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Changes in postharvest quality and antioxidant metabolism during development and ripening of sapodilla (*Manilkara zapota* L.)

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Abstract

Reactions involving reactive oxygen species (ROS) are intrinsic to fruit ripening and antioxidant system play an essential role during its development. The present research work describes the major changes in the antioxidant metabolism during development of sapodilla fruit from two cultivars. For both cultivars, ripening improved fruit physicochemical quality parameters. 'Sapoti Ipa-Curu' (BRS 227) at ripe stage presented more sweetness (20.87 ºBrix) in comparison to 'Sapota Tropical' (BRS 228). However, total vitamin C, yellow flavonoids and total soluble phenols (TSP) contents declined during development, which resulted in a lower total antioxidant activity (TAA). At ripe stage, the 'Sapota Tropical' (BRS 228) reached for yellow flavonoids (3.16 mg/100 g^{-1}), TSP (1.63 mg GAE/100 g^{-1}) and antioxidant activity (0.13 x 102 µM Trolox/g^{-1}). The activities of oxygen-scavenging enzymes also decreased with ripening; furthermore, the reduction in antioxidant enzymes activity was inversely correlated to membrane lipid peroxidation, indicating that sapodilla ripening is characterized by a progressive oxidative stress.

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Introduction

Fruit ripening is a complex developmental process, highly regulated and irreversible involving a series of physiological, biochemical and structural changes as well as changes in oxidative metabolism leading to an attractive, edible, and ripe fruit (Zhu *et al.*, 2008; Pal Singh *et al.*, 2012). Reactions involving reactive oxygen species (ROS), like H2O2, superoxide anion, are intrinsic to senescence and fruit ripening as they promote the oxidative process that contributes to a general deterioration of cellular metabolism (Jiménez *et al*., 2002).

During various metabolics activities ROS have been continuously produced especially in stress conditions initiating and enhancing degenerative processes associated with ripening and senescence, along with expression of several defense genes (Rogiers *et al.,* 1998). The generated ROS cause oxidative injury to lipid membrane, nucleic acids and proteins and such type of damage could be protected by the activation of different antioxidant defense enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) and also by non-enzymatic antioxidante system including water-soluble compounds such as glutathione, ascorbic acid and phenolics compounds, and lipid-soluble such as carotenoids and tocopherols (Pal Singh *et al.*, 2012). The antioxidants play an essential role not only in maintaining ROS production and neutralization rates in equilibrium under non-stressful conditions, but also determining the level of oxidative stress (Torres and Andrews, 2006).

In addition to changes in the chemical composition and physical characteristics of the fruit taking place during ripening other changes include important increases in respiration and, as a consequence, an enhancement in the ROS accumulation as well as changes in the antioxidante activities which promote oxidative stress that contributes to a

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general deterioration of cell metabolism (Camejo *et al*., 2010). The development of oxidative stress during ripening has been reported in a range of fruit as a result of gradual decrease in the activities of antioxidante enzymes, lower levels of antioxidants compounds or both (Camejo *et al.*, 2010; Oliveira *et al*., 2012). According Menichini *et al*. (2009) the content of phytochemicals as antioxidants in plants is influenced by numerous factors as weather, genotype, cultivation methods and maturity stage. In addition, previous studies have also shown that cultivars differing in antioxidant metabolism also differed in their potential storage or shelf-life and susceptibility to various physiological disorders (Hodges *et al*., 2001).

Sapodilla (*Manilkara zapota* L.) is a climateric tropical fruit, and ripening is controlled by the production of ethylene. The fruit is originated from Central America known for its sweet flavor, however and in spite of such potential marketing quality; its physiology has not been thoroughly investigated. When ripe, fruit present high phenolic content (4.5%) which are the main responsible for its great antioxidant capacity, for the contribution of L-ascorbic acid to sapodilla total antioxidant capacity is insignificant (<0.1%), due to its low levels (12 mg.100 g-1) (Leong and Shui, 2002).

Give the importance of antioxidant metabolism in postharvest fruit ripening; it's necessary to understand the dynamics of enzymatic and non-enzymatic antioxidant during fruit ripening stages. Thus, this study investigated changes in the postharvest quality and physiology of antioxidant metabolism during sapodilla development and ripening, thereby, also aimed at establishing the optimum harvest stage to ensure that fruit reach consumers or the food industry with maximum functional properties and quality attributes.

