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Harvest and post harvest

Antioxidant enzymes and antioxidant activity in two soursop selections (*Annona muricata* L.) from Nayarit, Mexico stored at 15 °C

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Abstract-The changes in concentration of vitamin C, enzymatic and antioxidant activity during the ripening of two soursop selections (G1 and G2) at room temperature (22 ºC) and refrigeration (15 ºC) with an HR 85% were evaluated. The content of soluble protein, the activity of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), the concentration of vitamin C, as well as the antioxidant activity was evaluated by DPPH, ABTS and FRAP methods. The initial soluble protein concentration of the G1 and G2 selections diminished at 22 and 15 °C during ripening. Fruits stored at 22 °C showed the highest CAT activity. The maximum activity of SOD was recorded on the sixth and fourth day in fruits stored at 22 and 15 ºC, respectively. Fruits stored at 22 °C recorded the highest amount of vitamin C. Fruits stored at 22 and 15 ºC showed the highest antioxidant activity on the fourth day. The fruits stored at 15 ºC was able to increase the shelf life up to 8 days without affecting the ripening process. Therefore, the enzymatic and antioxidant activity has an important role in the possible alteration that the fruit might suffer during its fruit ripening.
Index terms: *Annona muricata*, ripening, enzymatic activity, antioxidant activity, refrigeration.

Enzimas antioxidantes y actividad antioxidante en dos selecciones de Guanábana (*Annona muricata* L.) de Nayarit, México almacenadas a 15 °C

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Resumen-Se evaluaron los cambios en la concentración de vitamina C, actividad enzimática y antioxidante durante la maduración de dos selecciones (G1 y G2) de guanábana a temperatura ambiente (22 ºC) y refrigeración (15 ºC) con un HR 85%. Se determinó el contenido de proteína soluble, la actividad de catalasa (CAT), superóxido dismutasa (SOD), peroxidasa (POD), la concentración de vitamina C, así como la actividad antioxidante evaluada por los métodos de DPPH, ABTS y FRAP. La concentración de proteína soluble inicial de las selecciones G1 y G2 madurados a 22 y 15 ºC, disminuyó. Los frutos almacenados a 22 ºC mostraron mayor actividad de CAT. La máxima actividad de SOD se registró al sexto y cuarto día en los frutos almacenados a 22 y 15 ºC respectivamente. Los frutos almacenados a 22 ºC registraron la mayor cantidad de vitamina C. Los frutos almacenados a 22 y 15 ºC registraron la mayor actividad antioxidante al cuarto día. El almacenamiento a 15 ºC logró incrementar la vida de anaquel hasta por 8 días sin causar alteraciones en el proceso de maduración. Por lo tanto, la actividad enzimática y antioxidante tiene un papel importante en las posibles alteraciones que el fruto puede sufrir durante su maduración.
Palabras clave: *Annona muricata*, maduración, actividad enzimática, actividad antioxidante, refrigeración.

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Introduction

The worldwide production of soursop is concentrated in: Mexico, Brazil, Venezuela and Costa Rica (SÃO JOSÉ et al., 2014). In Mexico, there are 14 genera and 63 species of Annonaceae, in which the soursop fruits report the highest level of production in the market. At the national level, the state of Nayarit generates the 80% of the total volume of soursop production, being the leading worldwide producer of soursop, although 100% of its production is generated from ungrafted trees (AGUSTIN y HERNANDEZ, 2011, SIAP, 2016). However, fresh commercialization of soursop is limited due to the high perishability of the fruit. After the fruit is harvested, it reaches the maturity of consumption between 2 and 7 days depending on the stage of maturation in which were harvested (LIMA et al., 2011). In addition to the above, a large variability and quality exist in the soursop production due to the lack of commercial varieties, which difficult the postharvest handling. Recently, several efforts have been carried out to evaluate the morphological variability of soursop fruits and increase its shelf life in selections derivate from these investigations using postharvest technologies (ESPINOSA et al., 2013; JIMÉNEZ-ZURITA et al. 2016; JIMÉNEZ-ZURITA et al., 2017). Among the technologies to prolong the shelf life and maintain the quality of the fruit, the refrigeration is one of the most efficient and of general use. Nevertheless, soursop fruits possess cold sensibility, it suffers physiological damage when is storage at 4 to 18ºC (ALVES et al., 1997), detecting peel browning, increase of firmness of the pulp, loss of ripening capacity, pulp browning, loss of taste and ripening acceleration (BADRIE y SCHAUSS, 2010). However, diverse authors reported that fruits storage at 15ºC does not suffer cold damage, besides to extend the postharvest shelf life (ESPINOSA et al., 2013; JIMÉNEZ-ZURITA et al., 2017; SILVA et al., 2001).

