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Activity of oxidative enzymes involved in the browning of minimally processed sweet cassava (*Manihot esculenta* Crantz)

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Abstract

Recent studies report that the shaped minimally processed sweet cassava 'Rubiene shape', when submitted to turning, presents extended useful life compared to the not turned one, i.e., the 'Minitolete shape'. This behavior may be related to a greater participation of enzymes involved in protection against oxidative damage. The objective of this research was to evaluate the effect of the minimally processed sweet cassava shape on postharvest physiological deterioration with emphasis on the activity of oxidative enzymes involved in the darkening of tissues. Sweet cassava roots, cv. Mossoró, were produced in the experimental field of the Unidade Acadêmica de Serra Talhada, in Serra Talhada - PE, Brazil. After 14 months, they were harvested and minimally processed in Minitolete and Rubiene shapes. The product consisting of a package containing approximately 200 g was stored at 5 ± 2 °C and 90 ± 5% RH for 11 days. The experimental design was completely randomized using a 2x6 factorial design, referring to shapes and refrigerated storage times with 3 repetitions. Visual analysis of the processed product, total soluble phenols, polyphenol oxidase enzyme activity, peroxidase, superoxide dismutase, catalases and ascorbate peroxidases were evaluated. The reserve parenchyma cells, remaining on the Rubiene shape were less responsive to postharvest physiological deterioration, in contrast to the sensitivity observed in surface tissues of the 'Minitolete shape'. This indicates that changes in the phenolic and oxidative metabolism of sweet cassava roots are tissue-dependent. The Rubiene shape maintained its quality during the 11 days of storage at 5 ± 2 °C, 4 days more if compared to the 'Minitolete shape'.

Keywords: postharvest physiological deterioration; enzymatic browning; oxidative stress; quality; injury.
Abbreviations: APXs_ascorbate peroxidases, CATs_catalases, H_2O_2_Hydrogen peroxide, PAL_phenylalanine ammonia-lyase, PP0_polyphenol oxidase, POD_peroxidase, ROS_reactive oxygen species, SODs_superoxide dismutase, TSP_total soluble phenols.

Introduction

Estimated postharvest losses, in the case of sweet cassava, correspond from 5 to 25% of the production (Wenham, 1995). This happens mainly due to the postharvest physiological deterioration that begins 24 to 72 hours after harvest (Wheatley, 1987). The main symptoms for roots *in natura* and in minimally processed form are the formation of bluish streaks, vascular discoloration and darkening of tissues throughout the storage parenchyma (Marriot et al., 1978). Recent studies showed that sweet cassava roots have potential suitability for minimum processing (Brito et al., 2013; Junqueira et al., 2014), thus representing an alternative to their increased marketing. Brito et al. (2013) demonstrated that minimal processing and conservation procedures of sweet cassava have been adjusted as new formats of product presentation, such as the proposed 'Minitolete' and 'Rubiene', the latter being produced with the aid of a turner, whose technique is applied in other tubers, such as carrot (Simões et al., 2010) and potato (Pineli and Moretti, 2004). The abrasion, caused by turning, allows the removal of the surface tissue and it may consequently slow down some symptoms of DFP, as reported by Silva et al. (2013). Both shapes aim at constituting a new option for this root's agribusiness due to its high visual appeal, with an expected increase in the consumption by food service, delivery and hotel sectors. Minimal processing acts as a stress inductor in the plant tissue, as the mechanical damage caused by peeling, cutting, slicing and/or turning causes the breakage of tissue homeostasis. This causes the cells to decompartmentalise at injury sites, promoting increased oxidative processes catalyzed by polyphenol oxidase (PPO) and peroxidases (POD) that act in the oxidation of phenolic compounds producing insoluble and dark pigments (Tomás-Barberán and Spín, 2001). However, there is an increase in respiratory rate, which can lead to the accumulation of reactive oxygen species (Vieira et al., 2013), potentially harmful, responsible for oxidative damage to proteins, DNA and lipids, disrupting cell membranes, among other degenerative changes (Apel and Hirt, 2004; after Mollller et al., 2007, please insert Vieira et al., 2015). Recent researches address the negative effects and propose solutions to control the enzymatic browning and oxidative stress in sweet cassava roots (Zidenga et al., 2012; Xu et al., 2013), since the phenolic and oxidative metabolism directly affect the commercial quality of the tubers. According to Xu et al. (2013), there is a correlation between

