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Quality, bioactive compounds and enzymatic metabolism of cv. Vitoria pineapple during Maturation[1]

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ABSTRACT - Pineapple is a non-climacteric infructescence that needs to be harvested at the highest quality, and it is crucial to determine the ideal harvest point. This work evaluates changes in quality, bioactive compounds, expression and enzymatic activity and antioxidant metabolism during maturation of cv Vitória pineapple, recently introduced in commercial orchards, in order to define the harvest point. 'Vitória' pineapple infructescence were harvested from commercial planting in five maturity stages and five replications: 100% green (TG), break (B), 75% green and 25% orange (GO), 25% green and 75% orange (OG), 10% green and 90% orange (PO), and 100% orange (TO). In 'Vitória' pineapple during maturation, color evolution was clearly shown by the color index (CI) and firmness was higher in TG, G, BP stages. Soluble solids and titratable acidity increased as a function of the maturity stages. The ascorbic acid content was higher in the TG and G, while the yellow flavonoids, PET, and carotenoids higher from OG to PO maturity stages. Antioxidant activity by $ABTS^{\bullet+}$ and DDPH radicals were higher in the PO stage. The moleculr weight of the antioxidant enzymes regardless the maturity stages was estimated at 47 kDa POD, 28 kDa SOD, and 37 kDa APX. By gel electrophoresis, the SOD, APX, and POD accumulations were higher at earlier maturity stages, whereas the activities were higher at the TO maturity stage. Altogether, the highest quality and functional properties in 'Vitória' pineapple, as defined by the highest contents of bioactive compounds and higher antioxidant, activity, were found mainly in the maturity stage PO, which surely value fresh consumption.

Key words: *Ananas comosus*. Quality valorization. Functional potential. Enzymatic separation. Enzymatic activity.

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INTRODUCTION

Pineapple (*Ananas comosus* var. Comosus) is a non-climacteric fruit that must be harvested at the maximum quality stage (DING; SYAZWANI, 2016). Therefore, determining the ideal harvest point is crucial in valuing and obtaining a longer postharvest life and consumer acceptance (IKRAM *et al*., 2021). Determining changes during maturation enables defining maturity indices which are related to the maximum sensorial, nutritional, and functional quality of the fruit (BATISTA-SILVA *et al*., 2018). Therefore, this maturity stage must be well defined for new introduced cultivars in aiming for maximum postharvest life and quality enhancement.

The pineapple crop is highly cultivated and valued in tropical and subtropical regions, and its infructescences are recognized as important foods due to their antioxidant and anti-inflammatory properties (IKRAM *et al*., 2021). Mature *Ananas comosus* has vast nutritional and energy content with high carbohydrate, mineral and secondary metabolite levels, which includes bioactive compounds such as carotenoids, ascorbic acid, flavonoids, and phenolics that confer antioxidant activity (LÉCHAUDEL *et al*., 2018; SUN *et al*., 2016).

These compounds provide defenses against free radicals and reactive oxygen species, combined with the enzymatic antioxidant metabolism conferred by the activities of superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX) (SUN *et al*., 2016). However, the contents of these bioactive compounds, as well as the abundance and activity of these enzymes may be differentially expressed during infructescence maturation throughout the final development stages (DING; SYAZWANI, 2016; OGAWA *et al*., 2018).

Victoria cv. pineapple, originating from the cross between Primavera and Smooth Cayenne cv. pineapples, presents itself as an alternative to phytosanitary problems, as it is resistant to fusariosis, a crop-limiting disease, and is also resistant to transportation and storage, in addition to presenting or similar superior agronomic, nutritional and physiological characteristics to other cultivars already established on the market (OGAWA *et al*., 2018).

However, to the best of our knowledge there are no data describing changes in quality during the maturation of this cultivar, especially those relating to antioxidant metabolism and enzyme expression. This information is decisive in defining the harvesting point, aiming to valorize potential markets based on well-founded claims of antioxidant metabolism.

Thus, Brazilian pineapple farming is highly suitable for exploring new cultivars with promising agronomic characteristics, as well as differentiated quality attributes and functional potential, targeting the most competitive market niches that demand discriminating appeals, such as exports. Changes in pineapple peel color, soluble solids, acidity (MOURA *et al.*, 2024; BATISTA-SILVA *et al*., 2018; DING; SYAZWANI, 2016), in addition to bioactive compounds and antioxidant defenses (LÉCHAUDEL *et al*., 2018) are attributes which have been used as indicators for the pineapple harvesting stage aiming for fresh consumption. Therefore, it is necessary to establish parameters which characterize maturity evolution, indicating the harvest point at maturity stages with greater functional properties, attempting to add value by discriminating maturity that gives pineapples greater market potential.

In view of the above, the objective of the work was to evaluate changes in quality characteristics, bioactive compounds, antioxidant activity, expression, and antioxidant metabolism during the maturation of Victoria cv. pineapple, aiming to define the harvesting point which can be indicated as having the greatest functional appeal.

MATERIAL AND METHODS

Plant Material

Vitória pineapple cultivar infructescences were harvested from a commercial plantation carried out under usual cultural practices, at the Quandu Farm, Itapororoca, Paraíba, Brazil. Pineapples were selected using the following maturity stages as criteria: 100% green (TG); Break (B); 75% green and 25% orange (GO); 25% green, and 75% orange (OG); 10% green and 90% orange (PO); and 100% orange (TO) (Figure 1A). After harvesting, pineapples at the corresponding stages were packed in polyethylene boxes and transported to the laboratory, where were washed in running tap water and sanitized with a 50 ppm sodium hypochlorite solution.

Evaluations

Color Index (CI) - The color parameters *L**, *a**, and *b** were obtained from two objective readings in the equatorial region of the surface of the infructescence pulp using a Minolta digital colorimeter. The color index (CI) was calculated according to Motta *et al.* (2015) by the equation:

$$CI = \frac{2000a}{L\sqrt{(a)^2 + (b)^2}} \quad (1)$$

Firmness, soluble solids (SS), titratable acidity (TA) and SS/TA ratio - Firmness (N) was determined by a Magness Taylor Pressure Tester penetrometer in two equidistant readings in the equatorial region of the pineapples; soluble solids (SS) by direct reading

with an Abbe-type refractometer with temperature control (20 °C); titratable acidity (TA) by titration with 0.1M NaOH solution, with results expressed in g citric acid/100g of fresh pulp; SS/TA ratio obtained by dividing the SS by the TA contents (IAL, 2008).

Ascorbic acid - was determined by titration with DFI solution (2,6-dichlorophenolindophenol 0.002%) and expressed in mg 100 g^{-1} of fresh pulp (IAL, 2008).

Yellow flavonoids - were determined according to Francis (1982) and the results expressed in mg $100g^{-1}$.

Total carotenoids - extraction and determination were based on the method described by Wright and Kader (1997) and readings were taken at 454 nm, and expressed in μg β-carotene g^{-1}.