Ripening and senescence can be considered as oxidative phenomena, activated at the beginning of the senescence. In order to delay this phenomena, fruits have a non-enzymatic system, in which it is induced the synthesis of numerous secondary metabolites (phenolic acids, flavonoids, tannins, vitamin C and terpenoids) (YANISHLIEVA et al., 2006) that have an antioxidant activity and help to decrease the reactive oxygen species (ROS). On the other hand, fruits also possess an enzymatic antioxidant system that is located in several compartments of the cell (ZHENG y WANG, 2001), including superoxide dismutase (SOD) that converts the superoxide anion (O_2^{-}) to peroxide (H_2O_2); which converts the H_2O_2 in water and the catalase (CAT) that eliminates the H_2O_2 (OUESLATI et al. 2010). Therefore, if at least one of these enzymes is active, there is the possibility that the senesce damage in the fruits get delayed (BALOIS-MORALES et al., 2008).

Experimentally, it has been demonstrated that the activity of some enzymes such as CAT, SOD, and POD decreased the ROS concentration, and in the case of tropical and subtropical fruits is increased by the cold during the storage (AQUINO-BOLAÑOS y MERCADO-SILVA, 2004; BAQUERO et al., 2005).

Considering the previously mentioned, the objective of the present work was to evaluate the enzymatic and antioxidant activity in two soursop selections during cold storage with the potential of industrial commercialization and fresh product sale for obtaining basic information that helps in future works to increase the postharvest shelf life.

Materials and methods

Plant material and location

We previously harvested fruits from two soursop selections (G1 and G2) from ungrafted trees in an orchard of 15 years old located in Tepic, Nayarit, México (21°32'2.77"N, 104°58'39.73"O, 893 mamsl) (JIMÉNEZ-ZURITA et al., 2016). G1 selection fruits showed low acidity, higher pH and intermediate values of total soluble solids (Table 1). The fruits of the G2 selection showed a higher number of seeds, total mass, the mass of peel and pulp, besides the highest value of titratable acidity (Table 1).

Table 1. Average values of morphological and chemical variables of two soursop selections in Tepic, Nayarit (JIMÉNEZ-ZURITA et al., 2016).

	NS	WS	WP	WP[1]	L*	C*	h	M	pH	TSS	TA
G1	104.55	73.07	246.26	728.43	40.12	16.13	159.41	1361.71	3.79	10.97	0.6
G2	187.60	123.28	264.13	1027.08	37.70	16.31	158.40	1718.48	3.58	10.44	0.8

NS = Number of seeds, WS = Weight of seeds (g), WP: Weight of peel (g), WP[1] = Weight of pulp (g); L* = lightness intensity (0: black, 100: white); C* = chromaticity (of gray); h = hue angle (0: red; 180: green); M = Weight (g); TSS = Total soluble solids (ºBrix); TA = Titratable acidity (%).

We used the harvest index used by the producers. The fruits with more than 160 days after anthesis and slight green or slight yellow color in the epidermis of the fruit, are adequate for the harvest. Soursop fruits were harvested between 7 and 8 a.m. and selected with no physiological damage or by pathogens. After, the fruits were placed in plastic boxes and transported to the Laboratorio de Producción Agrícola de la Facultad de Ciencias Agropecuarias of the Universidad Autónoma del Estado de Morelos. A total of 36 fruits per each selection (G1 and G2) were acclimatized by 1 h at room temperature and then placed in controlled temperature chambers (OLG HOT TEMP No. OLG-800D). The temperature and relative humidity were registered with a data logger (HOBO® U12, USA).

Experimental organization

We designed four treatments as mentioned next: 1) G1 and G2 selections stored at 22ºC; 85 % HR for 8 days, 2) G1 and G2 selections stored at 15 °C for 4 days and 4 days at 22 °C; 85 % HR. Pulp from soursop fruits was used to quantify the enzymatic activity, antioxidant activity, and soluble protein. Determinations were carried out in the start of the experiment, 4th and 6th days in the fruits stored at 22ºC. In the fruits stored at 15ºC, we performed the evaluations on day 0 and 4 after being moved to 22ºC. We used a completely randomized design, the experimental unit was six fruits with six measures. Data were analyzed by ANOVA and means comparison with a Tukey test ($P \leq 0.05$) using SAS® version 9.2, with the GLM procedure as indicates Castillo (2011).

Soluble protein

Soluble protein was determined by the Bradford method (1976). We mix 1 g of pulp in fresh with 7 mL of cold 0.1 M of Tris-HCl (pH 7.1) buffer by 30 s with a homogenizer (T8 IKA® Staufen, Germany). The sample was centrifuged at 4ºC (Z326K Hermle, Wehingen, Germany) for 20 min at 18510 *g*. We took 0.1 mL of the supernatant and mixed with 5 mL of Bradford reagent (0.01 % de coomassie blue G-250, 4.7 % ethanol and 8.5 % de phosphoric acid) place on vortex and incubated at room temperature for 12 min. The absorbance was measured at 595 nm. A calibration curve was generated using bovine serum albumin (Chem Cruz™) for the quantification of the protein. Results were reported in milligram per gram in fresh weight (mg g^{-1} f.w).