the accumulation of ROS and enzymatic browning of sweet cassava roots. Reilly et al. (2004) confirm the dual role played by ROS, which act both in signaling cell death in response to injury and in the oxidation of phenol compounds to produce visible DFP symptoms. The deleterious effects of ROS production are minimized by activating a complex enzymatic defense mechanism for purposes of neutralizing the physiological and biochemical changes. At first, the superoxide dismutase (SODs) enzymes act, followed by catalases (CATs) (Apel and Hirt, 2004). Still, some peroxidases promote the removal of hydrogen atoms from alcohols, combining them with hydrogen peroxide, harmful to cells, to form water molecules such as ascorbate peroxidases (APXs) (Apel and Hirt, 2004). Xu et al. (2013) reported the key role of SODs and CATs in minimizing the negative effects of high ROS concentrations in sweet cassava roots. During the first 24 hours after harvest, the SOD's activity was severely increased, both in wild type plants and in transgenic ones. The SODs act in reducing the superoxide radical concentration by converting it to H_2O_2 and CATs. In turn, they are able to neutralize the toxic effect of H_2O_2 by converting it to water (Apel and Hirt, 2004). The visible symptoms of DFP may be closely related to the activity of SODs and CATs. In its initial phase, the activity of CATs and SODs is sufficient to promote cellular detoxification. However, when the total disposal capacity of CATs is exceeded, there is H_2O_2 accumulation, expressing therefore the DFP (Xu et al., 2013). Recent studies report that the Rubiene shape although having its tissue exposed to a higher intensity of mechanical injuries due to turning does not darken at the same speed if compared to the non-turned shape, i.e., 'Miniolete', thus keeping the product quality for consumption for a longer period (Silva et al., 2013). It is believed that this behavior may be related to a greater participation of enzymes involved in ROS elimination, as proposed by Xu et al. (2013) and Iyer et al. (2010). Thus, studying the relations of phenolic and oxidative metabolism in different shapes can bring additional information to what is known about the darkening of tissue cut from sweet cassava roots. Thus, the objective of this research is to evaluate the shape effect of minimally processed sweet cassava on postharvest physiological deterioration, with emphasis on the activity of oxidative enzymes involved in the darkening of tissues, aiming at a better understanding of the phenolic and oxidative metabolism.

Results and Discussion

The "Rubiene" shape has smaller change in phenolic metabolism and darkening less

Shapes have differences regarding incidence of darkening of their tissues (Fig. 1A). The "Minitolete" decreased significantly in appearance from the third day of conservation, mainly due to the presence of dark spots in the tissues of roots' system I (phellogen and phelloderm) (Carvalho et al., 2004). In the seventh day, the "Minitolete" underperformed the established limit score, being considered unfit for consumption. "Rubiene" shape darkened poorly throughout the conservation, resulting in scores above the accepted limit. The slight reduced quality was promoted mainly by the presence of small traces of yellowing, insufficient to depreciate the appearance below the limit. Thus, "Rubiene" was fit for consumption throughout the conservation period. This was a similar result to that reported by Silva et al. (2013), in which "Rubiene" could be consumed for up to 9 days. In general, the "Rubiene" shape became less dark if compared to the "Minitolete". This possibly occurred due to the removal