Phenolic extract and total extractable polyphenols (PET) - the phenolic extract for determining PET and antioxidant activity was obtained according to Dantas *et al.* (2015). PETs were obtained from a 200 μL mL^{-1} aliquot of the phenolic extract, with results calculated based on the gallic acid standard curve linearity (0 to 50 mg g^{-1}) and expressed as gallic acid equivalents (GAE) mg 100 g^{-1}.

Total antioxidant activity by $ABTS^{\bullet+}$ $DPPH^{\bullet}$ - Total antioxidant activity (AAT) was determined by capturing the free radical $ABTS^{\bullet+}$ according to Dantas *et al*., (2015). The samples were read at 734 nm in a spectrophotometer, after 6 minutes of addition of the radical. A Trolox standard curve was used and the results were expressed in μM of Trolox g of fresh $pulp^{-1}$ (DANTAS *et al.*, 2015).

The total antioxidant activity (AAT) by capturing the free radical $DPPH^{\bullet}$ (1,1'-diphenyl-2-picrylhydrazyl) was measured according to Dantas *et al.*, (2015). A 100 μL aliquot was used, to which 3.9 mL of the radical (0.06 mM) was added. For control, 100 μL of control solution (50% methyl alcohol + 70% acetone + water, ratio 4:4:2) was used instead of the phenolic extract. Readings were taken at 515 nm in a spectrophotometer, 30 minutes after addition of the $DPPH^{\bullet}$ radical. The total antioxidant activity (g of pulp. g $DPPH^{-1}$) was calculated by estimating EC50 (amount of sample necessary to reduce the initial concentration of the $DPPH^{\bullet}$ radical by 50%).

Each enzymatic assay and the protein electrophoretic profile were determined using the enzymatic extract obtained according to Lv *et al.* (2011), with modifications. Two grams of pulp were homogenized with 5 mL of 100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM ethylenediaminetetraacetic (EDTA) and 1% polyvinylpyrrolidone (PVP). The solution was centrifuged at 9000 rpm for 20 minutes at 4 °C and the supernatant was used for enzymatic analyses.

Electrophoretic profile of proteins by SDS-PAGE - was used to verify the separation of proteins from the sample based on the molecular weight of the proteins according to the method described by Laemmli (1970). For separation, an SDS gel with 12.5% polyacrylamide was used and for concentration, a gel with 3.5% polyacrylamide was used. The enzyme extract was treated with 10% SDS and b-mercaptoethanol for 10 min at 100 °C and then subjected to a denaturing condition using a 25 mA constant current for approximately 3 hours. The running buffer consisted of 0.25 M Tris-HCl buffer solution pH 8.3, containing 1.92 M glycine and 1% SDS. After the run, the gel was stained in Commassie brilliant blue R–250. Excess dye was removed from the gel with a decolorizing solution of acetic acid, methanol. and water (10:45:45 v/v). To estimate the molecular weight of proteins, 10 μL of a molecular marker from a wide range of known proteins was applied (Myosin, 200 kDa; β-galactosidase, 120 kDa; Bovine Serum, 91 kDa; Glutamate, 62 kDa; Ovalbumin, 47 kDa; Carbonic Anhydrase, 37 kDa; Myoglobin, 28 kDa; Lysozyme, 19 kDa; Aprotinin, 9 kDa).

Antioxidant enzyme activities

The protein concentration of the enzyme extracts was determined according to Bradford (1976), with readings at 595 nm and a bovine serum albumin-BSA standard curve (2.5 to 60 μg mL^{-1}).

The activity of superoxide dismutase (SOD, EC 1.15.1.1) was determined by the capability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) according to Lv *et al.* (2011). The reaction mixture (1.5 mL) contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 100 mM EDTA, 2 μM riboflavin, 75 μM NBT, and 50 μL of the enzyme extract. Riboflavin was added last, and the reaction was started by turning on two 30W lights, remaining for 10 minutes. Enzyme activity was measured at 560 nm and one unit of SOD was defined as the required amount of enzyme that inhibits NBT photoreduction by 50% (U mg^{-1} protein) under the experimental conditions.

Peroxidase activity (POD, EC 1.11.1.7) was determined by the oxidation of guaiacol using H_2O_2 and an extinction coefficient of 26.6 mM^{-1} cm^{-1}, according to Wu *et al.* (2010). The reaction mixture contained 1.2 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.1 mL of 0.5 M hydrogen peroxide, 0.1 mL of 3% guaiacol, and 0.1 mL of the enzyme extract. The increase in absorbance was monitored for 60 seconds at 25 °C and 470 nm. One unit of POD activity was defined as the amount of enzyme that catalyzes the peroxidation of 1 μmol of guaiacol per milligram of protein per minute (U mg^{-1} protein).

The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was determined according to Yang, Zheng, and Cao (2009). The assay mixture (1.5 mL) contained 50 mM potassium phosphate buffer (pH 7.0), 1 mM H_2O_2, 0.45 mM

ascorbic acid and 100 µL enzyme extract. The decrease in absorbance at 290 nm was monitored for 3 minutes and the enzymatic activity was calculated with an extinction coefficient of 2.8 mM^{-1} cm^{-1}. One unit of APX activity was defined as the amount of enzyme that oxidizes 1 µmol of ascorbic acid per minute per mg protein (U mg^{-1} protein), under the experimental conditions.

Statistical analysis

The experimental design used was the completely randomized, evaluating pineapples at six maturity stages. For physical evaluations (color and firmness), 50 pineapples from each maturity stage were used (50 replications). For physicochemical evaluations, bioactive compounds, protein electrophoretic profile, and enzymatic activity evaluations five replications of 10 pineapples were used.

The data were subjected to analysis of variance (ANOVA) and the means of the treatments (maturity stage) were compared by the Tukey test ($p \leq 0.05$), using the SISVAR® 9.3 software (2011) to carry out these analyses.

Principal Component Analysis (PCA) and Cluster Analysis were also carried out based on the scores calculated in PCA. Cluster analysis was performed using the R® cluster.Sim procedure in which the optimal number of groups is defined by combining different normalization formulas, distance measures, cluster methods, and the Calinski-Harabasz index.

RESULTS AND DISCUSSION

Color index (CI)