Catalase (EC. 1.11.1.6; CAT)

CAT activity was evaluated through the method of described by Alia et al. (2005) with some modifications, which consisted in mix 1 g of fresh pulp with 7 mL of a cold solution of 0.1 M Tris-HCl at pH 8.5, containing 0.1% polyvinylpyrrolidone, by 30 s in a homogenizer (T8 IKA® Staufen, Germany). Next, the samples were centrifuged at 4ºC for 20 min at 18510 *g* (Z326K Hermle, Wehingen, Germany). The measurement of the CAT activity was performed using quartz cell of 3 mL, containing 2.8 mL of 10 mM Tris-HCl (pH 8.5) and 0.1 mL of 0.88% of hydrogen peroxide in 100 mM of Tris-HCl (pH 8.5). In order to start the reaction, 0.1 mL of supernatant was added and the change in the absorbance was registered for 5 min in a spectrophotometer (HACH® DR 500, USA). The results were reported as units of enzymatic activity per milligram of protein (U mg^{-1} of protein). A unit (U) was defined as the change in the absorbance of 0.001 in one minute in 240 nm.

Superoxide dismutase (EC. 1.15.1.1; SOD)

One g of fresh pulp was homogenized by 30s with 7 mL of a cold solution of 0.05 M phosphate buffer, containing 0.1 M EDTA at pH 7.8, using homogenizer (T8 IKA® Staufen, Germany). Later, samples were centrifuged at 18510 *g* for 20 min at 4ºC. Enzymatic activity was measured according to the method of Beyer y Fridovih (1987). The reaction mixture was as described next: 27 mL of 0.05 M phosphate buffer at pH 7.8, containing 0.1 mM EDTA, 1.5 mL of L-methionine (30 mg mL^{-1}), 1 mL of nitro blue tetrazolium (1.41 mg mL^{-1}) and 0.75 mL of 1.0% of Triton X-100. 0.03 mL of riboflavin (4.4 mg 100 mL^{-1}) and 0.1 mL of supernatant were added to 3 mL of the reaction mixture previously mentioned. Then, the mixture was exposed to fluorescence lamps at 20 watts GroLux per seven minutes. After this time, lectures were taken at 560 nm. Enzymatic activity was reported as units of enzymatic activity per milligram of protein (U mg^{-1} of protein). U of SOD is equal to the supernatant quantity that photoinhibits in 50% the nitro blue tetrazolium farmazon formation.

Peroxidase (EC. 1.11.1.7; POD)

One g of soursop pulp was homogenized (T8 IKA® Staufen, Germany) in 7 mL of a cold solution of 100 mM Tris-HCl (pH 7.1) containing 1.0% of polyvinylpyrrolidone) and then centrifuged at 18510 *g* for 20 min at 4ºC. Enzymatic activity was quantified as reported by Flurkey y Jen (1978), which consisted in a reaction mixture of 2.5 mL of Tris-HCl (100 mM, pH 7.1), 0.25 mL of 0.1 M guaiacol, 0.1 mL of 0.25% hydrogen peroxide and 0.15 mL of the supernatant. The results were reported as units of enzymatic activity per milligram of protein (U mg^{-1} of protein). U was defined as the change in the absorbance in 0.001 min^{-1} at 460 nm.

Vitamin C

Quantification of vitamin C was performed by the method of Jagota y Dani (1982). We added 0.8 mL of 10 % (m/v) of trichloroacetic acid (TCA) to 0.2 mL of the sample obtained from homogenized pulp (7 g) with 6 mL of TCA. Then, the samples were placed in an ice bath for 5 min. Later, the samples were centrifuged at 18510 *g* for 10 min at 4ºC. We added 0.2 mL of Folin-Ciocalteu reagent (diluted 1:10) to aliquots of 0.5 mL of supernatant, diluted with 2 mL of double-distilled water. The mixture was incubated for 10 min and the absorbance was measured at 760 nm. A standard curve with ascorbic acid was performed to estimate the vitamin C content. Total concentration was expressed as mg ascorbic acid (AA) per 100 g of fresh weight (mg of AA 100/g.f.w).

Antioxidant activity by the DPPH method

In order to determinate the antioxidant activity, one g of pulp was homogenized with 10 mL of distilled water for 30 s and then centrifuged at 18510 *g* for 20 min at 4ºC. The supernatant was used to evaluate the antioxidant activity. The antioxidant capacity was determined by the DPPH method using the technique established by Brand-Williams et al. (1995). 100 μL of the supernatant was added to the DPPH solution of $6.1x10^{-5}$ M (Sigma Aldrich®, USA). The reaction mixture was incubated in darkness for 30 min and the absorbance was measured at 517 nm. Antioxidant activity was evaluated using a standard curve with ascorbic acid (0-100 mg L^{-1}). Data were expressed as mg of ascorbic acid equivalents (mg AAE/100 g.f.w).