of superficial tissues through turning, a characteristic step for obtaining this shape. The remaining tissues, despite being subjected to greater injuries, did not transmit the damage in the same proportion. This is possibly related to a lower activity of enzymes involved in the enzymatic browning, PPO and POD, resulting in quality maintenance for a longer period. The extended useful life found in this study is similar to that observed by Junqueira et al. (2014), whose "sticks" showed stability in the index of tissues, browning after 12 days of storage. The Minitolete shape of minimally processed sweet cassava promoted rapid product depreciation, even when stored at 5 ºC, being considered suitable for commercialization until the 7th day of conservation. For Rubiene, this period may be extended for up to 11 days (Fig. 1A). The "Rubiene" shape showed contents twice as high as the "Minitolete" at day 0 of storage, a tendency kept throughout the storage (Fig. 1B). When comparing average values, the same behavior was observed: "Rubiene" was twice as high (see Table 1). These results may be associated with a greater activation of the phenolic metabolism, especially for the "Rubiene", via the enzyme phenylalanine ammonia-lyase (PAL), that is, the first enzyme involved in the phenylpropanoid metabolism (Dixon and Paiva, 1995), which is responsible for the TSP biosynthesis. Furthermore, the lower activity of polyphenol (PPO) and peroxidase (POD) enzymes recorded for "Rubiene" (Fig. 2A and 2B) probably caused higher phenolic accumulation, since theses enzymes promote reduced TSP content in tissues by oxidation. It is believed that the higher levels of TSP in the "Rubiene" shape, as recorded throughout the preservation, compared to "Minitolete", are due to intense injury caused by turning, which stimulated PAL activity. Thus, despite the samples of TSP being collected only few hours after the turning, it was sufficient to alter the metabolism of phenylpropanoids. This was possibly due to stimulation promoted by abrasion of the tissues in lathe, which enhanced the production of an unknown elicitor responsible for intercellular communication; this promotes, in turn, increases in the PAL activity in tissues adjacent to injuries, as observed by Saltveit et al. (2005) and Choi et al. (2005), and/or through induction of reactive oxygen species (ROS) (Orozcocárdenas et al., 2001). Enzymatic browning is directly influenced by the type and concentration of the phenolic substrate (Martin-Belloso and Solive-Fortuny, 2006). The TSP concentration in tissues results from the balance between its synthesis and consumption (Reyes et al., 2007). The major phenolic compounds identified in cassava associated with the development of physiological deterioration include scopoletin, scopolamine esculin, proanthocyanidins, (+)-catechin and (+)-gallocatechin (Rickard, 1985). It is believed that a slight decrease between early and late evaluations for the 'Minitolete' occurred mainly due to scopoletin oxidation, which is primarily responsible for the vascular browning in cassava. This resulted in the production of dark and insoluble pigments, such as melanin, rapidly depreciating its quality (Fig. 1A). Regarding 'Rubiene', whose recorded decrease was 79%, the result in the production of dark compounds may not have been caused by oxidation of phenolic compounds, since this shape only showed signs of yellowing in their tissues. In addition, the decreased TSP in both shapes may also be related to the healing of injured tissues with a deviation toward the formation of insoluble phenols such as lignin (Reyes et al., 2007). This may explain the increase in roots' cooking time