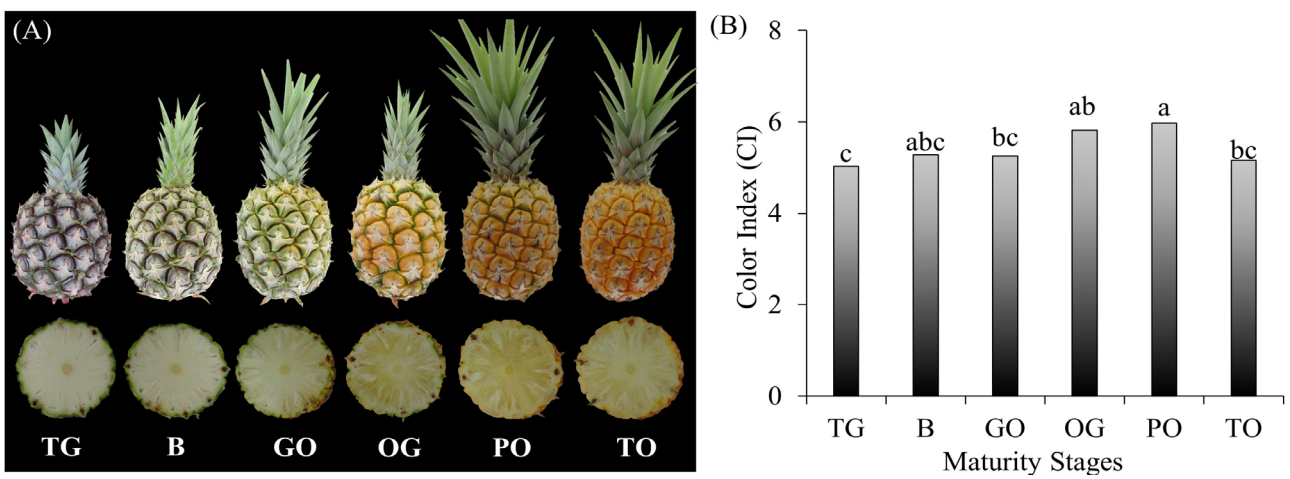
Changes in the 'Victoria' pineapple pulp color were described by the color index (CI), which indicates the degree of variation from green to yellow tones (MOTTA *et al*., 2015), presenting its highest CIs in fruit at the PO stage, which did not differ from the OG, while those from the totally green (TG) stage had the lowest color index (Figure 1B). It was observed that the CI tended to decline from the PO stage onwards as a result of the oxidation of the pigments from the advancing maturation. Pulp color is one of the main attributes related to pineapple acceptance, and is also an adequate maturity index (DING; SYAZWANI, 2016), which in this work indicated the OG stage as the most appropriate for 'Victoria' pineapple consumption based on the pulp color.

Firmness, soluble solids (SS), titratable acidity (TA), and SS/TA ratio

The firmness of the 'Victoria' pineapple was higher in the TG, break (B), and GO stages, which did not differ from each other. The OG, PO, and TO fruit maturity stages showed the lowest firmness, especially those of the TO, with a mean decline of 62%. These results indicate that full maturity in 'Victoria' pineapple may have been reached at the OG stage (Figure 2A). The decrease in pulp firmness with advancing maturity is due to changes in the cell wall polymer's structure resulting from degradation of pectic polysaccharides due to the action of cell wall enzymes such as pectinmethylesterases, pectatase, and galactosidase, among others (LIU; LIU, 2017).

The soluble solids (SS) of the 'Victoria' pineapple pulp increased during maturation, so that the OG, PO, and TO maturity stages presented the highest contents with a mean of 20%, which did not differ from each other, followed by the B and GO stages, which also did not differ. TG pineapples had the lowest SS content (65% lower, on average) when compared to the others (Figure 2B).

Figure 1 - Evolution of peel and pulp maturity (A) and color index – CI of pulp (B) of 'Vitória' pineapple at maturity stages: 100% green (TG); Break (B); 75% green and 25% orange (GO); 25% Green and 75% orange (OG); 90% orange and 10% green (PO); and 100% orange (TO)

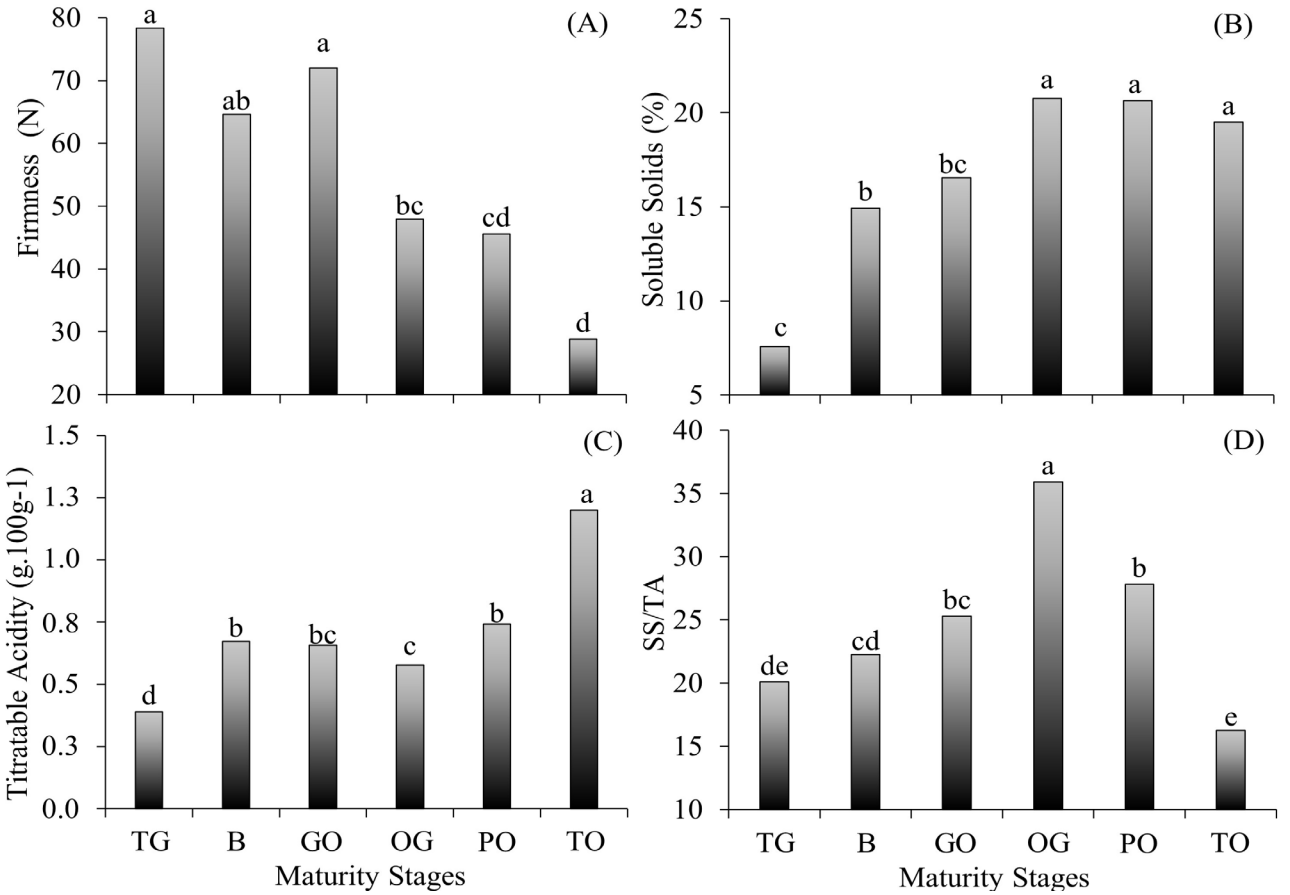


Means followed by the same lowercase letters do not differ by the Tukey test at 5% probability. n = 50 (B)

Similar SS contents to those of the TG stage were reported in 'Queen Victoria', which presented 6.5 and 11.5%; 'MD2' with SS of 5.3 and 7.2%; and 'Flhoran 41' of 6.0 and 7.0%, all those pineapples at TG and B maturity stages, respectively. However, the same pineapple cultivars at maturity stages equivalent to OG and PO presented SS contents of 14.9 and 15.6% ('Queen Victoria'), 12.2 and 13.0% ('MD2'), and 12.1 and 11.6% ('Flhoran 41') (LÉCHAUDEL *et al*., 2018), much lower than those reported herein for 'Victoria' pineapple at similar maturity stages, demonstrating the superiority of Vitória pineapple in accumulating sugars as maturation progresses. The increase in SS content during maturation results from the fruit development process, since starch (accumulated in the fruit growth phase) is converted into soluble sugars (OGAWA *et al*., 2018).