Antioxidant activity by the ABTS method

Antioxidant activity was performed using the technique reported by Re et al., 1999. We mixed 7 mM of ABTS and 2.45 mM potassium persulfate in equal proportions (1:1). The mixture was incubated during 16 h and then diluted with 20% ethanol until reach an absorbance of 0.7 ± 0.02 a 734 nm. 100 μL was mixed with 3 mL of ABTS, incubated for 15 min and then the absorbance was measured in the wavelength previously mentioned. Data were expressed as mg ascorbic acid equivalents (mg AAE/100 g.f.w).

Antioxidant activity by the FRAP method

Antioxidant activity was evaluated by ferric reducing antioxidant power (FRAP) as reported by Benzie y Strain (1996). We prepared the FRAP reagent (TPTZ, $FeCl_3$ and acetate buffer). After, 1.8 mL of FRAP reagent was mixed with 140 μL of distilled water and 60 μL of the sample (sample was obtained in the same way as mentioned in antioxidant activity). After the mixture was incubated for 30 min at 37ºC and then the absorbance was measured at 593 nm. Data were expressed as mg ascorbic acid equivalents (mg AAE/100 g.f.w).

Results and discussion

Soluble protein

The concentration of soluble protein diminished during ripening in both selections at 22ºC, in physiological maturity with values of 102.7 (G1) and 135.5 (G2) mg g^{-1} f.w and 17.7 (G1) and 15.6 (G2) mg g^{-1} f.w in consumption maturity (Figure 1A), statistically significant differences ($P \leq 0.05$) between the two selections were detected (Figure 1A). The fruits stored at 15ºC for 4 days and transferred to 22ºC showed a similar tendency than fruits stored constantly at 22ºC (Figure 1A). After the storage of low temperature, we quantified the highest concentration of soluble protein with average values of 50 mg g^{-1} f.w in both selections (Figure 1A). Four days after storage, we registered the minimum concentration with average values of 26.5 mg g^{-1} f.w. for both selections. No significant differences ($P > 0.05$) were found between both soursop selections (Figure 1A). The reduction of the protein levels could be related with the hydrolysis of the proteins for the formation of more simple compounds (aminoacids and peptides) that could be useful for the function and metabolism of the fruit (CASANO y TRIPPI, 1992). On the other hand, some authors have reported that can show a protein degradation by the effect of ROS associated with the oxidative stress generated during fruit ripening, which can be reacted with diverse molecules, including DNA and proteins (HODGES, 2003; BLOKHINA et al., 2003). Camargo et al., (2016) reported that the concentration of soluble protein in fruits of loquat decreased at stored temperatures of 6, 10 and 15ºC and then relocated to 27ºC. This is probably because several changes and metabolic routes that implies the synthesis of proteins, that could be inhibited due to the low temperature at which the fruit was exposed (DUEÑAS-GÓMEZ et al., 2008; ALIA et al., 2005). Zhou et al., (2015) mentioned that the low protein content found in the refrigerated fruits pulp can be attributed to the decreased of the metabolic rate originated by the postharvest treatments and stored in refrigeration.

Catalase (EC. 1.11.1.6; CAT)

CAT activity was significantly increased during fruit ripening at 22ºC in both soursop selections (Figure 1B). CAT activity increased 6.87 U in G1 and 3.13 U in G2 in physiological maturity, and 14.80 and 10.31 U in consumption maturity for G1 and G2, respectively (Figure 1B). G1 selection showed the highest activity ($P \leq 0.05$; Figure 1 B). The increase of the CAT activity might be due to the high respiration rate and others oxidative process that occurs during ripening (DJANAGUIRAMAN et al., 2010). Once the consumption maturity has been accomplished, the fruit continues with the oxidative process leading to senescence, causing the appearance of necrotic areas, which may be a consequence of the imbalance between the high concentration of oxygen and the low activity of CAT (FOYER y NOCTOR, 2005).

It is important to mention that the behavior of the CAT activity may be due to the fruit variety. A previous work reported an increase and diminution of CAT activity in orange fruits during its maturation (HUANG et al., 2007). Fruits stored at 15ºC of the G1 selection showed an increase in the CAT activity from 3.34 to 9.95 U after day four (Figure 1B). On the other side, in the G2 selection, CAT activity showed values between 5.19 and 3.92 U in the same period (Figure 1B). Clearly, the G1 selection showed the highest activity ($P \leq 0.05$) of after transferred the fruits to room temperature (Figure 1B). CAT activity diminished in refrigerated fruits of yellow pitaya and then transported to room temperature due to a possible alteration in the transcription of this enzyme, and as a consequence, an increase in the concentration of H_2O_2 is observed during the oxidative metabolism (DUEÑAS-GÓMEZ et al., 2008). CAT activity has been reported in mamey sapote (*Pouteria sapota*) and pitahaya (*Hylocereus undatus*) in refrigeration and then transferred to room temperature, indicating that the activity of this enzyme can be increased or maintained with no change during ripening (ALIA et al., 2005; BALOIS-MORALES et al., 2008). These differences might be dependent on the specie, time of harvest and storage, among others (BALOIS-MORALES et al., 2008).