Materials and Methods

Plant material

Sapodilla from 'Sapota Tropical' (BRS 228) and 'Sapoti Ipa-Curu' (BRS 227), proceeding from the Experimental Station of the Vale do Curu of the Embrapa Tropical Agroindustry, located at Paraipaba-CE, Brazil, were tagged after fruitset with 10 mm in transversal diameter and hand harvested after 90 after pegged and then at each 30 days as follow: 120, 150 and 180 days later (at physiological maturity). Parts of fruits harvested at physiological maturity were stored at ambient condition during 10 days at 25°C and 60% RH, until achieve the ripe stage. Then, fruit from all developmental stages were evaluated regarding their quality and components of antioxidant metabolism. Sapodilla pulp was processing using an omnimixer (Ultraturrax IKA®, Germany) diluted 1:1 in distilled water (w/v) and the homogenates were frozen at -80°C for further analysis.

Quality and non-enzymatic antioxidants

Fruit from each cultivars at different stages of development and mature were weighed on a balance of accuracy of 0.01 g. Titratable acidity (AT) of sapodilla pulp was evaluated as determined by AOAC (2005) using an automatic titrator (Mettler-Toledo® DL12, Columbus, USA) and results were expressed as percent of malic acid. The pH was measured using an automatic pH-meter (Labmeter PHS-3B®, Brazil) as recommended by AOAC (2005). Soluble solids (SS) content was determined by refractometry as described by AOAC (2005) using a digital refractometer (ATAGO® N1, Kirkland, USA) with an automatic temperature compensation. The results were expressed in oBrix (concentration of sucrose w/w).

The total vitamin C was determined by titration with 0.02% DFI as method described by Strohecker and Henning (1967) and results were expressed as milligrams per 100 g of fresh weight (FW). Total yellow flavonoid were extracted and determined as described by Francis (1982). The absorbance was measured at 374 nm for the total yellow flavonoid content using an absorption coefficient of 76.6 mol/cm. Results were expressed as mg/100 g-1 FW. The total phenol content of sapodilla was measured by a colorimetric assay using Folin-Ciocalteau reagent as described by Obanda and Owuor (1997). Before the colorimetric assay, the samples were subjected to extraction in 50% methanol and 70% acetone as described by Larrauri *et al.* (1997). Results were expressed as gallic acid equivalent (GAE), milligrams per 100 g^{-1} FW.

Total antioxidant activity (TAA) and lipid peroxidation degree

The TAA was determined using the 2,2-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) method as described by Rufino *et al*. (2006). Before the colorimetric assay, the samples were subjected to a procedure of extraction (Larrauri *et al*., 1997). Once the radical was formed, the reaction was started by adding 30 µL of extract in 3 mL of radical solution, absorbance was measured (734 nm) after 6 min, and the decrease in absorption was used to calculate the TAA. A calibration curve was prepared, and different Trolox concentrations (standard trolox solutions ranging from 100 to 2000 µM) were also

evaluated against the radical. Antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC), micromoles of Trolox/g-1 FW.

Lipid peroxidation was measured by the formation of malondialdehyde (MDA) based on the method of Zhu *et al*. (2008). Measurements of absorbance at 532 nm were corrected for unspecific turbidity by subtraction from the absorbance at 600 nm and TBARS (Thiobarbituric acid reactive substances) was calculated using an extinction coefficient of 155 M^{-1} cm^{-1}, expressed as nmol/TBARS g^{-1} FW.