Titratable acidity (TA) contents were higher in fruit at the TO ripening stage, which are similar to those reported by Lu *et al*. (2014) in 'Giant Kew' and 'Smooth Cayenne' pineapples, with mean acidities of 1.12 and 1.23 g 100 g^{-1} of citric acid, respectively. The higher TA of the TO stage in 'Victoria' pineapple indicates advanced ripeness. Fruits at the PO, GO, and B maturation stages presented similar TA contents which did not differ from each other, and close to those of 'Pattavia' and 'Sriracha' pineapples at maturation stages equivalent to the PO of 'Victoria' pineapple, which presented TA of around 0.85 and 0.81 g 100 g^{-1} of citric acid, respectively (LU *et al*., 2014). The TA of 'Victoria' pineapples at OG and GO stages also did not differ, but the TG had the lowest acidity contents (on average 55% lower) when compared to the others (Figure 2 C). The increase in acidity content during pineapple ripening is a result of citric acid accumulation through the action of phytohormones and key transcription factors that regulate the construction of infructescence quality (BATISTA-SILVA *et al*., 2018).

Figure 2 - Firmness between fruitlets of the peel (A), and soluble solids - SS (B), titratable acidity - AT (C), and SS/AT ratio (D) of pulp of 'Vitória' pineapple at maturity stages: 100% green (TG); Break (B); 75% green and 25% orange (GO); 25% Green and 75% orange (OG); 90% orange and 10% green (PO); and 100% orange (TO)



* Means with equal lowercase letters do not differ by the Tukey test at 5% probability. n = 5

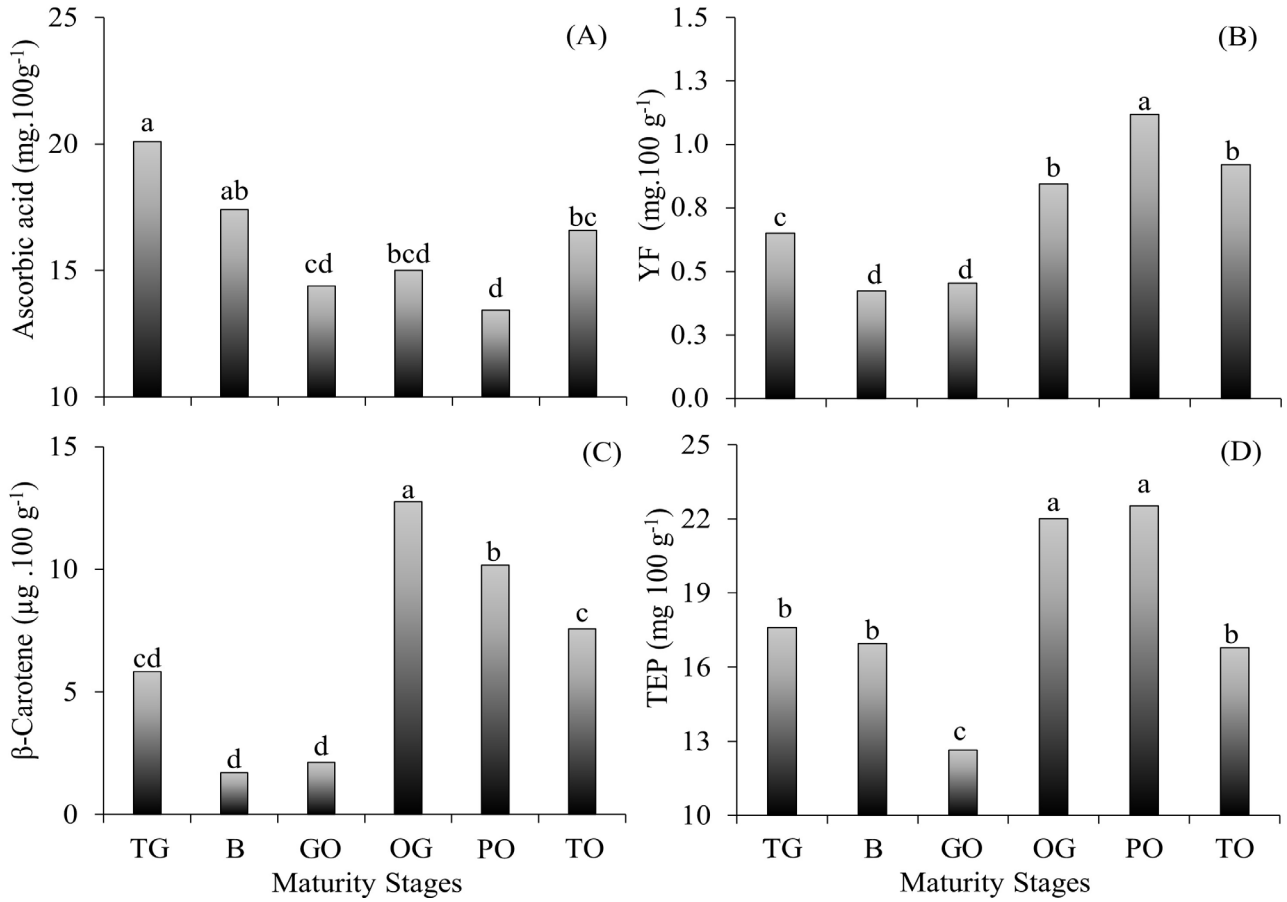
The SS/AT ratio expresses the balance between the sweetness and acidity of the fruits, partly simulating the fruit flavor which is perceived by the consumer, for which the OG stage presented the highest SS/AT ratio values, with an indication of greater perception of sweetness, followed by the PO and GO stages which did not differ. The GO and B stages did not differ from each other and presented a ratio varying between 22 and 25. The TG and TO fruit presented the lowest SS/AT ratios (Figure 2D) as a result of the higher TA (Figure 2C) and advanced fruit maturation. Similar results were reported in 'Victoria' pineapple which showed a low SS/AT ratio at the green maturity stage and increased as maturation progressed, declining at more advanced maturity stages (OGAWA *et al*., 2018).

