DANIELLE DA SILVA EUGÊNIO

PHOSPHATE FERTILIZATION AS A MODULATOR OF ENZYMATIC BROWNING IN MINIMALLY PROCESSED CASSAVA

Serra Talhada-PE

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Dissertação apresentada à Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Serra Talhada, como parte das exigências do Programa de Pós-Graduação em Produção Vegetal, para obtenção do título de Mestre em Produção Vegetal.

Orientador: Prof. Dr. Adriano do Nascimento Simões

Co-orientadora: Dra. Kelem Silva Fonseca

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APROVADA em 26/02/2021.

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À Deus e a todos que acreditaram em mim quando nem eu mesma acreditei, contribuindo direta ou indiretamente, para a realização deste sonho.

Dedico

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(Albert Einstein)

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APRESENTAÇÃO

A mandioca é uma das mais importantes raízes comestíveis básicas do mundo, principalmente em regiões tropicais e subtropicais do mundo. Além disso, o grande número de variedades existentes no Brasil permite a escolha de acordo com a região e a finalidade de exploração da cultura. O seu alto rendimento em carboidratos atrelado a baixa demanda por água e fertilidade do solo, a torna muito atraente aos agricultores e consumidores, exercendo papel importante tanto na segurança alimentar, quanto na crescente demanda industrial de alimento. Entretanto, um ponto agravante a ser abordado é sua alta perecibilidade, decorrente de um processo conhecido como deterioração fisiológica pós-colheita (DFP), tornando as raízes desagradáveis ao paladar e não comerciáveis. Redução na qualidade visual, sabor e odor são algumas das mudanças induzidas pela DFP, restringindo a aceitação comercial de mandioca.

Técnicas vêm sendo aprimoradas na busca de reduzir as perdas, bem como atender às demandas e mudanças de mercado. Raízes minimamente processadas vêm ganhando destaque desde quitandas até supermercados, surgindo como resposta à demanda por produtos de fácil preparo e maior conveniência. No entanto, o estresse abiótico, provocado pelo ferimento no tecido vegetal durante o processo, desencadeia uma série de reações, incluindo aumento da respiração, ocasionando deterioração fisiológica alguns dias após o processamento mínimo.

Pesquisas têm comprovado a eficiência de técnicas que interfiram de maneira positiva nas reações metabólicas durante o acondicionamento de raízes, como atmosfera controlada através da utilização de embalagens, aplicação de revestimentos comestíveis e uso de antioxidantes, constatando a preservação por diferentes períodos. Atrelado a isso, estudos do manejo de práticas relacionadas ao cultivo como poda, idade de colheita, densidade populacional e fertilização mineral também vêm se mostrado eficientes e elucidam a promoção destas na morfologia e atributos de qualidade pós-colheita.

Estudos recentes elucidam o ATP como sinalizador do estresse abiótico ocasionado por ferimento do tecido vegetal. Desse modo, o ATP e espécies reativas de oxigênio (EROS) atuam como moléculas de sinalização primária e secundária, respectivamente, ativando a NADPH oxidase, aumentando a produção de EROS. Essa produção de EROS ocorre de forma contínua, bem como sua modulação através de mecanismos enzimáticos antioxidantes, como as enzimas superóxido dismutase (SOD), catalase (CAT) e ascorbato peroxidade (APX). Sequências de reações são

desencadeadas ativando a fenilalanina amônia liase (PAL), precursora da via dos fenóis. Essa sinalização primária mediada pelo ATP pode levar não somente ao aumento de compostos fenólicos, mas, sua oxidação, através da atividade das enzimas polifenoloxidase (PPO) e peróxidase (POD) desencadeando o escurecimento enzimático ou deterioração fisiológica pós-colheita (DFP). Desse modo, levando em consideração a escassez de estudos científicos em relação a influência da prática agronômica de fertilização fosfatada na pós-colheita de raízes alimentícias minimamente processadas, bem como de toda cascata de sinalização desencadeada pela molécula ATP extravasada de células rompidas é que se faz necessário o estudo da influência do fósforo não somente na quantidade, como também na qualidade pós-colheita dos alimentos. E assim, servir de ferramenta para ajudar no controle do escurecimento enzimático de raízes, favorecendo uma maior eficiência no uso dos recursos.

