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FULL ARTICLE

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Association of preharvest management with oxidative protection and enzymatic browning in minimally processed cassava

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Abstract

The aim of this study was to examine oxidative protection and enzymatic browning in the storage of minimally processed cassava and their relationship with population density and harvest age. Population densities were 1.0, 1.25, 1.5, and 1.75 plants m^{-2}. After being harvested at 300, 360, or 420 days after planting, cassava were minimally processed and stored at 5 ± 2°C. It was observed that superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) play key roles in the tolerance of young roots to browning. Planting density, however, does not appear to be a key factor modulating the activity of the enzymes studied.

Practical applications

Younger harvested cassava roots, harvested at 300 days, are more tolerant to enzymatic browning. This appears to be in part due to enzymatic activity modulation of the SOD, CAT, and POD enzymes. In addition, it has been demonstrated that agronomic techniques aimed at increasing productivity, such as increasing the planting density of cassava, do not alter the biomarkers of postharvest quality. In summary, evidence that field management may be an efficient approach to improving the conservation of minimally processed cassava is provided. We believe that the findings of this paper will be of great interest regarding the influence of field management on the postharvest quality of freshly cut cassava and will also provide applicable results relating to its production chain.

KEYWORDS

harvest age, *Manihot esculenta* Crantz, oxidative protection, planting density, postharvest physiological deterioration

1 | INTRODUCTION

Cassava has major socioeconomic importance in the northeastern region of Brazil, where it is grown in small family-run agricultural areas and is fundamental both as a food source and a means of generating employment and income (Gonzalez, Perez, Cardoso, Andrade, & Johnson, 2011). Cassava, also called manioc or tapioca, is the most important staple root crop in the world. Its production ranks sixth after maize, rice, wheat, potatoes, and soybeans (FAOSTAT, 2013).

Minimal processing of fruits and vegetables physically changes the products without modifying their sensory characteristics. It is appropriate for cassava because of the high perishability of the roots,

low postharvest value-added marketing, and lengthy preparation (Barrett, Beaulieu, & Shewfelt, 2010; Uchechukwu-Agua, Caleb, & Opara, 2015). Nevertheless, handling during the stages of minimal processing promotes damage to the cassava roots, leading to an increased respiratory rate, and the accumulation of reactive oxygen species (ROS). ROS are produced continuously and removed by various antioxidant mechanisms, including the enzymes superoxide dismutase (SOD), which catalyzes the conversion of O_2^- into H_2O_2 and catalase (CAT), which in turn removes H_2O_2 and produces H_2O and O_2 (Apel & Hirt, 2004). However, under conditions of stress, there is an oxidative burst and a higher production of ROS induced by damage (Xu, Duan, Yang, Beeching, & Zhang, 2013). This induces blue/black or brown discoloration of the vascular parenchyma from phenol oxidation via the action of polyphenol oxidase (PPO) and peroxidase (POD), triggering the phenomenon known as enzymatic browning or postharvest physiological deterioration (PPD) (Djabou, Carvalho, Li, Niemenak, & Chen, 2017; Freire et al., 2015; Salcedo & Siritunga, 2011).

Reduced visual quality, increased cooking time and changes to the taste induced by PPD, are the major constraints regarding the commercial acceptance of minimally processed cassava (Blagbrough, Bayoumi, Rowan, & Beeching, 2010; Salcedo et al., 2010). Therefore, studies on how to improve the techniques and technologies applied in the minimal processing of cassava and other vegetables, have intensified, and these have contributed to the quality of the products. The technological goal is to minimize the metabolic reactions that cause senescence and quality loss by various techniques, including the use of antioxidants (Ramos, Sediyama, Viana, Pereira, & Finger, 2013), atmosphere modification (Limbo & Piergiovanni, 2006) and incorporating nanoparticles into packing materials (Luo, Wang, Jiang, & Xu, 2015). However, these techniques increase the cost of production and hinder implementation on an industrial scale (Sánchez et al., 2013). Another alternative is the genetic modification of plants, through the introduction of genes that confer greater resistance to root browning (Xu et al., 2013). Despite some advances, high expression of genes encoding the enzymes of ROS scavenging pathways, is in some cases associated with root yield losses, and there is some discussion about potential risks to the ecological balance (Paoletti & Pimentel, 1996; Zidenga, Leyva-Guerrero, Moon, Siritunga, & Sayre, 2012).