Bioactive compounds

The ascorbic acid content was higher in pineapples of the TG and B maturity stages, which did not differ, with contents ranging from 17.40 to 20.09 mg 100 g^{-1}. Fruit in the GO, OG, and TO stages presented similar contents, which did not differ from each other. In turn, GO, OG, and PO pineapples presented the lowest contents, varying between 13.43 and 15 mg 100 g^{-1} (Figure 3A). However, the ascorbic acid contents in 'Victoria' pineapples were lower than those reported by Ferreira *et al*. (2016) in 'Victoria' pineapples (OG), 75% orange, (35.88 mg 100 g^{-1}) and 'Queen Victoria', 'MD2', and 'Flhoran 41' pineapples in the PO and TO stages, with 23.3 and 25.0; 44.7, and 53.7; 21.6; and 20.5 mg 100 g^{-1}, respectively (LÉCHAUDEL *et al*., 2018). The great variability in ascorbic acid content may be due to factors such as cultivar, management, solar radiation, air temperature, and acidity (CALDERÓN *et al*., 2009).

The yellow flavonoid (YF) contents were higher in 'Victoria' pineapple at the partially (90%) orange (PO) maturity stage when compared to the others, with contents of 1.12 mg 100 g^{-1}. The YF contents in the OG and TO stages did not differ, with levels between 0.84 and 0.92 mg 100 g^{-1}, but they were the lowest in the TG, B, and GO stages when compared to the others (Figure 3B). The

Figure 3 - Contents of bioactive compounds, (A) ascorbic acid, (B) yellow flavonoids (YF), (C) β-carotene, and (D) total extractable polyphenols (TEP) of pulp of 'Vitória' pineapple at maturity stages: 100% green (TG); Break (B); 75% green and 25% orange (GO); 25% Green and 75% orange (OG); 90% orange and 10% green (PO), and 100% orange (TO)



* Means with equal lowercase letters do not differ by the Tukey test at 5% probability. n = 5

YF contents reported herein were much lower than the total flavonoids of pineapple cultivars evaluated by Lu *et al.* (2014), which ranged from 6.16 to 34.50 mg 100 g^{-1}. However, Dantas *et al*. (2015) reported YF for 'Victoria' pineapple from the same growing region at around 0.19 to 0.52 mg 100 g^{-1}, close to those reported herein.

The β-carotene contents of the 'Victoria' pineapple pulp at the OG maturity stage were higher when compared to the others, with values of 12.76 µg 100 g^{-1}, followed by the TO and TG stages which did not differ. Pineapples at TG, GO, and PO stages presented the lowest contents (Figure 3C). The β-carotene contents of 'Victoria' pineapples were much higher than those of 'Phulae' and 'Nanglae' pineapples with 3.35 and 1.41 µg 100 g^{-1}, respectively (KONGSUWAN *et al*., 2009).

These variations in β-carotene contents can be caused by factors such as cultivation conditions, variety or cultivar, and maturity stages, as Freitas *et al*. (2015) found higher β-carotene contents in the OG pineapple maturity stage than the TG stage. The β-carotene content in 'MD-2' pineapple pulp increased with maturation (DING; SYAZWANI, 2016). Another factor that can cause this variation is the biosynthesis of carotenoids, which can continue after harvest due to the action of enzymes responsible for carotenogenesis and also due to possibly activating genes in the isoprenoid pathway responsible for the biosynthesis of enzymes associated with this process (FREITAS *et al*., 2015).

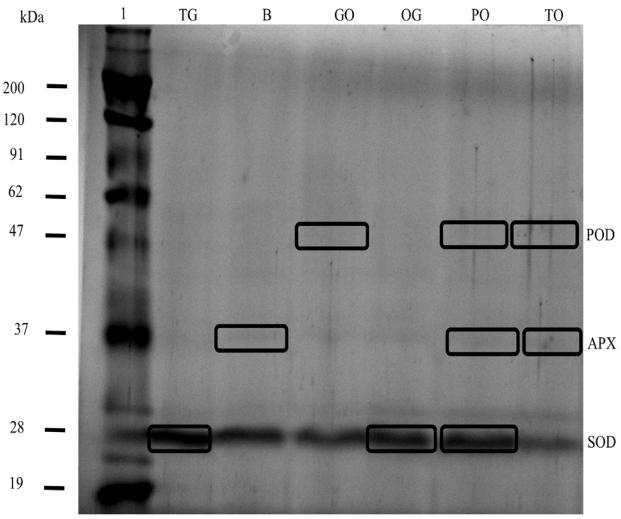
The total extractable polyphenols (TEP) contents at the OG and PO stages were higher than in the other stages, but did not differ, with values around 22.02 to 22.53 mg 100 g^{-1}, followed by the TG, B, and TO stages which did not differ. The pineapple in the GO stage showed the lowest TEP values when compared to the others (Figure 3D). This result may be due to the influence of maturity evolution on the expression and consequent antioxidant activity of the fruit, since the transition from green color to the beginning of pigmentation (stage B) requires a greater amount of energy to support the dynamism of physiological cell processes (OGAWA *et al*., 2018). Variations in TEP levels can also be influenced by factors such as temperature, humidity, soil, fertilization, maturation, and cultivar (DANTAS *et al*., 2015). Sun *et al*. (2016) reported changes and variations in TEP contents in different pineapple cultivars, which at a similar stage to PO presented average TEP contents in 'MD-2' of 72.57 mg 100 g^{-1}, 'Tainung4' of 65.64 mg 100 g^{-1}, 'Fresh Premium' of 46.76 mg 100 g^{-1}, and 'Smooth Cayenne' of 40.42 mg 100 g^{-1}, all higher than those reported herein. However, the 'Red Pineapple' cultivar at a similar maturation stage presented TEP levels of around 25.51 mg 100g^{-1} (SUN *et al*., 2016), which is close to those reported herein.

Separation of enzymes by SDS-PAGE

In the present study, the molecular weight (MW) of Peroxidase (POD) was estimated at 47 kDa by SDS-PAGE (Figure 4), considering that POD presents MWs in the range of 30 to 60 kDa in several vegetables (PANDEY *et al*., 2017). In this sense, the POD MW in jack fruit was estimated at 48 kDa by SDS-PAGE (TAO *et al*., 2018) and in beans it was estimated at 45 kDa (KÖKTEPE *et al*., 2017). In addition, the POD MW for 'Queen' pineapple was estimated to range between 37 and 50 kDa (DEBNATH *et al*., 2019) and for 'Perola' pineapple approximately 28 KDa (MOURA *et al*., 2024). The variations and differences observed in the MW of peroxidases are attributed to post-translational modifications of the polypeptide chain, including the number and composition of post-added glycan chains, which are present in plant peroxidases (AL-BAGMI *et al*., 2019). The POD stain intensity increased during maturation of the 'Victoria' pineapple in the GO stage, then in the PO, and more markedly in the TO, possibly due to the greater production of reactive oxygen species as maturation progresses (PANDEY *et al*., 2017).

