

The speed of weight loss increased, independently of the storage temperature, and was significantly lower in fruits stored at 16 ±2 °C (5-11%) than in those stored at 25 ±2 °C (13-24%). This was to be expected, due to the reduction of all the physiological processes that are affected by the storage temperature. The TF+TR treatment, followed by the TR, were those that showed the least weight loss compared to the other treatments. Auxiliadora *et al*. (2004); Tovar-Gómez *et al*. (2011) used waxes in combination with 1-MCP and obtained a 23% decrease in weight loss, compared to the control. The firmness decreased significantly during ripening at the two storage temperatures. At both storage temperatures, the hydrothermal treatment preserved firmness for a longer time. This effect was more evident in fruits stored at 16 ± 2 °C, where the firmness was greater up to nine days of storage, compared to six days in the stored at 25 ± 2 °C. No significant differences were found in the rest of the treatments. These results were similar to the trials in guavas with hydrothermal treatment of 47 °C for 6 min, stored at 8 and 22 °C, in which a delay in the loss of pulp firmness was verified (Vieira *et al*., 2008). This suggests that the effect of hydrothermal treatments depends on different factors, such as temperature, exposure time and species, among others.

The content of total soluble solids increased during the first six days, independently of the storage temperature. The concentration increased from 9.7 to 18.83 °Brix at 25 ±2 °C and 16.96 °Brix at 16 ±2 °C, without finding a significant difference between treatments during ripening. The increase in total soluble solids content is attributed to the activity of enzymes that hydrolyze starch to simpler carbohydrates during ripening (Kader, 2002). The treatments TR and TF+TR at 16 ±2 °C and 25 ±2 °C respectively, retarded the evolution of the total soluble solids. Acidity

was not affected by hydrothermal treatment, fungicide, coating and combinations ($p \leq 0.05$). The pH decreased during the ripening of the fruits, independently of the storage temperature. The values went from an initial average value of 4.6, intermediate of 3.7 and final of 3.1 to 25 +2 °C and 16 +2 °C, during storage.

The luminosity was affected by the effect of temperature and treatments. In general, fruits at 16 ±2 °C with average values of 41.53 at the beginning, 48.76 at the intermediate and 46.24 at the end of storage had higher luminosity values than those stored at 25 ±2 °C, with average values of 41.53 at start, 43.79 to the intermediate and 36.73 to the end of storage. The °Hue showed no significant differences between both temperatures (25 ±2 °C and 16 ± 2 °C) and varied from 102 to 90°. In the Chroma it changed from 30.29 to 6.86 at 25 ±2 ºC and from 30.29 to 17.59 at 16 ±2 ºC. Both variables decreased during ripening due to the darkening of the epidermis that characterizes these fruits in senescence. In this range is the change from green to yellow until it reaches dimming.

The coating retained the visual characteristics of the fruits. The hydrothermal treatment (TH), its combinations with fungicide (TH+TF) and the fungicide with edible coating (TH+TF+TR) produced a greater darkening, which was similar to that found in the control fruits. The high temperature at which this treatment was applied and the storage temperature (25 ±2 °C) favored the darkening of the shell. The results indicate that as the weight loss increased during maturation and senescence, the darkening of the husk increased.

No differences were found in the activity of the PME during the ripening of the fruits at both storage temperatures. The activity of the PME in the pulp increased during the first days of maturation. In fruits stored at 25 ±2 °C, the maximum activity of PME was found after two days. In those stored at 16 ±2 °C, the control and treatment with edible coating exhibited the maximum PME activity at six days, while in the fruits with the TH+TF+TR treatment, the maximum activity was measured at three days. On the contrary, in the rest of the treatments this was delayed, occurring after nine days of storage.

The increase in activity of PME coincided with the formation of the maximum peak of ethylene production, showing that the activity of PME is regulated by this hormone. Fruits stored at 16 ±2 °C generally had lower PFO activity compared to those stored at 25 ±2 °C during ripening. At the end of the maturation the activity was 25-38 UAE $g^{-1}$ $min^{-1}$ for fruits stored at 25 ±2 °C and 10-18 UAE $g^{-1}$ $min^{-1}$ in the stored at 16 ±2 °C in shell. Studies conducted by Oliveira *et al*. (1994) showed that the activity of the PFO decreased with the progress of soursop maturation.