Factors related to cultivation that can easily be modified, such as the harvest age and population density, promote changes in plants, both in morphology and postharvest quality attributes (Andrade et al., 2017; Leskovar, Agehara, Yoo, & Pascual-Seva, 2012; Simões et al., 2010). Recently, some field strategies have been reported to delay PPD in unprocessed cassava roots (Zainuddin et al., 2018). In this study, the authors reviewed superficially the effects of pruning, which increases the sugar/starch ratio and limits scopoletin accumulation, and furthermore they suggested that PPD should be evaluated at different harvest ages, indicating that this factor affects the postharvest quality of the roots. Andrade et al. (2017) demonstrated that at high harvest ages, the freshly harvested roots had lower enzyme activities and lower metabolite contents related to oxidative protection, such as SOD, CAT, and phenolics. However, how the age of harvesting and other management issues in the field affect PPD in the conservation of minimally processed cassava roots, remains to be determined. Thus, the objective of this study was to evaluate the biochemical changes related to oxidative protection and enzymatic browning in minimally processed cassava and the relationship between these changes and the population density and harvest age of the roots.

2 | MATERIALS AND METHODS

Cultivation was conducted in the experimental area of the Federal Rural University of Pernambuco, in the Academic Unit of Serra Talhada (UAST), Brazil. The vegetative propagules used in the planting were of the Mossoró variety, originating from the Active Germplasm Bank of the Federal Rural University of the Semi-Arid Region (UFERSA), Rio Grande do Norte, Brazil. Planting was carried out at densities of 1.0 plants m^{-2} (1 m between rows × 1 m between plants), 1.25 plants m^{-2} (1 m between rows × 0.80 m between plants), 1.5 plants m^{-2} (1 m between rows × 0.66 m between plants), and 1.75 plants m^{-2} (1 m between rows × 0.57 m between plants). Harvests occurred in July 2014 at 300 days (d) after planting, in September 2014 at 360 days after planting and in November 2014 at 420 days after planting.

The experimental design used randomized blocks in split plots, where the plots corresponded to the three ages at harvest (300, 360, and 420 days after planting) and the subplots corresponded to population densities (10,000, 12,500, 15,000, and 17,500 plants ha^{-1}), with three repetitions (blocks).

Harvested cassava roots were transported to the laboratory of the Postgraduate Program in Plant Production of the Unidade Acadêmica de Serra Talhada (PPGPV/UAST), where minimal processing was carried out in accordance with the process flowchart proposed by Freire, Simões, Vieira, Barros Júnior, and Costa (2014). The roots were then packed in 150 × 200 × 0.0006 mm polypropylene bags and stored at 5 ± 2°C and 90 ± 5% RH for 15 days, with three repetitions (bags of 150 g) of each harvest age, density and days of evaluation.

Over the 15 days of storage, roots were visually assessed every 3 days, at 0, 3, 6, 9, 12, and 15 days. At these times, ±2 mm-thick samples were collected for evaluation of soluble proteins, SOD, CAT, total soluble phenols (TSP), PPO, and POD. The samples were stored at −80°C.

2.1 | Visual assessment (general appearance)

Visual quality scoring was performed by four trained evaluators, to determine the presence or absence of dark spots on the tissue surface, yellow-green discoloration and a sticky consistency, all of which are characteristic of *Pseudomonas* spp. contamination. In addition, the presence of a fermented odor and whitening of the pieces, related to starch precipitation, was recorded. These characteristics were subjectively scored on a scale ranging from 1 to 5, based on

that described by Coelho, Andrade, Mélo Neto, Ferreira-Silva, and Simões (2017). Each piece received a score, and the average value was calculated for each sample.