during the conservation (data not shown). The responses found for "Rubiene" throughout the conservation, in this

Table 1. Sensory acceptance, Phenolics compounds, Polyphenol oxidase and Peroxidase activity in roots of sweet cassava cv. Mossoró harvested at 14 months and minimally processed in Minitolete and Rubiene shapes stored at 90 ± 5 % RH for 0, 3, 5, 7, 9 and 11 days.

Shapes	Visual score (1-5)	Phenolic compounds (mg Kg^{-1} FW)	Polyphenol oxidases activity (EU $min^{-1}mg^{-1}$ FW)	Peroxidases activity (EU $min^{-1}mg^{-1}$ FW)
Minitolete	3.70 b	19.90 b	4.90 a	7.28 a
Rubiene	4.44 a	39.97 a	2.57 b	5.41 b
C.V. (%)	9.23	11.17	24.21	14.27

Means followed by the same letter between lines do not differ significantly by Tukey test at 5 % probability. C.V. which means coefficient of variation.

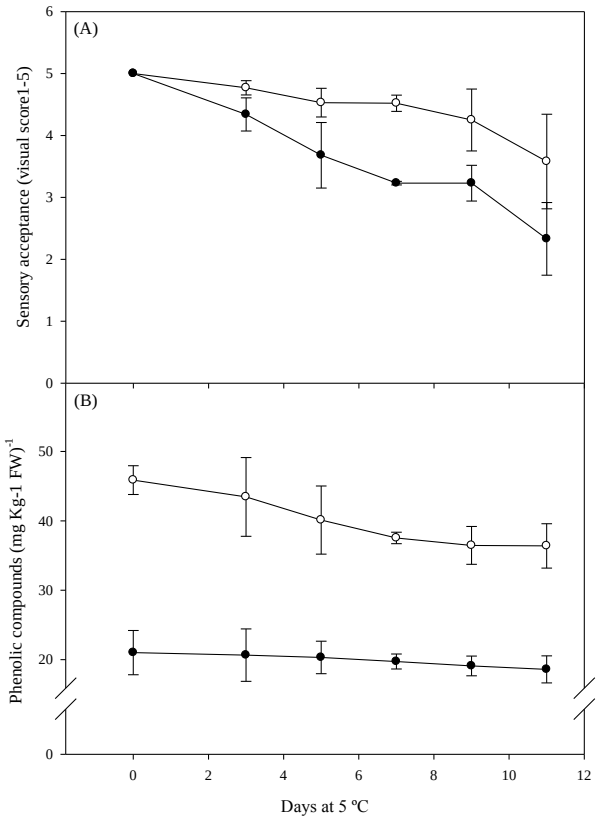

Fig 1. Scores on Appearance (A) and Phenolics compounds (B) in roots of cassava cv. Mossoró harvested at 14 months and minimally processed in Minitolete (-●-) and Rubiene (-○-) shapes stored at 5 ± 2 ºC and 90 ± 5 % RH for 0, 3, 5, 7, 9 and 11 days. The vertical bars represent the standard deviation from the mean and the minimal significant difference (MSD) at 5 %. Data for three replications.

work, are in accordance with that reported by Junqueira et al. (2014) with minimally processed sweet cassava in stick shape and stored at 5 ± 2 ºC, in which decreased TSP contents was also observed during the 12 evaluation days, but with no darkening being reported. It was observed that, on day 0, the "Minitolete" shape had approximately four times the activity of the "Rubiene" regarding PPO activity (Fig. 2A), and about twice as higher as POD (Fig. 2B). This tendency was maintained during the 11 conservation days and confirmed by the difference of almost 50% among shapes, with a higher activity of PPO for "Minitolete", while for POD this difference was close to 34% (Table 1). Besides, during evaluation days, increases were observed in PPO and POD activity. For "Minitolete", PPO activity increased by 33%, and for "Rubiene" it was four times higher (Fig. 2A). For POD, this increase was 21% in "Minitolete" and 4% in "Rubiene" (Fig. 2B). The increased PPO and POD activity during the 11 days of refrigerated storage may be associated with decreased TSP contents (Fig. 1B). This is because TSPs are substrates for these enzymes (Campos and Silveira, 2003). Increases in PPO and POD activity during storage seems to happen in roots that became dark, such as with sweet cassava (Junqueira et al., 2014) and Peruvian carrot (Nunes et al., 2010). The POD activity may have a synergistic action with PPO (Subramanian et al., 1999). The higher the oxidation of some soluble phenolic compounds by means of PPO, the greater the production of hydrogen peroxide (H_2O_2), a substrate for POD (Subramanian et al., 1999). Moreover, the injuries associated with minimal processing can stimulate oxidative stress by increasing the tissues respiratory rate, resulting in increased levels of reactive oxygen species (ROS) such as H_2O_2, as in plants subjected to adverse environmental conditions (Mittler, 2002). Thus, there is stimulation of peroxidases acting in the removal of hydrogen atoms from alcohols, combining them with H_2O_2 to form water molecules (Salisbury and Ross, 1992). The processing of stick cassava required the removal of tissues from the periderm to central portions of central xylem (Junqueira et al., 2014). In this shape, browning was not observed despite TSP decreases observed during the conservation period (Junqueira et al., 2014). These results corroborate what was evidenced in the present study, in which more internal parenchymal cells, despite having been exposed by abrasion promoted through turning, did not

respond proportionally to the damage if compared to tissues that were not turned. It is emphasized that, in both shapes, sampled tissues were removed from the most superficial layer with 5 mm. This suggests that responses to injuries depend on the tissue region; therefore, the enzymatic browning evolution was differentiated between shapes. The "Minitolete" became intensely dark, while the "Rubiene" showed only yellowish spots (Fig. 1). Therefore, the "Minitolete" shape showed a higher PPO and POD activity than "Rubiene", which can be directly related to susceptibility to tissues' browning.

"Minitolete" shape is more activity oxidative protection system enzyme compared to Rubiene shape