Superoxide dismutase (SOD, EC 1.15.1.1) regulates oxidative stress in cells, causing dismutation of superoxide radicals in oxygen to hydrogen peroxide (SUJIWATTANARAT *et al*., 2016). Several vegetables have

Figure 4 - Separation of enzymes by polyacrylamide gel, SDS-PAGE, stained with Commassie blue, from extracts of 'Vitória' pineapple pulp at maturity stages: 100% green (TG); Break (B); 75% green and 25% orange (GO); 25% Green and 75% orange (OG); 90% orange and 10% green (PO); and 100% orange (TO). **(Molecular Markers:** (Myosin, 200 kDa; β-galactosidase, 120 kDa; Bovine Serum, 91 kDa; Glutamate, 62 kDa; Ovalbumin, 47 kDa; Carbonic Anhydrase, 37 kDa; Mioglobin, 28 kDa; Lysozyme, 19 kDa; Aprotinin, 9 kDa)



SOD MW in the range of 14 to 31 kDa. The SOD MW in garlic was approximately 28 kDa (LIU *et al*., 2011), while SOD for black soybeans exhibited a MW of 31.0 kDa (WANG *et al*., 2012) and the SOD MW in olives was estimated at 25 kDa (LOPEZ-HUERTAS; DEL RÍO, 2014).

Superoxide dismutase (SOD, EC 1.15.1.1) regulates oxidative stress in cells, causing dismutation of superoxide radicals to hydrogen peroxide (SUJIWATTANARAT *et al*., 2016). Several vegetables have SOD MW in the range of 14 to 31 kDa. The SOD MW in garlic was approximately 28 kDa (LIU *et al*., 2011), while SOD for black soybeans exhibited a MW of 31.0 kDa (WANG *et al*., 2012), and the SOD MW in olives was estimated at 25 kDa (LOPEZ-HUERTAS; DEL RÍO, 2014). Therefore, the SOD MW in 'Victoria pineapple in the present study was estimated at 28 kDa by SDS-PAGE (Figure 4). In turn, the SOD stain intensity in 'Victoria' pineapple pulp was greater in the TG stage, declining in subsequent stages, then increasing again in the OG and GO stages (full maturation), and declining in the TO (advanced maturation), indicating the uncoupling of the enzymatic antioxidant system at this advanced maturity stage; moreover, greater expression of SOD genes activated in response to the accumulation of free radicals (NUKUNTORNPRAKIT *et al*., 2015) have been observed, aiming to activate the enzymatic antioxidant system (LIU; LIU, 2017).

Ascorbate peroxidase (APX, EC 1.1.1.11) is one of the most important enzymes in cells, as it is responsible for eliminating and maintaining H_2O_2 content (PANDEY *et al*., 2017). The APX molecular weight in 'Victoria' pineapple during ripening was estimated at 37 kDa by SDS-PAGE (Figure 4). Several plants have APX molecular weights (MW) in the range of 25 to 58 kDa. The APX MW in Pallavicinia was approximately 28 kDa (RAJAN; MURUGAN, 2010), in soybeans it ranged from 30 to 45 kDa (KUSAR *et al*., 2012), in beans it was found to be 25 kDa (TORRES *et al*., 2007), and in olive fruit ranged from 30 to 45 kDa (LOPEZ-HUERTAS; DEL RÍO, 2014), with the highest APX stain intensity presented herein falling within this range. In this study, during the maturation of the 'Victoria' pineapple, the APX stain intensity began to increase in stage B (with higher ascorbic acid content (Figure 3)), then continued in the PO, and also more markedly in the TO (in parallel with the reduction in the stain intensity of the SOD at this same maturity stage), probably due to the greater generation of free radicals with the advancing maturation (NUKUNTORNPRAKIT *et al*., 2015), which signals the increased expression of other antioxidant enzyme genes (FERNÁNDEZ-OCAÑA *et al*., 2011) for tissue detoxification (ZHANG *et al*., 2015).

Activity of antioxidant enzymes

'Victoria' pineapple in the GO and TO maturity stages showed the highest superoxide dismutase (SOD) activities when compared to the other stages, which did not differ (Figure 5A), and the highest activity occurred following the stages that showed the highest stain intensity of this enzyme (Figure 4). The B, OG, and PO maturity stages presented close values of the SOD activity that did not differ, followed by the TG stage, which presented the lowest SOD activity.

SOD activity was similar to that of 'Pattavia' and 'Trad-See-Thong' pineapples with 70 to 150 U mg^{-1} protein (NUKUNTORNPRAKIT *et al*., 2015). SOD activity is directly linked to the regulation of fruit development and maturation processes, and its activity may differ according to the plant species, development stage, and environmental stress conditions (FERNÁNDEZ-OCAÑA *et al*., 2011). Therefore, it is important for consumers' health to eat evenly ripened pineapples, as they have lower SOD activity, which neutralizes oxidative stress, interrupting chain oxidant reactions, and minimizing damage caused by free radicals (LIU *et al*., 2011), such as pineapple in the PO stage from this work, which presents indications of lower oxidative stress compared to the TO stage.

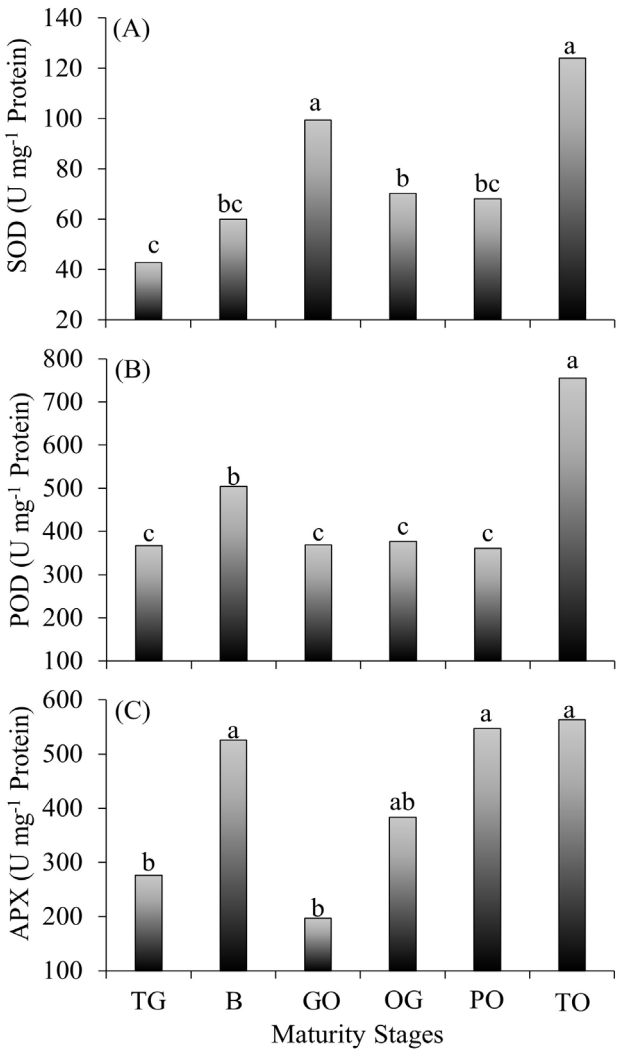
Peroxidase activity (POD) was higher in 'Victoria' pineapple at the TO maturity stage when compared to the others, followed by the B stage (Figure 5B), also following the greater stain intensity in the electrophoretic separation observed for this enzyme (Figure 4). POD activity in the TG, GO, OG, and PO stages did not differ and was lower than the others. 'Comte de Paris' pineapple presented lower POD activity than those reported herein, with values of 200 U mg^{-1} protein (ZHANG *et al*., 2015), as well as 'Queen' pineapple with an average POD activity of 150 to 200 U mg^{-1} protein. Such differences can be attributed to the variety and ripeness degree of the fruits, among other factors (CALDERÓN *et al*., 2009).