Activity of antioxidants enzymes

Two grams of fruit pulp was homogenized in 10 mL of 0.1 M potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA for 1 min, followed by centrifugation at 3248 x g for 40 min at 4°C (Yang *et al.*, 2009). The supernatant fraction was used as a crude extract for the enzyme activity assays, and all procedures were performed at 4°C. The total protein content was determined according to the method of Bradford (1976) using bovine serum albumin (BSA) as a standard. Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined spectrophotometrically on the basis of the inhibition of the photochemical reduction of NBT (Giannopolitis and Ries, 1977) The reaction mixture absorbance was measured by a Spectrum SP 2000UV spectrophotometer at 560 nm, and 1 unit of SOD activity (UA) was defined as the amount of enzyme required to cause a 50% reduction in the NBT photoreduction rate. Thus, results were expressed as UA per milligram of protein (P). Catalase (CAT, EC 1.11.1.6) activity was measured according to the method of Beers and Sizer (1952). The reaction started by adding the enzyme extract, and then the decrease in hydrogen peroxide (H_2O_2) was monitored through absorbance at 240 nm and quantified by its molar extinction coefficient (36 M^{-1} cm^{-1}). One unit of CAT activity (UA) was defined as the amount of enzyme required to decompose H_2O_2 (μmol H2O2/min) and the results were expressed as UA per milligram of P. Ascorbate peroxidase (APX, EC 1.11.1.1) activity was assayed according to the method of Nakano and Asada (1981). Enzyme activity was measured using the molar extinction coefficient for ascorbate (2.8 M^{-1} cm^{-1}), considering that 1 mol of ascorbate is required for a reduction of 1 mol of H_2O_2. Results expressed as UA per milligram of P, when a unit of enzyme activity (UA) is micromoles of H_2O_2 per minute.

Statistical analysis

The experimental design was completely randomized in factorial 2 x 5 (cultivars x developmental stages) with four replications of five fruits. The data obtained were subjected to analysis of variance (ANOVA) using a computer program (SISVAR 3.01) and the averages were compared by Tukey´s test at 5% probability. The Pearson's correlation coefficient was calculated for all variables at 1% and 5% significance levels.

Results and Discussion

Important changes in physicochemical attributes occurred in both sapodilla cultivars during fruit development and ripening, when 'Sapota Tropical' and. 'Sapoti Ipa-Curu' weight increased significantly although no significant differences were found between cultivars (Table 1). The main weight gain happened at the end of growth and beginning of maturation stages, when fruit doubled their weight and is characterized by cell growth and great increase in volume, hence similar results were reported previously (Brito and Narain, 2002; Miranda *et al.*, 2008).

Ripening resulted in a statistically significant increase in soluble solid content (SS) in both sapodilla cultivars with statistical differences between cultivars (Table 1). During ripening of fruit in general, soluble solids are mainly consisted of sugars produced as starch is hydrolyzed, and which are used as substrates in respiration process needed for maintenance of cells metabolism, hence such depolymerization is greatly responsible for taste development due to this increase in sweetness (Prasanna *et al*., 2007). Sapoti Ipa-Curu' (BRS 227) at ripe stage presented more sweetness (20.87 ºBrix) in comparison to 'Sapota Tropical' (BRS 228). Silva *et al.* (2016) evaluating the harvesting period of Murici (Byrsonima crassifolia Kunth) fruit in relation to physical and chemical parameters, observed that a maximum peak for SS at 35 days after anthesis (DAA), with mean values of 27 ºBrix. After this period of significant increase, there was a drastic decrease starting at 42 DAA (~10 ºBrix) until the end of the maturation process at 49 DAA, which was related to the degradation of sugars that are metabolized for energy production as a result of the respiratory process.

The average fruit titratable acidity for two sapodilla cultivars is shown on Table 1. Cultivars exhibited a similar pattern with a significant reduction during ripening, although no differences were found between them. The decrease in acidity coincided with the increase in SS content. According Kulkarni and Aradhya (2005), this is an inherent process during ripening that imparts a characteristic flavour. These authors observed a gradual decrease in acidity,

Table 1. Postharvest quality of sapodilla fruit cv.'Sapoti Ipa-curu' and cv.'Sapota Tropical' during the development and ripening.