Superoxide dismutase (EC. 1.15.1.1; SOD)

SOD activity was significantly increased during the ripening of the soursop selections at 22ºC. We detected an increase from 6.73 to 47 U for the G1 selection, while the G2 selection showed an increase in the activity from 5.69 to 44 U (Figure 1C). Conversely, fruits stored at 15ºC for 4 days and then transferred to 22ºC showed the same tendency to increase the SOD activity (Figure 1C). No significant differences ($P > 0.05$) were detected between the soursop selections evaluated (Figure 1C). During the maturation of the fruit, ROS generate an oxidative damage, and its level can be regulated by the activity of antioxidant enzymes such as SOD and CAT (FERREIRA-SILVA et al., 2012). The resistance of the fruit to low temperatures and oxidative damage is associated with the activity of these enzymes more than other physiological factors, being involved in the ROS elimination produced by the oxidative stress (POLATA et al., 2009). Besides, the behavior of the enzymatic enzyme varies according to the maturity stage and the type of the fruit (CAMEJO et al., 2010). It has been reported the increase of the SOD activity in the different stages of fruit ripening, such as in cherry and guava, indicating that SOD plays a key role in the senescence-related with the free radicals (MONDAL et al., 2009). JIMÉNEZ et al., (2002) in tomato (*Lycopersicum esculentum* Mill.) reports the increase of SOD activity during the overripe stage, however, these enhance is not correlated with the gene expression in SOD. The previously mentioned coincides with the established by Cuadra-Crespo y del Amor (2010) that evaluated the SOD activity in red pepper stored under cold conditions (5ºC) and high relative humidity (95%), founding an increase in its activity with no damage. With the results obtained in this investigation, we can assume that the increase of the SOD activity during the storage at 22ºC and 15ºC, might confer a protection to the integrity of the cellular membrane.

Peroxidase (EC. 1.11.1.7; POD)

POD activity in soursop fruits of G1 selection stored at 22ºC diminished from 48.84 U to 23.31 U on the fourth day of maturation. After, an increase of 33.96 U on the sixth day was observed (Figure 1D). On the other hand, in the G2 selection, POD activity increased from 19.50 U to 98.10 U at the fourth day and it was maintained in 83.26 U at the sixth day of ripening (Figure 1D). The activity of POD was higher in the G2 selection ($P \leq 0.05$; Figure 1 D). POD catalyzes the oxidation of phenols to quinones, producing brown colors, however, this also can degrade ROS, specifically the H_2O_2, nevertheless no generation of brown color is observed, which can be classified as an antioxidant enzyme (CHEN et al., 2016). POD activity in soursop fruits stored at 26ºC has been reported by Lima et al. (2002), recording the highest activity on the third day and a huge decrease on the fifth day of storage. The decrease in the POD activity to the end of the storage can be related with the senescence of the fruits, in that the products of the enzymatic reaction can inhibit the enzymatic activity of POD (DUEÑAS-GÓMEZ et al., 2008). POD enzymatic activity of fruits stored at 15ºC was increased after storage, from 7.25 to 67.40 U in the G1 selection and maintains between 100.41 and 119.24 U in the G2 selection (Figure 1D). Soursop fruits of the G2 selection showed more POD activity ($P \leq 0.05$; Figure 1 D).

Previous reports have been demonstrated the increase of the POD activity during the storage of fruits in refrigeration and then moved to room temperature (ALIA et al., 2005; DUEÑAS-GÓMEZ et al., 2008; BALOIS-MORALES et al., 2007). These increases can be related with the generation of an oxidative stress induced by the exposition to low temperatures, which can cause a liberation of the POD to the cytoplasm, increasing its activity (MARTÍNEZ-DAMIÁN et al., 2013).