The increase in POD activity at the TO maturity stage with advanced ripening can be attributed to fruit stress caused by the process which triggers senescence. Increased POD activity is related to deteriorating changes in flavor, oxidation of pigments compromising color, texture, and degradation of vitamins, which causes loss of nutritional quality in fruits and vegetables, which are indicative of senescence (CALDERÓN *et al*., 2009).

Ascorbate peroxidase (APX) activities in 'Victoria' pineapple at B, PO, TO, and OG ripening stages were highest and did not differ, followed by TG and GO, which also did not differ and presented the lowest APX activities (Figure 5C). Similar results were reported by Léchaudel *et al*. (2018) in three pineapple cultivars ('Queen Victoria', 'MD2', and 'Flhoran 41'), which showed a decrease in APX enzymatic activity as maturation progressed, and also showed an increase in the APX electrophoretic stain intensity in the mature stage (equivalent to PO), as also observed herein for

the PO and TO stages (Figure 4). However, 'Pattavia' and 'Trad-See-Thong' pineapple cultivars at similar maturity stages showed enzymatic activity of 7 and 11 U mg^{-1} protein, respectively (NUKUNTORNPRAKIT *et al*., 2015), being lower than those reported herein. In this sense, in parallel with the reduction in ascorbic acid levels (Figure 3A), 'Victoria' pineapple at PO and TO stages have a high capacity for removing reactive oxygen species generated with maturity advancement, since APX is one of the enzymes responsible for removing H_2O_2 from cells using ascorbate as a substrate (PANDEY *et al*., 2017).

Figure 5 - Superoxide dismutase (SOD) (A), peroxidase (POD) (B), and ascorbate peroxidase (APX) (C) activities of 'Vitória' pineapple pulp at maturity stages: 100% green (TG); Break (B); 75% green and 25% orange (GO); 25% Green and 75% orange (OG); 90% orange and 10% green (PO), and 100% orange (TO)



* Means with equal lowercase letters do not differ by the Tukey test at 5% probability. n = 5

Total antioxidant activity (TAA) by the capture of $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals

Antioxidant activity was higher in pineapple pulp at B, GO, and PO stages, which did not differ, with higher $ABTS^{\bullet+}$ capture values, followed by fruit at TO and OG stages. The TG stage showed the lowest $ABTS^{\bullet+}$ capture values (Figure 6A). Ding and Syazwani (2016) reported an increase in antioxidant activity in 'MD-2' pineapple at maturity stages equivalent to TG (1) and GO (3) and a decrease as maturation progressed. However, Fawole and Opara (2013) attributed this variation in antioxidant activity, in cases of more mature fruits with high AAT, to be due to the increase in antioxidant defense against the effects caused by oxidative stress resulting from advancing maturation. The $ABTS^{\bullet+}$ capture values reported herein were close to those of the 'MD-2' pineapple at the GO equivalent stage, with values of around 1.71 µM Trolox g^{-1} (MARTÍNEZ *et al*., 2012).

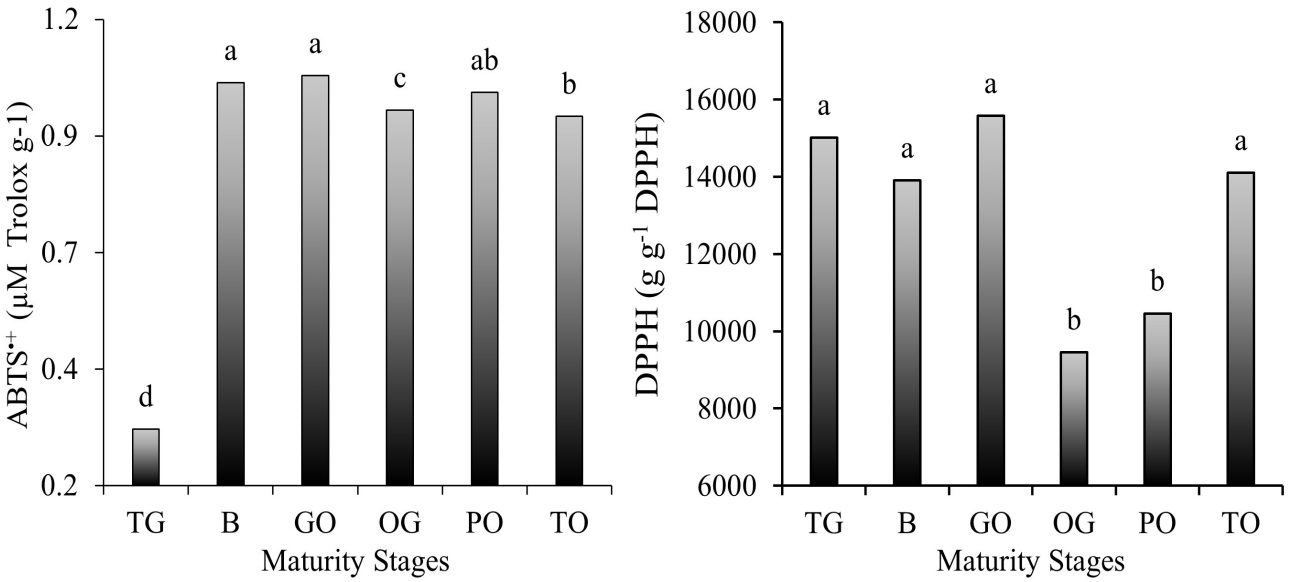
The antioxidant activity determined by the $DPPH^{\bullet}$ ($DPPH^{\bullet}$) radical capture was expressed in g of pulp g^{-1} of $DPPH^{\bullet}$, meaning the smaller the number of grams of pulp needed to reduce grams of DPPH•, the greater the antioxidant activity. Thus, the OG and PO stages presented twice the highest antioxidant activity on average when compared to the other maturation stages. In turn, TG, B, GO, and TO stages did not differ and presented the lowest antioxidant activity by this method (Figure 6B). This variation seems to be related to the total extractable phenolic (TEP) content, since fruit at maturity stages that presented low phenolic content also showed a lower $DPPH^{\bullet}$ sequestration capacity (Figure 3D). Phenolic compounds are generally the phytochemicals with the greatest contribution to the antioxidant activity of plants. These compounds contain several hydroxyl groups that may be responsible for the ability to eliminate free radicals (MARTÍNEZ *et al*., 2012). Nonetheless, Paz *et al*. (2015) found low efficiency of phenolic extracts from pineapple pulp in scavenging free radicals, and attributed this fact to phenolic compounds being linked to other molecules, such as carbohydrates, which can considerably reduce antioxidant activity. The variation in the antioxidant activity of pineapples may be due to the phenolic content in the extracts, climatic conditions, and postharvest handling of the infructescences (DANTAS *et al*., 2015). In this sense, the antioxidant activities of the pineapples studied cannot solely be attributed to their phenolic content, but also to the actions of different antioxidant compounds present in the fruit, such as ascorbic acid and β-carotene.