DH (Days)[1]	Cultivars	Weight (g)	Soluble solids (SS) °Brix	Titratable acidity (AT) % malic acid	pH	SS/AT
90	Sapota	30.99 ± 1.08^{Aa}	8.20 ± 0.29^{Aa}	0.29 ± 0.02^{Da}	5.21 ± 0.01^{Aa}	27.89 ± 1.77^{Aa}
	Sapoti	26.17 ± 2.22^{Aa}	8.87 ± 0.04^{ABa}	0.31 ± 0.01^{Da}	5.43 ± 0.03^{Bb}	28.21 ± 0.87^{Aa}
120	Sapota	61.78 ± 5.77^{Aa}	8.35 ± 1.23^{Aa}	0.23 ± 0.01^{Ca}	5.47 ± 0.07^{Ba}	36.59 ± 5.68^{Aa}
	Sapoti	41.63 ± 3.29^{Aa}	7.65 ± 0.39^{Aa}	0.22 ± 0.01^{Ca}	5.55 ± 0.04^{Ba}	34.79 ± 2.12^{Aa}
150	Sapota	102.42 ± 4.27^{Ba}	9.60 ± 0.22^{ABa}	0.11 ± 0.01^{Aa}	5.58 ± 0.04^{Bb}	91.38 ± 10.72^{Ba}
	Sapoti	84.44 ± 9.50^{Ba}	9.62 ± 1.10^{Ba}	0.10 ± 0.01^{Aa}	5.42 ± 0.12^{Ba}	92.92 ± 8.09^{Ba}
180	Sapota	121.46 ± 30.87^{BCa}	11.22 ± 0.27^{Ba}	0.12 ± 0.02^{Aa}	5.56 ± 0.05^{Bb}	96.70 ± 11.77^{Ba}
	Sapoti	115.34 ± 9.67^{BCa}	11.05 ± 0.30^{ABa}	0.12 ± 0.02^{Aa}	5.37 ± 0.05^{Ba}	99.14 ± 15.42^{Ba}
Ripe	Sapota	150.95 ± 24.70^{Ca}	20.87 ± 0.48^{Ca}	0.17 ± 0.02^{Ba}	5.09 ± 0.05^{Aa}	125.31 ± 13.61^{Ca}
	Sapoti	142.93 ± 7.52^{Ca}	23.92 ± 0.85^{Cb}	0.16 ± 0.02^{Ba}	5.23 ± 0.05^{Ab}	150.15 ± 17.52^{Cb}

For each parameter and cultivar, different capital letters indicate significant differences at $P < 0.05$ between harvest dates. For each parameter and harvest date, different lowercase letters indicate significant differences at $P < 0.05$ among cultivars.

concomitant with increased SS and total sugar content during ripening of pomegranate. Pimentel *et al.* (2013) explained the reduction of acidity and an increase in the SS during ripening occurs because of the strong relationship between acidity in the fruits and metabolic behavior, and they indicated that certain species can exhibit a decrease in acidity levels if their organic acids are used for energy production during the respiration process, which is common.

Pulp pH decreased slightly for both cultivars. However and despite the decrease for 'Sapota Tropical', there was no significant difference between stages 90 days and ripe (Table 1). Significant differences were found between cultivars regarding pH values during ripening, 'Sapoti Ipa-Curu' was significantly higher (5.23) than 'Sapota Tropical' (5.09) with ripening. As Brito and Narain (2002), the sweet and pleasant taste of the sapodilla fruit ripe is related to the reduced values of titratable acidity and pH.

The ratio between SS and titratable acidity defines the "taste" of the fruit, consequently increased during the observed period of ripening. There were also differences in the SS/TA ratio between the ripe cultivars (Table 1). The low acidity associated with the high SS content resulted in an elevated SS/TA ratio, characteristic of sapodilla fruit. 'Sapoti Ipa-Curu' was significantly higher than 'Sapota Tropical' with ripening, thus the taste of this cultivar is more sugared and less acidic than that of other.

The yellow flavonoids, ascorbic acid (AA), total soluble phenolics (TSP) content and total antioxidant activity (TAA) in sapodilla fruit at different maturity stages are presented on Table 2. Ripening results in significant decrease of yellow flavonoids content in both sapodilla cultivars, there were significant differences between them only at 90 days. Sapodilla showed significantly high content of yellow flavonoids at the initial development stage (90 days after tagged), presenting significant differences between cultivars. A reduction in flavonoid content with ripening was also reported in grape fruit, 96.61% (Doshi *et al*., 2006). These phenolics represent a large group found in plants presenting antioxidant and anticancer properties and also contributing to quality characteristics of fresh and processed food products including astringency, texture, taste and color.