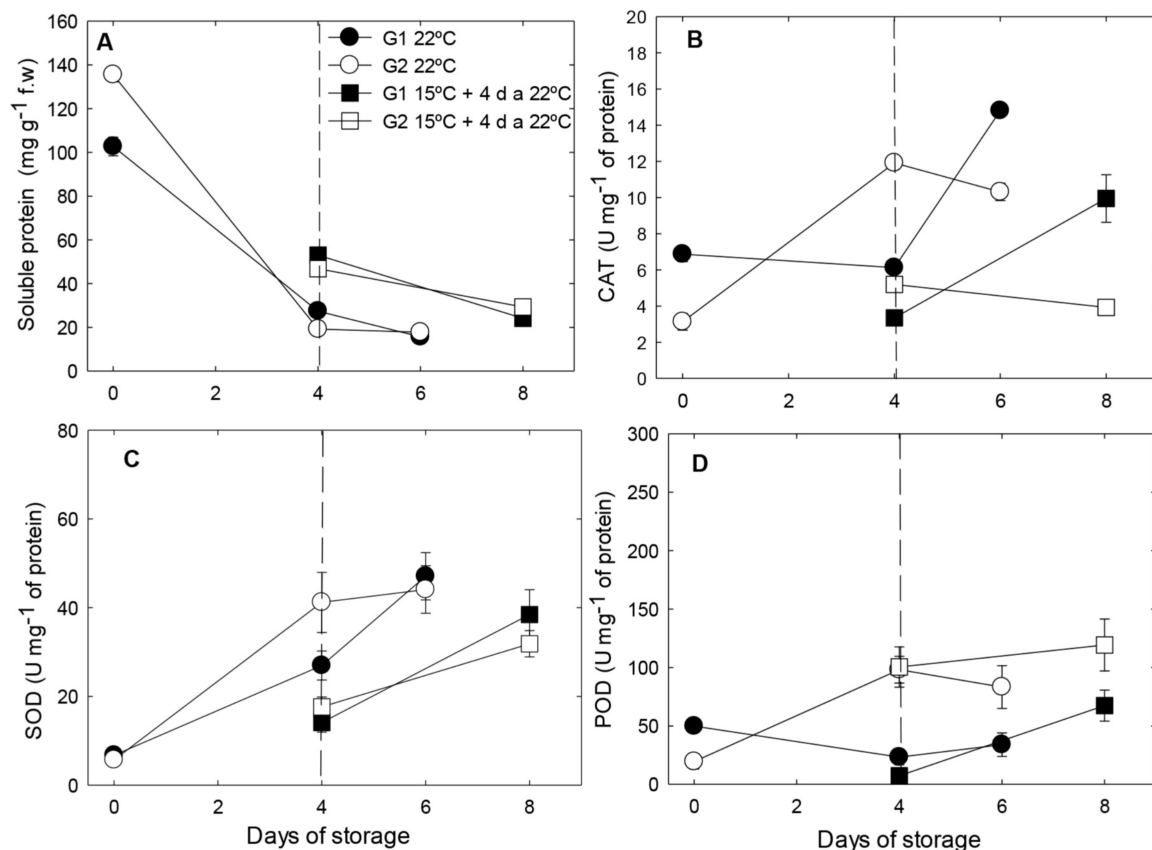

Figure 1. Soluble protein (A), CAT (B), SOD (C) y POD (D) in fruits of two soursop selections (G1 y G2) stored at 22 and 15 °C (± 2). Each point indicates the mean of six observations ± standard error. Honest minimum significant difference (DSMH) = 6.54 (A), 2.24 (B), 2035.9 (C), 57.29 (D). The dotted line indicates the transfer to 22 °C.

Vitamin C

Vitamin C content was increased in the fruits exposed to 22ºC after the initial day, specifically the G1 selection, that reached 44.2 mg AA/100 g.f.w on the sixth day of evaluation (Figure 2A). The G1 selection was higher ($P \leq 0.05$) in comparison with the G2 selection (Figure 2A). The increase in the concentration of vitamin C during soursop ripening stored at room temperature can be attributed to three possible causes: 1) the catabolism of the starch and carbohydrates of the cell wall, 2) the transformation of acid salts in free form and 3) the low utilization of organic acids during respiration (PAULL, 1982). No significant changes were observed ($P > 0.05$) in the vitamin C content when the fruits were stored at 15ºC and then transferred to 22ºC (Figure 2A). It has been reported that in tropical fruits such as kiwi and guava the storage of low temperatures and then transferred to its ripening to room temperature, it has been efficient in the reduction or loss of vitamin C (TAVARINI et al., 2008; SUÁREZ et al., 2009). Nevertheless, a published article indicates that soursop stored at 16ºC can suffer cold damage, producing a reduction in the synthesis of this compound (MORENO-HERNÁNDEZ et al., 2014). Vitamin C is a non-enzymatic compound that contributes to the antioxidant capacity, therefore, its conservation is important during the postharvest handling (SHIVASHANKARA et al., 2004). The results obtained in the present investigation coincides with the previously mentioned because both selections showed a diminution in the vitamin C content in refrigeration.