2.2 | Total soluble proteins

Soluble proteins were extracted following the methods described by An, Yang, and Zhang (2012). We homogenized 0.25 g of tissue in 1.5 ml of potassium phosphate buffer (0.1 M, pH 7.0). The extract was centrifuged (MIKRO 220, Hettich, Berlin, Germany) at 10,000 × g for 21 min at 4°C. The test to quantify the content of soluble proteins was performed using methods taken from Bradford (1976), where 2.5 ml of Bradford reagent was added to 100 µl of the extract. The tubes were then agitated on a vortex mixer and remained at ambient temperature for 15 min. Readings were taken using a spectrophotometer (Libra S8, Biochrom, London, United Kingdom) at 595 nm, and the quantity of protein was calculated using the standard curve for bovine serum albumin and expressed in mg g^{-1} fresh weight.

2.3 | Total soluble phenols

The content of TSP was determined using methods in accordance with those of Freire et al. (2015). The extraction was performed by macerating 0.3 g of tissue in a mortar containing 1.5 ml of methanol. Then, the samples remained at rest for 20 hr in the dark at 4°C. Following this period, they were centrifuged at 10,000 × g at 2°C for 21 min

The assay was performed using 150 µl of supernatant, 2,400 µl of distilled water and 150 µl of Folin–Ciocalteu reagent (0.75 M). The mixture was homogenized for 3 min, 300 µl of $CaCO_3$ (2 M) were added and the tubes were kept in the dark at ambient temperature for 2 hr. In the blank control, 150 µl of methanol replaced the supernatant. The readings were taken using a spectrophotometer at 725 nm, and the results were expressed in mmol of TSP kg^{-1} of fresh matter, quantified using the standard curve of gallic acid.

2.4 | SOD activity

SOD activity was determined using methods described by Giannopolitis and Ries (1977). Aliquots of 100 µl of supernatant were added to 1.6 ml of potassium phosphate buffer, (50 mM, pH 7.8), containing 1 µM EDTA and 13 mM of methionine, 200 µl of nitro blue tetrazolium chloride (NBT) (0.75 mM), and 40 µl of riboflavin (1 mM).

The reaction was conducted in a light camera, under two 18-W fluorescent lamps, for 5 min Readings were taken at 540 nm. Activity was determined based on the inhibition of NBT reduction, with a unit of activity defined as the amount of enzyme needed to inhibit 50% of the photoreduction. The result was expressed in a specific activity unit (U mg protein^{-1} min^{-1}).

2.5 | CAT activity

The assay for CAT activity was conducted in accordance with Havir and McHale (1987). Aliquots of 300 µl of supernatant were added to 2.7 ml of potassium phosphate buffer (50 mM, pH 7.0), containing H_2O_2 (20 mM) previously kept at 30°C in a dry bath. The reaction occurred at 30°C and was accompanied by the decay of the absorbance at 240 nm for 3 min, with successive readings every 30 s. The CAT activity was calculated based on the molar extinction coefficient of 36 m^{-1} cm^{-1} for H_2O_2 and was expressed in µmol H_2O_2 mg protein^{-1} min^{-1}.

2.6 | PPO and POD activity

The extraction and determination of PPO activity and the extraction of POD was performed according to Freire et al. (2015), with homogenization of 0.25 g of superficial tissue (±2 mm) in 1.5 ml of potassium phosphate buffer (0.2 M, pH 6.0) previously maintained at 4°C. The extract was centrifuged at 10,000 × g for 21 min at 4°C.