The content of total soluble phenolics (TSP) showed a similar pattern for both sapodilla cultivars, with progressive reduction throughout the ripening process. The higher content of TSP was observed in fruits with 90 days presenting significant differences between cultivars. According Kulkarni and Aradhya (2005), a decrease in the total phenolics during ripening reduces the astringency of fruit, which associated with the degree of polymerization, being a desirable sensory quality. The decrease of astringency during fruit maturation resulted of the phenolics polymerization and in sapodilla ripening, there is predominance of polymeric forms justifying the reduction, in part, the astringency found in these fruits (Miranda *et al.*, 2008). A decrease in phenolic compounds during ripening was also reported during the advancement of ripening of two blackberries Brazilian varieties (*Rubus* spp) and a higher concentration was founded for unripe fruit of both varieties (Zielinsk *et al*., 2015). Azevedo *et al*. (2015) observed a decrease of phenolic compounds during maturation of cambuci fruits *(Campomanesia phaea)* that was attributed to the fact of fruits are cultivate in different locations. Shui *et al.* (2004) attributed the antioxidant capacity of *Manilkara zapota* L. to the polyphenolics blocks of gallocatechin or catechin or both.

Table 2. Antioxidants compounds and total antioxidant activity of sapodilla fruit cv.'Sapoti Ipa-curu' and cv.'Sapota Tropical' during the development and ripening.

DS (Days)[1]	Cultivars	Yellow flavonoids mg/100 g^{-1} FW	TSP 10^2 mg GAE/100 g^{-1} FW	Ascorbic acid mg/100 g^{-1} FW	TAA 10^2 μM Trolox/g FW
90	Sapota	11.44 ± 3.40 [Bb]	16.64 ± 1.61 [Da]	23.62 ± 1.97 [Ca]	23.83 ± 4.22 [Ad]
	Sapoti	6.91 ± 0.73 [Ba]	20.91 ± 1.22 [Db]	25.23 ± 1.71 [Ba]	24.82 ± 1.12 [Ad]
120	Sapota	5.35 ± 2.25 [Aa]	13.15 ± 1.15 [Ca]	21.84 ± 0.55 [Ca]	14.95 ± 0.60 [Ac]
	Sapoti	3.26 ± 1.40 [ABa]	13.22 ± 1.20 [Ca]	24.85 ± 2.11 [Bb]	15.36 ± 2.09 [Ac]
150	Sapota	6.62 ± 0.64 [Aa]	10.31 ± 2.41 [Cb]	17.99 ± 2.10 [Ba]	6.49 ± 0.17 [Ab]
	Sapoti	4.08 ± 1.83 [ABa]	7.70 ± 1.33 [Ba]	21.84 ± 0.55 [Bb]	6.79 ± 0.30 [Ab]
180	Sapota	5.59 ± 1.35 [Aa]	6.20 ± 0.26 [Ba]	16.08 ± 1.55 [Ba]	4.24 ± 1.25 [Ab]
	Sapoti	3.24 ± 1.84 [ABa]	5.29 ± 0.68 [Ba]	14.33 ± 2.35 [Ba]	3.38 ± 0.44 [Ab]
Ripe	Sapota	3.16 ± 1.69 [Aa]	1.63 ± 0.22 [Aa]	11.40 ± 1.69 [Aa]	0.13 ± 0.02 [Aa]
	Sapoti	1.70 ± 0.88 [Aa]	0.67 ± 0.69 [Aa]	12.16 ± 0.88 [Aa]	0.12 ± 0.03 [Aa]

For each parameter and cultivar, different capital letters indicate significant differences at $P < 0.05$ between harvest dates. For each parameter and harvest date, different lowercase letters indicate significant differences at $P < 0.05$ among cultivars.

Ascorbic acid (vitamin C) is abundant in plant cells and has many biological functions in fruits as enzymatic cofactors, redox control and antioxidant activity (Shwartz *et al*., 2009). Sapodilla cultivars showed a similar trend with significant decrease in AA content with ripening. The highest AA content was recorded at 90 days with no significant differences between them. As shown, mature sapodilla is not a good source of vitamin C. A significant reduction in AA content during maturation was also found in guava, mango and banana (Bashir and Abu-Goukh, 2003).