Shortly after minimal processing, SODs activity was 19% higher for "Minitolete" when compared to "Rubiene" (Fig. 3A). This behavior was maintained throughout the storage; however, the significant differences between the shapes occurred from the third evaluation day (Fig. 3A). During storage, SOD activity was increased regardless of shape. By the 11th conservation day, increases of about three times were noted for the "Minitolete" compared to that recorded at day 0. The increase for "Rubiene" was less intense (Fig. 3A). Cassava roots of wild and transgenic types exhibited, 24 hours after harvesting, a higher SOD activity, maintaining their high levels up to 96 hours (Xu et al., 2013). This resulted in an increased H_2O_2 concentration, mainly in transgenic plants. In this study, both formats had SOD levels increased over time, reaching a peak at about 11 conservation days, i.e., 264 hours. The observed increases reflect a plan tissue detoxification by removal of the radical O_2^- with the consequent production of H_2O_2 (Apel and Hirt, 2004). As observed for enzymes involved in the enzymatic browning, PPO and POD (Figure 2A and 2B), the lower SOD activity in "Rubiene" compared to the "Minitolete" is possibly due to different sampled tissues. For the "Minitolete", 5 mm of superficial layer was collected without the periderm. For the "Rubiene", the pieces underwent a further abrasion process caused by the turning, thus removing more cell layers from the surface. This confirms that different surface cells respond differently to oxidative damage. Immediately after minimal processing and until the seventh conservation day, the CAT activity was similar in both shapes studied. At 11 evaluation days, the difference observed between the shapes was 35%, being "Minitolete" higher than "Rubiene" (Fig. 3B). The Minitolete shape had as result APX activity higher than Rubiene's, at 69%, in all evaluations (Fig. 3C). On day 0 of conservation, "Minitolete" was higher than "Rubiene" at 72%. This shows that the "Minitolete" has more susceptible tissues to transient increases in APX activity if compared to those of "Rubiene". An increased activity of the APX during storage was also found. For "Minitolete", 52%, when compared to the values observed by the 11th day after the minimal processing in relation to day 0. For "Rubiene", such increase was 55%. This behavior was observed for SODs (Fig. 3A) and CATs (Fig. 3B), which also act in ROS removal. In general, it was found that stimulating SOD activity (Figure 3A), especially in "Minitolete", generates a higher H_2O_2 concentration in their tissues, promoting a greater action of secondary enzymes involved in neutralization, such as CATs (Figure 3B) and APXs (Figure 3C). As observed by Xu et al. (2013) for wild type and transgenic cassava, whose increase in CATs was observed 48 hours after harvesting, the ROS accumulation and enzymatic browning in sweet cassava seem to be correlated events; in which ROS act possibly in two ways: signaling molecules

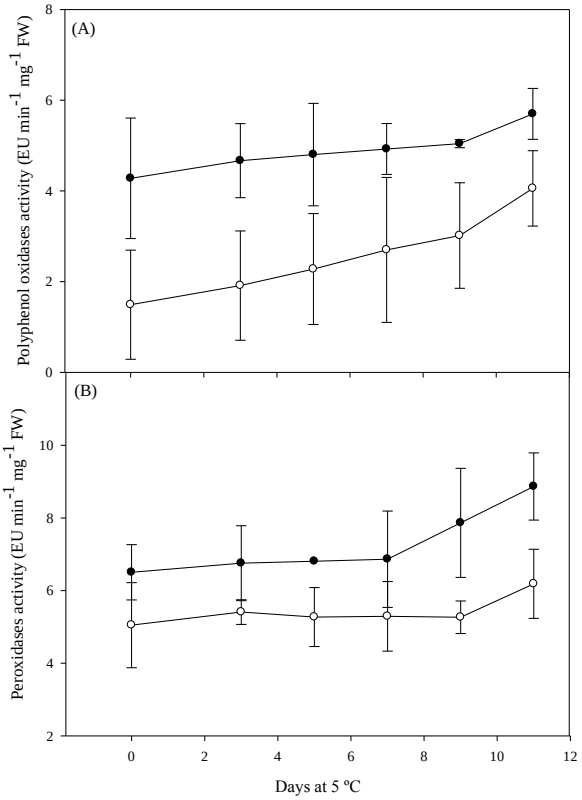

Fig 2. Polyphenol oxidases (A) and Peroxidases activity (B) in roots of cassava cv. Mossoró harvested at 14 months and minimally processed in Minitolete (-●-) and Rubiene (-○-) shapes stored at 90 ± 5 % RH for 0, 3, 5, 7, 9 and 11 days. The vertical bars represent the standard deviation from the mean and the minimal significant difference (MSD) at 5 %. Data for three replications.