In the assay for PPO, 100 µl of supernatant was added to a reaction medium containing 1.5 ml of potassium phosphate buffer (0.2 M, pH 6.0) and 1.3 ml of catechol (0.2 M), previously maintained at 25°C in a dry bath. The readings were taken using a spectrophotometer at 425 nm, at a temperature of 25°C, for 2 min, with a 10-s interval between readings. PPO activity was calculated based on the molar extinction coefficient of 3,400 M/cm for catechol and was expressed in µmol catechol mg protein^{-1} min^{-1}.

The assay for POD was conducted in accordance with Simões, Moreira, Mosquim, Soares, and Puschmann (2015), with 100 µl of supernatant added to a reaction medium containing 1 ml of potassium phosphate buffer (0.2 M, pH 6.0), 100 µl of guaiacol (40 mM) and 100 µl of hydrogen peroxide (23 mM), previously maintained at 25°C in a dry bath. Absorbances were obtained using a spectrophotometer at 470 nm, at a temperature of 25°C, for 3 min, with a 30-s interval between readings. POD activity was calculated based on the molar extinction coefficient of 26.6 mM^{-1} cm^{-1} for guaiacol and expressed in nmol mg protein^{-1} min^{-1}.

2.7 | Statistical analyses

The experimental design was completely randomized, with a factorial of 3 × 4 × 6 (300, 360, and 420 days after planting × 1.0, 1.25, 1.5, and 1.75 plants m^{-2} × 0, 3, 6, 9, 12, and 15 days of storage), with three repetitions. Biochemical assays were performed in technical triplicates. The data were analyzed using normality tests and analysis of variance, using the software Assistat v. 7.7 beta (Silva & Azevedo, 2016). The means were presented, including standard errors.

3 | RESULTS AND DISCUSSION

Statistical analyses (Table 1) show significant and non-significant effects of density of planting, harvest age and conservation times on the analyzed physiological and biochemical parameters. There was a triple interaction effect (with the exception of SOD), for visual assessment (VA), soluble proteins (SP), total soluble phenolics (TSP), CAT, PPO, and POD activities (Table 1). This shows statistically that

TABLE 1 Summary of the analysis of variance (ANOVA) for the effects of harvest ages (300, 360, and 420 days), plant densities (1.0, 1.25, 1.5, and 1.75 plants m^{-2}) and conservation times (0, 3, 6, 9, 12, and 15 days of storage) and their interactions on Visual assessment (VA), Soluble proteins (SP), Total Soluble Phenolics (TSP); Superoxide dismutase (SOD), Catalase (CAT), Polyphenoloxidase (PPO), and Peroxidase activities (POD)

		F value						
SV	*df*	VA	SP	TSP	SOD	CAT	PPO	POD
Harvest age (HA)	2	177.37*	174.88*	66.07*	7.98*	12.86*	167.37*	117.53*
Densities (D)	3	88.43*	21.88*	70.84*	5.71*	3.29*	5.73*	26.53*
Conservation (C)	5	80.39*	1.22ns	6.52*	0.79ns	6.78*	2.29*	4.18*
HA × D	6	65.16*	11.71*	5.95*	3.45*	0.69ns	36.18*	9.87*
HA × C	10	15.47*	1.84ns	3.48*	2.09*	3.73*	1.49ns	3.67*
D × C	15	7.04*	4.30*	9.21*	2.77*	0.77ns	3.54*	1.22ns
HA × D × C	30	5.02*	2.84*	3.26*	1.42ns	1.62*	3.94*	1.79*
Error	216							
Total	287							
CV (%)		3.58	11.84	20.34	32.74	36.36	20.49	22.75

Abbreviations: CV, coefficient of variation; SV, source of variation; degree of freedom.
*Significative at 5% of probability.

the preharvest management parameters used, especially the harvest age, influences the postharvest quality of minimally processed cassava roots.

There was a slight decrease in the scores during the storage of the roots from plants grown with densities of 1.5 and 1.75 plants per m^{-2}, as a consequence of an increased browning in the roots, although this was above grade 3, which is the limit of acceptance (Figure 1a).