The total antioxidant activity (TAA) of sapodilla extracts was in accordance with the results for flavonoids, total phenolic and ascorbic acid content for the two cultivars studied. The differences in antioxidant compounds and maturity stages in sapodilla fruit reflected the differences observed for the ABTS antioxidant assay (Table 2). The TAA of 'Sapota Tropical' as well as 'Sapoti Ipa-Curu' decreased during development, without significant differences between them. The highest TAA for both sapodilla cultivars was observed in 90 days. According to Leong and Shui (2002), unripe sapodilla possesses an extremely high antioxidant capacity which is not attributed to ascorbic acid, a constituent that is partly responsible for the antioxidant capacity of many fruits. In sapodilla fruit the contribution of ascorbic acid to the total antioxidant capacity is very low (<0.1%), which suggested that most of the antioxidant activity in this fruit could be due to polyphenolic compounds. Lopes *et al.* (2012) evaluating the bioactive compounds and antioxidant activity of four cashew apples clones *(Anacardium occidentale* L.) also observed a decrease for all cashews clones evaluated that was mainly attributed to decrease of the polyphenol content. Shui *et al.* (2004) founded that the best time for one to consume *Manilkara zapota* L. fruits at a flavorful stage with high amounts of antioxidants was with ABTS values ranging from 600 to 1200 mg/100 g FW. Due to the complexity of the composition of foods, their antioxidant power depends on the synergistic effects and redox interaction between the different nutrient and "non nutrient" molecules, which together contribute to the possible health benefits.

Fruit ripening has been described as an oxidative phenomenon. To determine whether the increasing oxidative stress accompanying fruit ripening was associated with reduced ability to enzymatically catabolize ROS, we have measured changes in the activities of significant antioxidant enzymes (SOD, CAT and APX) at different stages of the development and ripening of sapodilla fruit (Table 3).

SOD, CAT and APX activities showed progressive decrease with the sapodilla fruit ripening. In both cultivars, the highest SOD activity was recorded at 90 days, with significant differences between them. Results on SOD activity were in accordance with others fruits such orange (Huang *et al*., 2007) and tomato (Mondal *et al.*, 2004). CAT activity in 'Sapota Tropical' and 'Sapoti Ipa-Curu' declined substantially as fruit matured. However, for cv. 'Sapota Tropical', a significant increase was observed in CAT activity in fruits harvested at 180 days, although not statistically different from that presented by the fruits at 120 and 150 days. Similar to SOD and CAT, a decline in APX activity occurred in 'Sapota Tropical' and 'Sapoti Ipa-Curu'. According Huang *et al*. (2007), the decline APX activity as orange ripened may be

Table 3. Antioxidants enzymes activity and lipid peroxidation of sapodilla fruit cv.'Sapoti Ipa-curu' e cv.'Sapota Tropical' during the development and ripening.

DS (Days)[1]	Cultivars	SOD 10^2 UA/mg P	CAT 10^1 UA/mg P	APX UA/mg P	LP nmol/TBARS g^{-1} FW
90	Sapota	82.76 ± 8.78 ^{Ca}	34.98 ± 5.45 ^{Ba}	82.63 ± 9.92 ^{Cb}	60.03 ± 4.40 ^{ABa}
	Sapoti	95.90 ± 2.25 ^{Db}	37.01 ± 4.42 ^{BCa}	64.15 ± 2.71 ^{Ba}	96.50 ± 9.04 ^{Bb}
120	Sapota	62.28 ± 16.20 ^{Ba}	62.78 ± 11.88 ^{Cb}	81.09 ± 6.26 ^{Cb}	80.55 ± 19.56 ^{BCa}
	Sapoti	66.30 ± 6.42 ^{Ca}	25.32 ± 2.78 ^{Ba}	68.29 ± 3.89 ^{Ba}	72.96 ± 12.17 ^{Ba}
150	Sapota	10.71 ± 1.53 ^{Aa}	48.02 ± 10.63 ^{BCa}	55.07 ± 4.90 ^{Ba}	42.85 ± 4.43 ^{Aa}
	Sapoti	12.65 ± 0.38 ^{Aa}	50.85 ± 13.21 ^{Ca}	58.96 ± 1.53 ^{Ba}	31.83 ± 3.94 ^{Aa}
180	Sapota	23.21 ± 1.94 ^{Aa}	67.42 ± 15.19 ^{Cb}	62.02 ± 2.79 ^{Ba}	38.61 ± 6.03 ^{Aa}
	Sapoti	25.11 ± 3.72 ^{Aa}	26.12 ± 3.16 ^{Ba}	67.68 ± 6.98 ^{Ba}	28.78 ± 6.20 ^{Aa}
Ripe	Sapota	26.38 ± 4.20 ^{Aa}	0.80 ± 0.09 ^{Aa}	1.50 ± 0.34 ^{Aa}	104.25 ± 13.31 ^{Ca}
	Sapoti	45.38 ± 10.11^{Bb}	1.62 ± 0.35 ^{Aa}	3.47 ± 2.65 ^{Aa}	153.93 ± 15.57 ^{Cb}

For each parameter and cultivar, different capital letters indicate significant differences at $P < 0.05$ between harvest dates. For each parameter and harvest date, different lowercase letters indicate significant differences at $P < 0.05$ among cultivars.

due to disappearance of different isoforms in fruit pulp during ripening.