Antioxidant activity by the DPPH method

The antiradical activity evaluated by the DPPH method in fruits stored at 22ºC increased after four days, showing maximum values of 67.67 (G1) and 56.86 (G2) mg AAE/100 g.f.w on the fourth day. Later, in the G1 selection, the activity decreased to 44.71 mg AAE/100 g.f.w (G1). On the G2 selection, the activity reached values of 53.19 mg AAE/100 g.f.w (Figure 2B). No significant differences ($P > 0.05$) were found between the two selections (Figure 2B). The soursop is a fruit that shows antioxidant properties with potentially useful applications (SYAHIDA et al., 2012). Akomolafe y Ajayi (2015) mentioned that the capacity to catch the DPPH radical could be involved in the phenolic compounds of the soursop fruits, which are capable to neutralize the free radicals. Kuskoski et al. (2005) reported the antiradical activity of soursop fruits in 46.66 ± 1.6 mg AA/100 g, which is similar with the values showed in this investigation. Melo et al., (2008) mentioned that the

antioxidant activity (DPPH) of soursop is moderate in comparison with other fruits such as passion fruit. The fruits exposed to 15ºC increased its antiradical activity, because both groups showed initial values of 56.86 (G1) and 40.80 mg AAE/100 g.f.w (G2) at the end of the storage. These values increased after four days at room temperature with values of 138.35 and 76.77 mg AAE/100 g.f.w, (G1 and G2, respectively) (Figure 2B). Statistical significant differences were found between selections ($P \leq 0.05$) indicating that G1 selection, showed the highest antioxidant activity by this method (Figure 2 B). The antioxidant activity of the soursop fruits may be due the presence of hydroxide groups of the phenolic compounds (AKOMOLAFE y AJAYI, 2015), in addition to the fact that low storage temperatures can induce the expression of these compounds, as reported by Jin et al., (2011), indicating that the antioxidant capacity and the production of phenolic compounds was increased in strawberries stored at 10ºC.

Antioxidant activity by the ABTS method

The antioxidant activity by ABTS showed initial values of 35.36 and 45.30 mg AAE/100 g. f.w, in fruits stored at 22ºC, four days after and increased between 77.07 y 86.28 mg AAE/100 g.f.w was observed, and finally, in the sixth day, a decreased was observed with values of 47.41 and 62.98 mg EAA/100 g.f.w (Figure 2C). No significant differences were found ($P > 0.05$) between varieties (Figure 2C). The increase of the antioxidant activity on the ABTS radical could be related with the high metabolic activity in ripening and the climacteric period, besides of the ascorbic acid action, which presents a high concentration the days after harvest (PROTEGGENTE et al., 2002). Huang et al. (2007) mentioned that the diminution of the antioxidant activity (ABTS) can be related with the decrease of the phenolic compounds towards the end of ripening. This agrees with the reported by Jiménez-Zurita et al., (2017) who found a diminution of phenolic compounds in the final stage of maturation for the same selections of soursop. The results found in this investigation coincides with the reported by Kuskoski et al., (2005), who exhibited values of 76.8 ± 4.0 mg AA/100 g in soursop peel. G1 and G2 selections stored for four days at 15ºC and then transferred to 22ºC, showed a differential behavior, due to the G2 selection significantly increased its activity of 67.60 mg AAE/100 g.f.w to 110.88 mg AAE/100 g.f.w until day fourth after storage in comparison with the G1 selection (Figure 2C). The increase of this activity can be attributed to the low temperature in that the soursop fruits were stored. This is due to the reactions that continue after the harvest, forming responsible compounds of this increase. For example, reactions produced through the phenolic metabolism or reaction produced by the cellular rupture caused by the low temperature (PILJAC-ŽEGARAC y ŠAMEC, 2011; DE ANCOS et al., 2000). Besides of the previously mentioned, there are several factors that have a direct effect on antioxidant activity, such as the cultivar, harvest season, genetic or environmental factors, storage temperatures, and maturity (JIN et al., 2011).

Antioxidant activity by the FRAP method

The fruits stored at 22ºC showed a significant increase during the first four days, reaching values of 119.28 and 108.95 mg AAE/100 g.f.w (Figure 2D), however, no significant differences ($P > 0.05$) between selections was detected. The antioxidant activity by FRAP method has also been reported in soursop fruits matured at room temperature (22-25 ºC) (PADMINI et al., 2014; AKOMOLAFE y AJAYI, 2015.). FRAP can be related with the high metabolic activity of the ripening and the climacteric period, the action of the ascorbic acid, which presents high concentration days after harvest, besides of the decrease or the increase of the phenolic compounds (HUANG et al., 2007). Nevertheless, the antioxidant activity (FRAP) also can depend on the diverse types of substances with antioxidant activity in the fruit and different action sites of these substances (SUCUPIRA et al., 2012). G2 selection stored at 15ºC for four days showed a similar tendency to the fruits exposed at 22ºC because the reducing capacity was increased from 40.99 to 73.64 mg AAE/100 g.f.w (Figure 2 D). On the other hand, G1 selection fruits showed a decrease from 50.81 to 37.14 mg AAE/100 g.f.w in the reducing capacity (Figure 2D). Significant differences were detected between selections ($P \leq 0.05$) (Figure 2D). Moreno-Hernández et al. (2014) reported less antioxidant activity for soursop fruits stored at 16ºC, proposing that this activity can be related to the amount of vitamin C present in the fruit. Chongchatuporn et al. (2013) stated that in two mango cultivars (cvs. Nam Dok Mai y Choke Anan) stored at low temperatures (4 y 12 ºC), the antioxidant activity of the Nam cultivar was higher than the Choke Anan cultivar. This is because antioxidant compounds are synthesized in response to the stress generated by low temperatures (SOMOGYI et al., 2007). These results coincide with the reported in this investigation since the G2 selection presented a greater antioxidant activity than G1 selection. The antioxidant activity may depend on genetic factors, varieties, postharvest handling and, therefore, the evaluation during fruit ripening may be affected by storage conditions, temperature, among others (JAVANMARDI y KUBOTA, 2006).