This trend also occurred for the harvest times, in that roots harvested at 360 and 420 days resulted in slices with higher visually observed PPD symptoms than those harvested at 300 days (Figure 1b). Tumuhimbise, Melis, and Shanahan (2015), evaluating 12 contrasting cassava genotypes for tolerance of root slices to PPD, also observed a trend of increasing symptoms with increasing harvest time. Interestingly, in our study, all averages up to 15 days were above grade 3 (Figure 1a, b), which is the limit of commercial acceptance according to Coelho et al. (2017). This suggests that all recommended procedures for maintaining the quality of minimally processed cassava, for example, a cold chain, the use of sharp knives and appropriate packaging and temperatures for storage (Silva, Soares, & Geraldine, 2003), were correctly conducted.

The content of soluble proteins ranged from 1.3 to 2.2 mg/g of fresh mass (Figure 2a, b). This is similar to the findings of Schmitz, Magalhães Andrade, Valle, Labate, and Nascimento (2016), who observed values close to 1.0 mg/g of protein in tuberous roots of six cassava varieties.

Protein content decreased during storage (Figure 2a, b). Generally, protein levels decrease during the process of senescence in plants (Eason, Vré, Somerfield, & Heyes, 1997), due in part to the use of proteins in respiration (Halevy & Mayak, 1981) and the action of specific proteases (Shahri, Tahir, Islam, & Bhat, 2011), because the turnover favors degradation rather than synthesis. In this study, there was no change in protein content as a function of population density (Figure 2a), but as time to harvest increased, the content of soluble proteins decreased, following the trend of the enzymes SOD, CAT and PPO (Figures 4b, 5b and 6b). Protein content can be used

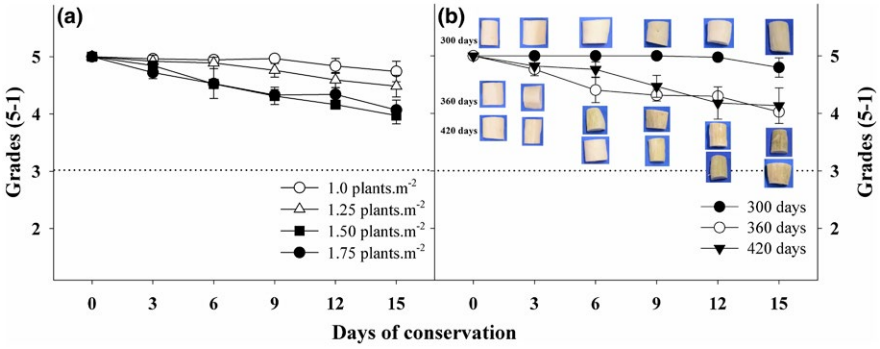

FIGURE 1 Visual scores of minimally processed cassava planted at different densities (a) and harvested at different ages (b). All were stored at 5°C for 15 days. The dotted line represents the score limit of commercial acceptance. In b, figures are representative of general appearance of minimally processed cassava harvest at 300, 360, and 420 days and stored for 0, 3, 6, 9, 12, and 15 days at 5°C

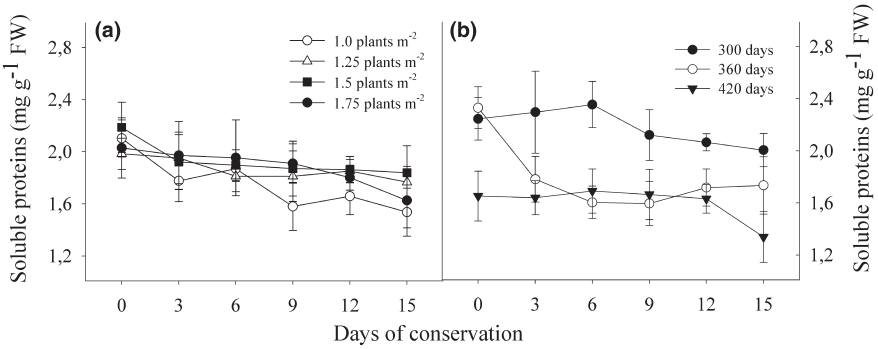

FIGURE 2 Soluble proteins in minimally processed cassava planted in different densities (a) and harvested at different ages (b). All were stored at 5°C for 15 days

as an indicator of deterioration in cassava, because low values are related to root senescence during storage (Uarrota et al., 2015).