Mondal *et al.* (2004) observed lower activities of these antioxidant enzymes in a tomato cultivar with short shelf life and affirmed that a reduced ability to scavenge free radicals mediates biochemical changes leading to fast ripening process in this cultivar. Our data showed that maturation and ripening of the fruit from these two sapodilla cultivars were accompanied by decrease in the activities of oxygen-scavenging enzymes (SOD, CAT and APX). So, due to a substantial increase in respiratory rate in climacteric fruits, probably have an increase in ROS production during ripening and the concomitant decline in antioxidant enzymes activities could contribute to accumulation of ROS increased oxidative stress. Thus, the increasing oxidative stress is evidently needed to facilitate many of the changes associated with ripening of sapodilla fruit.

Changes in oxidative processes occurring during sapodilla fruit ripening were measured by the extent of lipid peroxidation (Table 3), since cell membrane lipids and free fatty acids are highly susceptible to oxidation and increasing oxidative stress is thus indicated by the accumulation of lipid peroxidation products as malondialdehyde (MDA) (Yang *et al.*, 2008). Ripening of the two sapodilla fruit cultivars was accompanied by substantial increases the MDA content. Cell membrane deterioration was associated with ripening in banana fruit (Yang *et al.*, 2008) and tomato fruit (Mondal *et al.*, 2004). According Koc *et al.* (2004), the oxidation and peroxidation of membrane lipid and proteins could be caused by ROS. In that case, this increase in peroxidation of membrane lipids of ripe sapodilla fruit could be explained by non-enzymatic oxidation reactions carried out by ROS also confirmed from a decrease in antioxidant enzymes activities throughout the ripening processes.

Table 4. Pearson correlation coefficients of lipid peroxidation degree versus antioxidant parameters of sapodilla fruit cv.'Sapoti Ipa-curu' e cv.'Sapota Tropical' during the development and ripening.

Parameters[1]	Correlation Coeficient[2] 'Sapota Tropical'	'Sapoti Ipa-curu'
CAT	- 0.605*	- 0.468*
APX	- 0.525**	- 0.507*
SOD	-	- 0.706**
Yellow Flavonoids	-	-
Total Phenols	-	-
Total Vitamin C	-	-
TAA	-	-

[1]Antioxidant properties evaluated during fruit development.
[2] Lipid peroxidation (nmol/g FW) vs. Parameters. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$

The most characteristic alteration that fruits undergo during ripening is softening. There is evidence that fruit softening involves oxidative degradation, through the membrane deterioration by ROS as well as hydrolytic associated with degradation of cell wall and middle lamellae by several enzymes, including polygalacturonases (PGs), endoβ-1,4-glucanases and pectatelyases (PLs). Earlier studies showed a reduction in sapodilla firmness during ripening, which indicated the occurrence of fruit softening resulting from an increase in intercellular spaces, the decline of cell turgor and mostly from modifications on primary cell wall carbohydrate

metabolism and structure (Miranda *et al*., 2008). Our results support a relationship existed between the peroxidation of membrane lipids and firmness decreased of the sapodilla fruit with ripening, since data presented by Miranda *et al.* (2008) have confirmed higher reduction in firmness as fruits ripen. When lipid peroxidation results were correlated to the antioxidant of enzymatic and non-enzymatic nature (Table 4), the two cultivars were evidently dependent on antioxidant enzyme protection against cell membrane peroxidation and loss of integrity, whereas a negative correlation was observed between lipid peroxidation and antioxidants enzymes.

Conclusion

Our results demonstrated that ripening of sapodilla was accompanied by progressive increase in oxidative stress and peroxidative evidenced by the reduced of bioactive compounds as polyphenols and ascorbic acid and especially of antioxidant enzymes activity as well as increased in the lipid peroxidation. Thus, these events are responsible for promoting increase in oxidative stress and cause many metabolic changes associated with sapodilla fruit ripening which contributes to its postharvest quality.

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