promoting programmed cell death and oxidation of soluble phenols (Xu et al., 2013). During the initial phase of DFP in cassava, the immediate conversion of radical O_2 occurs, in which available CATs are sufficient to catalyze the conversion of these ROS to water and oxygen, reducing the H_2O_2 concentration at 24 hours after harvesting (Xu et al., 2013). After that, the gradual increase of CAT activity was insufficient to monitor H_2O_2 accumulation. The authors attributed this fact to a possible explanation for the early symptoms of postharvest physiological deterioration. The results found in this study indicate that the increase of the activity of SODs, CATs and APXS, obtained mainly for the Minitolete shape, were not proportional in order to minimize the cytotoxic effects from the ROS accumulation. This has resulted in DFP symptoms with consequent apparent depreciation. Similar results were found by Reilly et al. (2001) and Owiti et al. (2011), who reported an increased activity for SODs and CATs. However, increases were late and did not prevent the emergence of visible symptoms of postharvest physiological deterioration. Thus, the Minitolete shape showed higher enzymatic oxidative protection system (SODs, CATs and APXs) activity in the phenolic metabolism enzymes (PPO and POD) when compared to the Rubiene shape. This system became more active during storage, but the increased activity of cellular detoxification did not prevent the depreciation of the roots' quality. The results of the present study indicate that the injury intensity caused by the turning of root pieces of sweet cassava root did not result

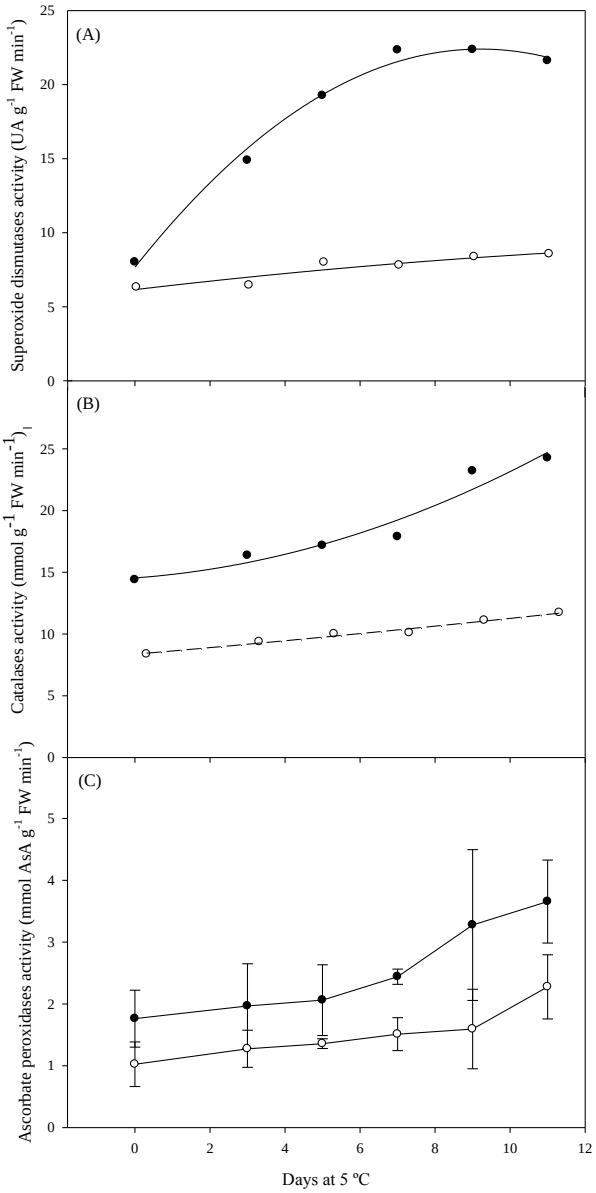

Fig 3. Superoxide dismutases (A), Catalases (B) and Ascorbate peroxidases activity (C) in roots of cassava cv. Mossoró harvested at 14 months and minimally processed in Minitolete (●) and Rubiene (○) shapes stored at 90 ± 5 % RH for 0, 3, 5, 7, 9 and 11 days. Adjustment of equation for graphic A, Minitolete (●) $y^2 = 58,30+183,83x^{0,5}$ $R^2=0,94$** and Rubiene (○) $y^2 = 43,43+3,55x$ $R^2 = 0,87$**. Adjustment of equation for graphic B, Minitolete (●) $y^2 = 214,11+3,20x^2$ $R^2=0,94$** and Rubiene (○) $y^{-1} = 0,066-0,001x$ $R^2 = 0,98$**. The vertical bars in graphic C represent the standard deviation from the mean and the minimal significant difference (MSD) at 5 %. Data for three replications.

in an increased intensity of the browning and the activation of the oxidative metabolism of remaining superficial tissue. This implies that changes in phenolic and oxidative metabolism in roots of sweet cassava are tissue-dependent, because the cells of the reserve parenchyma, that remained in Rubiene shape, were less responsive to postharvest physiological deterioration, in contrast to the sensitivity observed for superficial tissues of "Minitolete" shape. Thus, the Rubiene shape maintained its quality during the 11 conservation days at 5 ± 2 °C, 4 days more if compared to "Minitolete".