A reduction in TSP of approximately 1.3–1.6 times was observed after 15 days of storage, in relation to day 0 (Figure 3a, b). This may be related to the oxidation of these compounds, or to the formation of insoluble phenols used in healing tissue damage resulting from minimal processing (Freire et al., 2015; Reyes, Villarreal, & Cisneros-Zevallos, 2007).

A reduction in the phenol content during storage of minimally processed cassava was also observed by Junqueira, Simões, Sediyama, Côrrea, and Puschmann (2014). Uarrota and Maraschin (2015) verified that the content of phenolic compounds in cassava increased at the beginning of storage, which was related to a defense response associated with increased activity of phenylalanine ammonia lyase and was followed by an abrupt reduction over the course of storage. In general, phenolic-compound content decreased significantly by the third day and remained stable over the remaining 12 days of storage, regardless of population density (Figure 3a).

Later harvest promoted a reduction in the content of phenolic compounds (Figure 3b). This is likely to be related to the visually observed browning of roots harvested at 360 and 420 days (Figure 3b). Similar results were observed by Pimenta and Vilela (2003), who

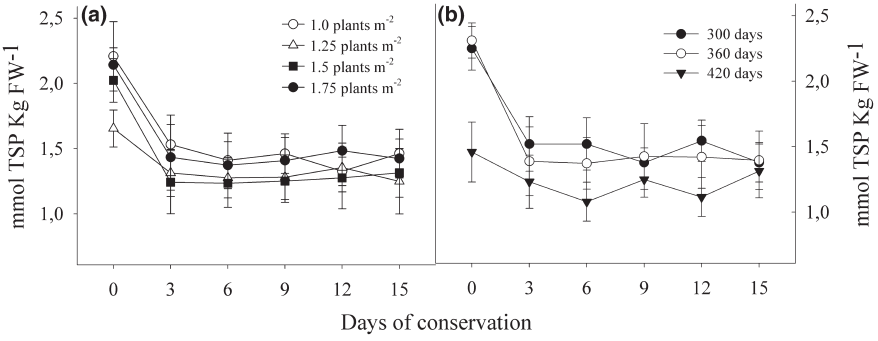

FIGURE 3 Total soluble phenols (TSP) in minimally processed cassava planted in different densities (a) and harvested at different ages (b). All were stored at 5°C for 15 days

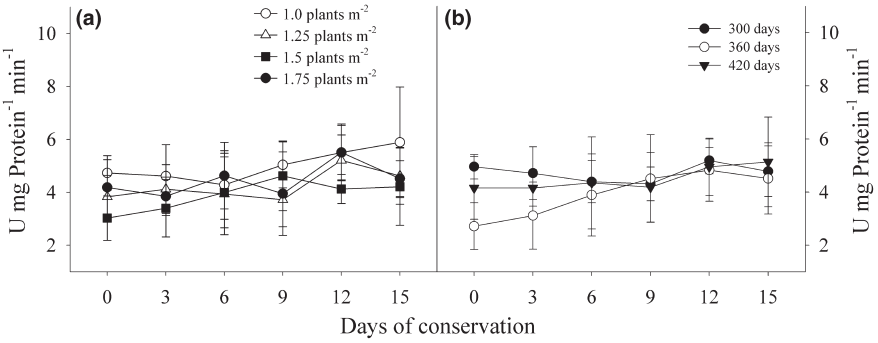

FIGURE 4 Superoxide dismutase activity (SOD) in minimally processed cassava planted in different densities (a) and harvested at different ages (b). All were stored at 5°C for 15 days