The breathing pattern shows two peaks at both storage temperatures. In fruits stored at 25 ±2 °C, the first peak of respiration reached values between 35 and 67 mL $CO_2$ $kg^{-1}$ $h^{-1}$ and at 16 ±2 °C, between 26 and 47 mL $CO_2$ $kg^{-1}$ $h^{-1}$ at second day after the harvest. This was probably induced by the physiological stress caused by the harvest, associated with the increase of carboxylates as respiratory substrates, mainly malic acid (Castillo *et al*., 2005). The second respiratory peak happened on the sixth day for the temperature of 25 ±2 °C with values of 21

to 40 mL $CO_2$ $kg^{-1}$ $h^{-1}$ and the ninth day for the temperature of 16 ±2 °C between 29 and 55 mL $CO_2$ $kg^{-1}$ $h^{-1}$. The latter coincided with a marked increase in the production of ethylene, which defines a climacteric behavior with the production of high amounts of $CO_2$ in comparison with other climacteric fruits. This is a determining factor for their post-harvest life, and indicates that the fruit enters the stage of senescence. Paull (1982) reported a maximum respiration rate of 108 mL $CO_2$ $kg^{-1}$ $h^{-1}$ during soursop maturation. On the other hand, Desmond *et al*. (1994) obtained values of 100 to 350 mL $CO_2$ $kg^{-1}$ $h^{-1}$ at 25-30 °C.

Ethylene production was lower in fruits stored at 16 ±2 °C that reached values of 9 to 34 µL ethylene $kg^{-1}$ $h^{-1}$ at the end of storage, compared to those stored at 25 ±2 °C that reached values of 9 to 31 µL $kg^{-1}$ $h^{-1}$. The increase in ethylene production was more evident in fruits stored at 25 ±2 °C than in those stored at 16 ±2 °C. A small peak of ethylene production occurred at two and three days of storage at 25 ±2 and 16 ±2 °C respectively. In the same order, a second increase in ethylene production began at 6 and 9 days of storage, when the second increase in the respiratory climacteric peak was exhibited, which leads us to propose that this increase is a response that begins when the ethylene has exceeded a certain threshold and is associated with the process of ripening and senescence of fruits (Worrell *et al*., 1994; Kader, 2002).

Several soursop studies have reported respiratory activities in which the ethylene release peak is located from four to six days after harvest, with rates ranging from 80 to 350 µL $kg^{-1}$ $h^{-1}$ (Paull, 1982; Worrell *et al*.,1994).

The treatments TH+TR, TF and TF+TR resulted in a lower incidence of decay in both storage temperatures, having 26.7, 33.3 and 36.7% incidence. With respect to severity, the fruits with TH+TF and TF treatments showed values of 38.5 and 46.2% (Table 2). These results indicate that the effect of the fungicide is greater when combined with hot water.

**Table 2. Incidence and severity of decay detected in soursop skin.**

| Treatments | Incidence (%) | Severity (%) |
|---|---|---|
| Control | 43.3 | 92.3 |
| (TH) | 46.7 | 61.5 |
| (TF) | 33.3 | 46.2 |
| (TR) | 40 | 61.5 |
| (TH)+(TF) | 43.3 | 38.5 |
| (TH)+(TR) | 26.7 | 53.8 |
| (TF)+(TR) | 36.7 | 53.8 |
| (TH+TF+TR) | 60 | 84.6 |

The combination between the hydrothermal treatment and the application of fungicides satisfactorily control perennial rots (Alahakoon *et al*., 1994). Aular *et al.* (2001) noted that the combination of these treatments satisfactorily controlled the rots in the passion fruit rind. There is a synergistic effect with the hydrothermal treatment and the fungicide, which is useful in the control of phytopathogenic fungi, since the spores of the same ones are in latent form at superficial level,

or between the first layers of the cells below the skin of the fruits. However, the lack of residual protection when hydrothermal treatment is applied allows recontamination by pathogens (Alahakoon *et al*., 1994). The effect of the hydrothermal treatment (TH) was similar to that produced by the edible cover (TR).

All treatments generated severity values lower than those of the control. Similar results were obtained by Alache *et al*. (1998), who submitted mango fruits in water at 46 °C for 65 minutes with the application of an edible coating, which were stored at 19.6 °C and 70% HR, where they could observe that the weight loss was reduced and the fruits reached 21 days of storage at room temperature, extending their useful life.

## Conclusions

The temperature of 16 ±2 °C was adequate for the storage of the fruits, since it allowed them to mature normally and reach a shelf life of 9 days compared with the control that obtained 6 days. The treatments with coating (TR) and the combination of the coating-fungicide (TR+TF) were the best. These helped retard the darkening of the fruit peel and reduced the weight loss by 11% compared to the control, but did not retard the severity of decay. The treatments that showed greater control in the severity of decay and anthracnose disease were those with fungicide (TF) and the hydrothermal-fungicidal treatment (TH+TF) with 46 and 38.5%. The hydrothermal treatment caused an accelerated darkening of the shell, causing loss of quality in its appearance.

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