Material and Methods

Plant materials, Harvesting, minimal processing and storage

Sweet cassava roots (*Manihot esculenta* Crantz) cv. Mossoró from useful cultivation area were harvested at 14 months of age. They were cooled in cold storage at 5 ° ± 2 ° C and 90 ± 5% relative humidity for 24 hours. The "Minitolete" and "Rubiene" shapes were obtained by Freire et al. (2014). Both shapes were immersed in cold water for 10 seconds (initial rinse); sanitized with 3% dehydrated sodium dichloroiso-cyanurate (in concentrations of 200 and 5 ppm of active chlorine, respectively, for 10 min); centrifuged with angular velocity of 2800 rpm in domestic centrifuge for 60 seconds; packed in polypropylene bags (150 x 200 mm and 0.4 µm thick) and kept under cold storage at 5 ± 2 ºC and 90 ± 5% of RH for 11 days.

Visual analysis (appearance)

The visual assessment was performed by two previously trained testers. They framed the samples in a sensory panel, whose criteria are specified below in accord to Brito et al. (2013). Staggered subjective scores contained in them range from 5 to 1, maximum and minimum scores, respectively. Score 3 was considered as the limit for acceptance of the processed product. Score 5 = characteristic white surface, excellent appearance and odor for consumption, having enough quality for commercialization; 4 = slight signs of browning; 3 = moderate browning intensity, limit of acceptance; 2 = greenish-yellow coloring on the surface, characteristic of Pseudomonas spp., sticky surface; 1 = all symptoms described above, and alcoholic odor, whitening on the surface, with dehydration, being totally unfit for consumption.

Total soluble phenols (TSP)

It was determined according to the methodology proposed by Reyes et al. (2007) with some modifications. 2g samples, taken from the surface tissue of minimally processed roots (± 5 mm of the surface tissue), were macerated in a mortar containing 10 mL of pure methanol and kept in the dark under cold storage at 5 °C for 24 hours. The extract was centrifuged (Hettich, model Universal 320 R) at 7960 *g* for 21 minutes, 2 °C. In the test, 150 µL of the supernatant extract diluted in 2400 µL of distilled water were used. 150 µL Folin Cioucauteu (0.25 N) was then added. The mixture was homogenized in shaker tubes for 3 minutes. 300 mL of calcium carbonate (1 N) were added. The preparation of the blank consisted of the replacement of the supernatant for 150 µL of pure methanol, keeping other analytical procedures. The reaction, with a final volume of 3.0 mL, was kept protected from light and at room temperature for 2 hours. Readings were carried out under spectrophotometer (model libra S8; Biochrom) at 725 nm. From the readings, the TSP was quantified with the aid of a standard curve using gallic acid in which a regression curve is generated for each shape.

Polyphenol oxidase (PPO, EC 1.10.3.1) and Peroxidase (POD, EC 1.11.17)

The extraction and activity of PPO and POD were performed according to Junqueira et al. (2014). 1 g samples were taken from the surface tissue (± 5 mm of the surface tissue) of minimally processed roots, macerated in a mortar, in ice bath, containing 6 mL of sodium phosphate buffer [0.2 M; pH 6.0 and polyvinylpyrrolidone (PVPP at 1%)]. Then the extract was centrifuged (Hettich, model Universal 320 R) at 7690 *g* for 23 minutes at 4 °C. Regarding PPO activity, the reaction, with a final volume of 2.9 mL, contained 100 µL of the supernatant enzyme extract and 1.5 mL of sodium phosphate buffer (102.45 mM, pH 6.0 and PVPP at 1%). The mixture was kept for 1 minute at room temperature (20 ± 5 ° C) for temperature stabilization. Then, 1.3 mL of catechol (89.65 mM) was added. The reaction was monitored under spectrophotometer (Biochrom; model libra S8) by change in absorbance at 425 nm for 2 minutes, with readings every 30 seconds. The activity was expressed in absorbance change per minute per milligram of fresh mass. The POD activity was performed with a reaction of final volume of 1.5 mL, composed of 300 µL of supernatant extract and 1 mL of sodium phosphate buffer (133.33 mM, PVPP to 1%, pH 6.0). The mixture was kept for 1.5 minutes at room temperature (20 ± 5°C) for temperature stabilization. Then 100 µL of hydrogen peroxide and 100 µl of guaiacol were added. The reaction was monitored in a spectrophotometer (Biochrom; model libra S8) by change in absorbance at 470 nm for 2 minutes, with readings every 30 seconds. The activity was expressed in absorbance change per minute per milligram of fresh mass. Extract enzyme supernatant volume replaced by the same volume of phosphate monobasic buffer was used as a blank in both enzymes comprising other analytical procedures.