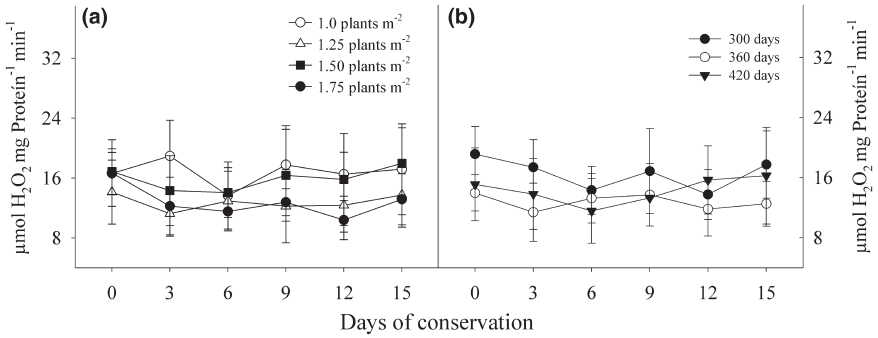

FIGURE 5 Catalase (CAT) in minimally processed cassava planted in different densities (a) and harvested at different ages (b). All were stored at 5°C for 15 days

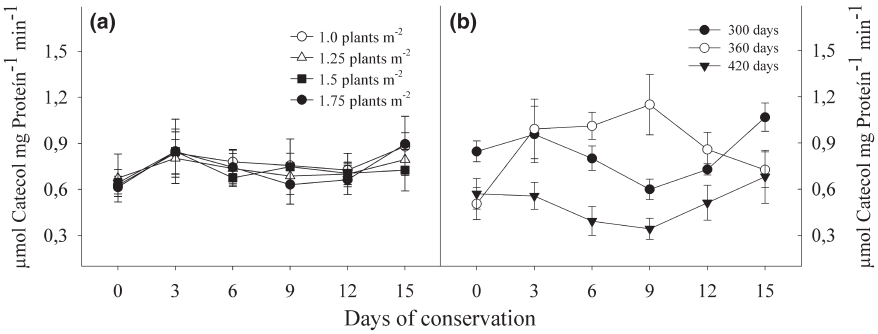

FIGURE 6 Polyphenoloxidase (PPO) in minimally processed cassava planted in different densities (a) and harvested at different ages (b). All were stored at 5°C for 15 days

noted that the fruit of the coffee tree harvested later after planting had a lower content of phenols, and by Junqueira et al. (2014) for minimally processed cassava. The phenolic compounds, besides being indicative of enzymatic browning, are important from a nutritional point of view because of their role in the prevention of premature aging and cancer (Liu, 2004). Thus, harvesting at 300 days was recommended, to maintain the TSP content in minimally processed cassava.

The SOD activity showed little variation during the storage period, irrespective of planting density, and age at harvest (Figure 4). However, it was observed that roots harvested at 360 days had lower SOD activity at day 0, which then increased during the storage period (Figure 4b).

Initially (at day 0) and after 3 days of storage, the SOD activity in plants harvested at 300 days was 1.20–1.78 times greater than in those harvested at different ages (Figure 4b). This may have been of fundamental importance for the reduced browning observed for this harvest age, since SOD provides the first line of defense against the toxic effects of elevated ROS, giving greater resistance to browning by removing the superoxide radical, an ROS with several harmful effects on tissues (Xu et al., 2013).

CAT activity remained constant regardless of storage time (Figure 5a, b). Comparing harvest ages, CAT activity in young roots was always greater than in older roots (Figure 5b). This may indicate that the protection conferred by CAT extended beyond 3 days, in contrast to SOD (Figure 5b).