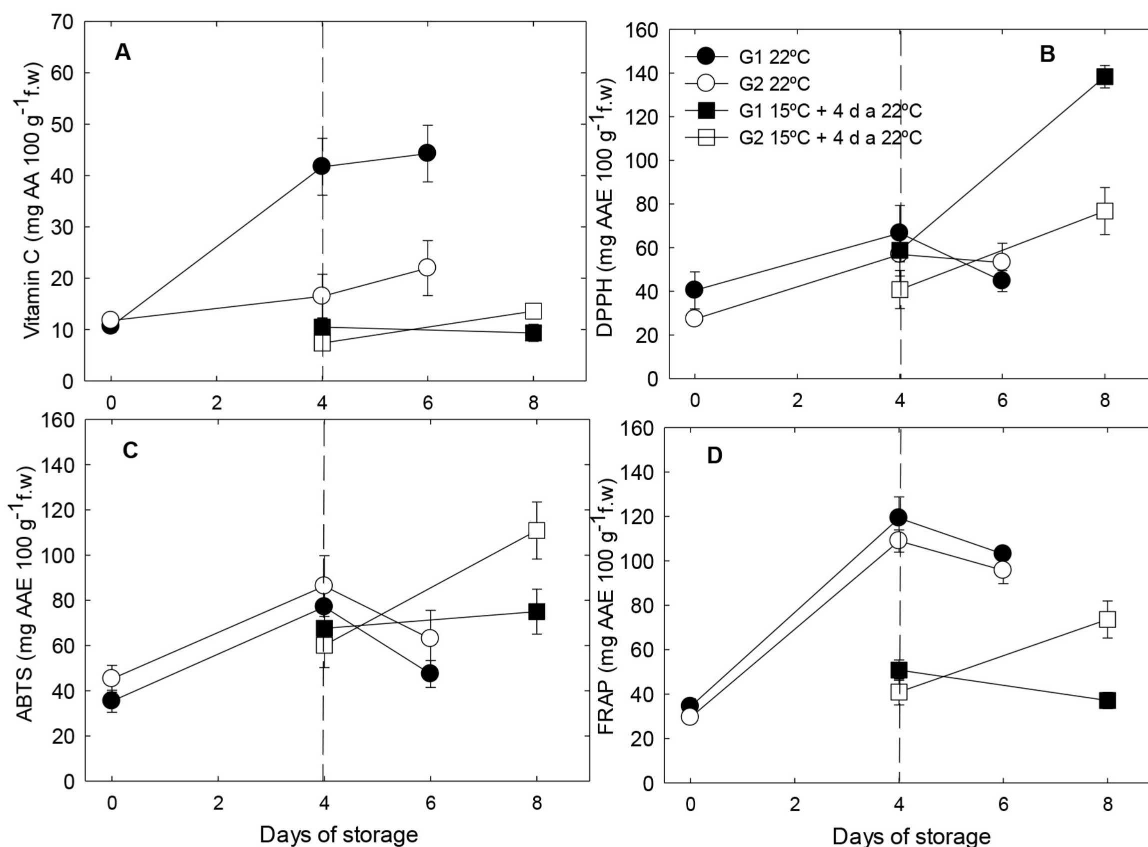

Figure 2. Vitamin C (A), antioxidant activity by the DPPH method (B), ABTS (C) and FRAP (D) in two soursop selections (G1 y G2) stored at 22 and 15 °C (± 2). Honest minimum significant difference (DSMH) = 9.47 (A), 21.81 (B), 23.27 (C), 13.18 (D). Each point indicates the mean of six observations ± standard error. The dotted line indicates the transfer to 22 °C

Conclusions

The maturation of soursop fruits presents a decrease in the concentration of soluble protein, as well as an increase in enzymatic activity. Also, an increase in the concentration of vitamin C and antioxidant activities (DPPH, ABTS, and FRAP) were observed. The synthesis of vitamin C was affected in a significant manner by the fruits stored at 15ºC. G1 selection showed a low POD activity, probably due to the high CAT activity, vitamin C and DPPH. The storage at 15ºC does not affect the enzymatic activity of CAT, SOD, and POD, however, antioxidant activity was increased. Fruits stored at 15ºC was not affected in its ripening, due to the enzymatic and antioxidant activity.

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