Principal component analysis

Principal component analysis explained 72.85% of the variance among maturity stages with two principal

components (PCs). Table 1 shows the contributions of the variables to the first two PCs, which in turn contribute to discriminating the maturity stages into four groups, simultaneously considering their effects on the physical, physicochemical, functional potential, and activities of antioxidant enzyme variables.

Figure 6 - Total antioxidant activity by capturing (A) $ABTS^{\bullet+}$ and (B) $DPPH^{\bullet}$ radicals of 'Vitória' pineapple pulp at maturity stages: 100% green (TG); Break (B); 75% green and 25% orange (GO); 25% Green and 75% orange (OG); 90% orange and 10% green (PO), and 100% orange (TO)



* Means with equal lowercase letters do not differ by the Tukey test at 5% probability. n = 5

Table 1 - Eigenvectors of the two main components (CP1 and CP2) for variables related to physical, physicochemical characteristics, functional potential, and enzymatic and non-enzymatic antioxidant activities of 'Vitória' pineapple pulp at maturity stages: 100% green (TG); Break (B); 75% green and 25% orange (GO); 25% Green and 75% orange (OG); 90% orange and 10% green (PO), and 100% orange (TO)

Variables	PC 1	PC 2
CI	0.323	-0.283
Firmness	-0.350	-0.203
SS	0.385	0.046
TA	0.222	0.411
SS/TA	0.183	-0.387
AA	-0.304	0.063
YF	0.306	-0.043
β-Carotene	0.097	-0.069
TEP	0.259	-0.280
DPPH	-0.322	0.277
ABTS	0.273	0.143
APX	0.260	0.167
SOD	0.159	0.382
POD	0.089	0.445
Eigenvalue	6.074	4.126
Accumulated variance (%)	43.38	72.85

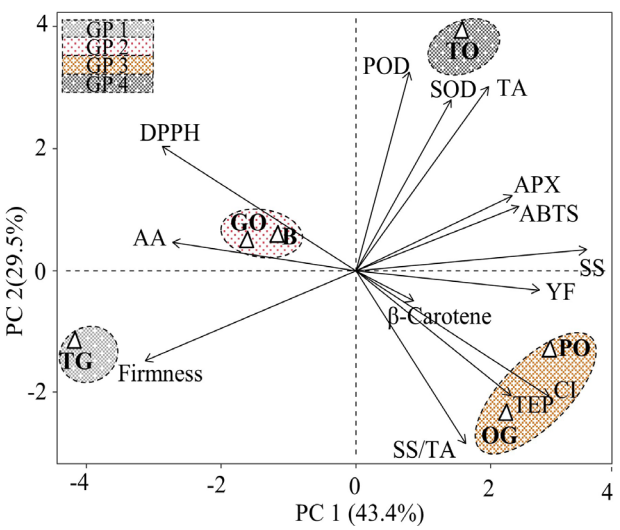
Values in bold represent eigenvectors with significant contribution (correlation greater than ± 0.6) to the component in question determined according to Wairegi and Van Asten (2011)

PC1 explains 43.38% of the total variance of the original data set. The variables in PC1 with positive eigenvectors are color index (CI), soluble solids (SS), yellow flavonoids (YF), total extractable polyphenols (TEP), ABTS, and the APX enzyme activity, while firmness, ascorbic acid (AA), and DPPH presented negative eigenvectors (Table 1).

PC2 explains 29.47% of the total variance and the variables which contributed to this component were TA, SOD, and POD enzyme activities, which presented positive eigenvectors, as well as the SS/AT variable, which presented a negative eigenvector (Table 1).

Figure 7 shows the circle of eigenvectors for the studied variables and the grouping of the 'Victoria' pineapple pulp maturity stages based on the variables correlated with PC1 and PC2. In this sense, group 1 was composed of the TG stage, which presented a negative and high score in PC1, mainly characterized by high firmness, low antioxidant activity by ABTS, as well as lower soluble solids (SS), titratable acidity (TA), flavonoids (YF), and color index (CI) levels. In turn, group 2 brought together the GO and B stages. This group was close to the centroid, indicating values close to the general mean for most of the original variables under study. However, group 2 was also characterized by maturity stages with higher ascorbic acid (AA) levels (stage B) and low antioxidant activity by DPPH (GO and B).

Figure 7 - Biplot based on the values of the variables and scores for the treatments of the first two main components (CP1 and CP2) originating from the physical, physicochemical characteristics, functional potential, and enzymatic and non-enzymatic antioxidant activities of 'Vitória' pineapple pulp at maturity stages: 100% green (TG); Break (B); 75% green and 25% orange (GO); 25% Green and 75% orange (OG); 90% orange and 10% green (PO), and 100% orange (TO)



Group 3 was formed by the PO and OG stages, and presented positive scores in PC1 and negative in PC2. In this sense, pineapples in the PO and OG stages have a high total extractable polyphenols (TEP) content, greater antioxidant activity by DPPH and ABTS, in addition to a higher SS/AT ratio, higher CI, low POD and SOD enzyme activities, and low titratable acidity, which highlight them as those with quality and functional characteristics that are most appealing to consumers. Finally, in group 4, the TO stage differed from the others, presenting a high and positive score in PC2 mainly characterized by high POD and SOD enzyme activities, high acidity and lower SS/AT ratio, characterizing it as an advanced ripening pineapple.

CONCLUSIONS

In infructescences of 'Vitoria' pineapples:

1. Changes in color during fruit maturation were clearly demonstrated by the CI;
2. The initial maturity stages (TG and B) showed greater firmness, and the SS and TA contents and the SS/AT ratio increase, depending on the maturity degree;
3. The ascorbic acid content was highest in the initial stages (TG and B), which progressively decreased, while the YF and TEP contents increased as maturation progressed, with the highest contents in the partially orange stage (PO); the highest β-carotene levels were in the OG and TO stages;
4. The antioxidant activity by capturing the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals was greater in fruit at the PO maturity stage;
5. The molecular weight of the antioxidant enzymes was estimated at 47 kDa POD, 28 kDa SOD, and 37 kDa APX, with more intense staining in the PO and TO stages (POD); TG, OD, and PO (SOD); and TO (APX);
6. The GO and TO maturity stages showed the highest superoxide dismutase (SOD) activities;
7. Peroxidase activity (POD) was higher in pineapple at the TO maturation stage;
8. The B, PO, and TO maturity stages showed the highest ascorbate peroxidase (APX) activities;
9. The TO stage is characterized as advanced maturation based on the bioactive compound content, antioxidant activity, and enzymatic activity;
10. The OG and PO maturation stages are characterized as being of maximum quality, lower oxidative stress, greater contribution of bioactive compounds and functional potential, and thereby valuing these stages of the 'Victoria' pineapple for fresh consumption.

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