CAT activity was not modulated by different planting densities (Figure 5a). However, the behavior in relation to harvest age was similar to that observed for SOD, where roots harvested 300 days after planting showed higher CAT activity during storage (Figure 5b). Our results suggest that SOD and CAT act together to minimize oxidative damage by scavenging ROS and play a key role in delaying PPD symptoms. Proteomic approaches by Qin et al. (2017) indicate that SOD, in combination with CAT activity, would be the first line of defense against PPD in supporting PPD-tolerant cassava varieties. In addition, results given by Uarrota et al. (2014) on unprocessed cassava slices also suggest the importance of CAT and SOD for the removal of ROS, since increased activity of these enzymes was found in PPD-tolerant genotypes in the early stages of evaluation (fresh samples and after 3 days at 25°C). In this study, we demonstrate that SOD has an important role in minimizing early events associated with the onset of PPD, whereas CAT seems to prevent the development of PPD symptoms when roots are harvested at a younger age. Furthermore, these enzymes can serve as good biomarkers for detecting the propensity to develop PPD during the storage of minimally processed cassava.

The activity of PPO remained stable during storage and did not differ between planting densities (Figure 6a).

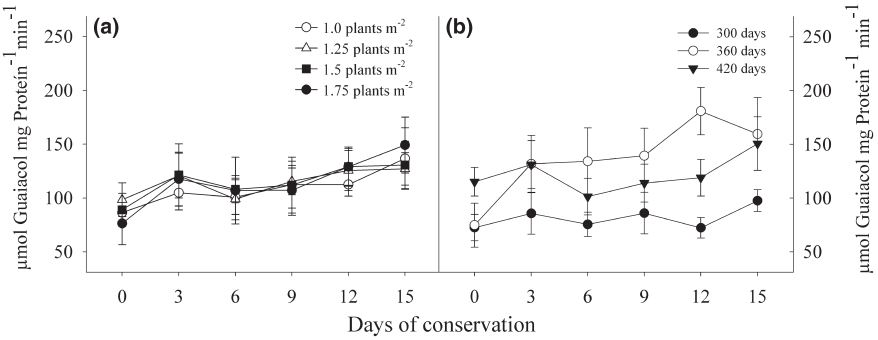

FIGURE 7 Peroxidase (POD) in minimally processed cassava planted in different densities (a) and harvested at different ages (b). All were stored at 5°C for 15 days

Regarding harvest age, it was verified that at 420 days after planting, the activity of PPO was lower than for other harvest ages (Figure 6b). This was a function of the lower content of phenolic compounds present at this age, since these are substrates for PPO (Figure 6b) (Tomás-Barberán & Espín, 2001). In this study, the most intense enzymatic browning, recorded by visual analysis at 420 days post-planting, could not be attributed to the activity of PPO.

In general, the activity of POD increased during storage, in association with the planting density (Figure 7a) and harvest age (Figure 7b). Planting density did not modify the specific activity of POD (Figure 7a). On the other hand, harvesting at 300 days after planting resulted in roots with lower POD activity than harvesting at 360 and 420 days (Figure 7b), and this was reflected in less browning observed by VA (Figure 1a).

Different planting densities did not modify the specific activity of POD (Figure 7a). On the other hand, harvesting at 300 days after planting resulted in roots with lower POD activity than harvesting at 360 and 420 days (Figure 7b), and this was reflected in less browning observed by VA (Figure 1a).

These results show that SOD and CAT enzymes are involved in the scavenging of ROS and are associated with POD, which plays a key role in the tolerance of young roots to browning. On the other hand, planting density does not appear to be a key factor modulating the activity of the enzymes studied. These results also show that anticipation of the cassava harvest can be beneficial to the cassava producer for minimal processing purposes. Furthermore, a delayed cassava harvest ties up land that could otherwise be used for farming. Extended ground storage also decreases starch quality and content, and stored roots become more fibrous, which increases cooking time.

4 | CONCLUSIONS

Our results demonstrate that SOD, CAT, and POD together play important roles in delaying the development of PPD symptoms when roots are harvested younger (300 days in this study) and minimally processed. Additionally, planting density did not modulate the accumulation of metabolites or the activity of the oxidative enzymes studied.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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