Superoxide dismutase (SOD, EC 1.15.1.1)

The SOD activity was determined according to Giannopolitis and Ries (1977) with adaptations. 2 g samples taken from the surface tissue (± 5 mm of the surface tissue) of minimally processed roots were macerated in a mortar in ice bath, containing 8 mL of potassium phosphate monobasic buffer (0.1 M; pH 7.0). They were centrifuged at 7960 *g* for 12 minutes at 4 °C. The activity of SODs was determined according to Giannopolitis and Ries (1977), with adaptations. The reaction, with a final volume of 3.0 mL, consisted of 100 µL of enzyme extract supernatant, 1.5 mL of potassium phosphate monobasic buffer (50 mM, 0.1 µM EDTA, pH 7.8), 995 µL methionine (16.58 mM), 375 µL Nitro blue tetrazolium chloride (NBT) at 75 µM and finally 3 µL riboflavin (2 µM). The reaction was exposed to light (fluorescent lamp, 45 W) for 15 minutes, then read under a spectrophotometer (Biochrom; model libra S8) at 560 nm. Tubes without extract, i.e., replaced with monobasic potassium phosphate buffer, exposed and not exposed to light, were considered as blanks in reaction. One unit of SOD activity (U) was defined as the amount of enzyme required to inhibit 50% of NBT reduction, and the activity was expressed in U per gram of fresh mass per minute.

Catalase (CAT. EC 1.11.1.6)

The CAT activity was determined according to Beers Júnior and Sizer (1952), with adaptations. The reaction, with a final volume of 1.75 mL, consisted of 350 µL of the enzyme extract supernatant, 1.0 mL of potassium phosphate monobasic buffer [57.14 mM; 0.1 µM EDTA; pH 7.0; 40 ºC] and 400 µL of hydrogen peroxide (H_2O_2) to 114.28 mM. The reaction was monitored under a spectrophotometer (Biochrom; model libra S8) at 240 nm by means of degradation of H_2O_2 for 1 minute, with readings every 30 seconds. The molar extinction coefficient used was 36 M^{-1} cm^{-1}. The result was expressed in µmol H_2O_2 per gram of fresh mass per minute.

Ascorbate peroxidase (APX. EC 1.11.1.1)

The activity of APXs was determined according to the methodology proposed by Nakano and Asada (1981), with adaptations. The reaction, with a final volume of 1.79 mL, consisted of 90 µL of the enzyme extract supernatant, 1.2 mL of potassium phosphate monobasic buffer (33.52 mM; 0.1 µM EDTA; pH 6.0), 250 µL ascorbate (1.40 mM) and 250 µL of H_2O_2 (13.97 mM). The reaction was monitored under a spectrophotometer (Biochrom; model libra S8) at 290 nm and 1-minute interval, with readings every 30 seconds. The molar extinction coefficient used was 2.8 mM^{-1} cm^{-1} and the result was expressed in µmol ascorbate per gram of fresh mass per minute. The enzyme extract supernatant volume replaced by the same volume of monobasic potassium phosphate buffer was used as a blank in CATs and APXS enzymes, comprising other analytical procedures related to each enzyme.

Experimental design and statistical analysis

The overall experimental design was completely randomized, with a 2 x 6 factorial design, corresponding, respectively, to the shapes under study (Minitolete and Rubiene) and the refrigerated storage times (0, 3, 5, 7, 9 and 11 days after minimal processing). Data were subjected to analysis of variance; when significant, means between shapes were compared by Tukey test at 5% probability using the computer software Sisvar. With respect to the times of refrigerated storage, when possible, the equation of linear or nonlinear regression was adjusted to a 5% significance using the Table Curve software.

Conclusions

The injury severity did not result in increased enzyme activity of the phenolic metabolism and cellular detoxification; in other words, the changes in metabolisms of sweet cassavas are tissue-dependent. It seems that reserve parenchyma cells, remaining in the Rubiene shape, are less responsive to postharvest physiological deterioration, being opposed to the sensitivity observed in superficial tissues of "Minitolete".

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