PHOSPHATE FERTILIZATION AS A MODULATOR OF ENZYMATIC BROWNING IN MINIMALLY PROCESSED CASSAVA

ABSTRACT

Cuts made during minimal processing trigger a cell signaling network initiated by ATP, stimulating the synthesis of phenolic compounds. In cassava, cutting also stimulates the polyphenoloxidase (PPO) and peroxidase (POD) activities, which leads to enzymatic browning. However, phosphate fertilization can also be a modulator of browning in minimally processed cassava. This study proposes to relate the increase in phosphorus (P) supply in the soil, via phosphate fertilization, to oxidative damage and protection, phenylpropanoid metabolism and enzymatic browning in minimally processed cassava roots. The roots were grown at three levels of P (0, 60 and 120 kg ha^{-1} P_2O_5). After nine months, the roots were harvested and yield and the P content in the root, stem and leaves were quantified. The roots were minimally processed and stored for 12 days at 5 ± 2 °C and 90 ± 5% relative humidity. The higher supply of P in the soil increased the P content of roots and stems, but commercial and agro-industrial yields remained significantly similar. Roots grown at 120 kg ha^{-1} P_2O_5 showed higher detection of hydrogen peroxide (H_2O_2) at the beginning and at 12 days of storage, which was accompanied by the peak activities of the enzymes superoxide dismutase, catalase and ascorbate peroxidase. In addition, phosphate fertilization increased total phenolic compounds; total soluble quinones; caffeic acid; the activity of the PPO and POD enzymes; and antioxidant capacity, by the DPPH and FRAP methods. The present study thus demonstrates another role of phosphorus application, in fertilization, in the quality of minimally processed cassava with browning potential.

Keywords: *Manihot esculenta* Crantz, ATP, ROS, phenolic compounds, PPO, POD, quinones, enzymatic browning.

RESUMO

O corte durante o processamento mínimo desencadeia uma rede de sinalização celular iniciada pelo ATP, estimulando a síntese de compostos fenólicos. O corte em mandioca também estimulam as atividades das polifenoloxidase (PPO) e peroxidase (POD), resultando em escurecimento enzimático. Todavia a adubação fosfatada também pode ser um modulador do escurecimento em mandioca, minimamente processada. Objetivou-se com esse estudo relacionar o aumento da oferta de fósforo (P) no solo, mediante adubação fosfatada, com os danos e proteções oxidativas, metabolismo dos fenilpropanóides e escurecimento enzimático em raízes de mandioca, minimamente processadas. As raízes foram cultivadas em três níveis de fósforo 0, 60 e 120 kg ha^{-1} P_2O_5. Após nove meses, as raízes foram colhidas, quantificados a produtividade e o teor de fósforo na raiz, caule e folhas. Foram minimamente processadas e mantidas a 5 ± 2 °C, 90 ± 5 UR por 12 dias. A maior oferta de fósforo no solo incrementou o conteúdo de fósforo nas raízes e nos caules, todavia manteve significativamente semelhantes a produtividade e o rendimento agroindustrial. Raízes cultivadas com 120 kg ha^{-1} P_2O_5 apresentaram uma maior detecção de peróxido de hidrogênio (H_2O_2) no início e aos 12 dias, acompanhados com máximos nas atividades das enzimas superóxido dismutase, catalase e ascorbato peroxidase. Além disso, a adubação fosfatada incrementou compostos fenólicos totais, quinonas solúveis totais, ácido caféico, bem como, maior atividade das enzimas polifenoloxidase e peroxidase, e maior capacidade antioxidante, pelos métodos DPPH e FRAP. Portanto, o presente estudo demonstra mais um papel da aplicação de fósforo na adubação, na qualidade de mandioca minimamente processada com potencial de escurecimento.

Palavras-chaves: *Manihot esculenta* Crantz, ATP, ROS, compostos fenólicos, PPO, POD, quinonas, escurecimento enzimático.

Highlights:

1. Phosphate fertilization increased phenolic compounds and quinone levels;
2. Phosphate fertilization resulted in greater oxidative damage and protection;
3. Phosphate fertilization increased the PAL, PPO and POD enzyme activities;
4. High phosphate fertilization rates intensify browning.

1 INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a food crop whose importance has grown significantly in world agriculture. The crop plays an important role in food security, mainly in less developed countries, thanks to properties such as tolerance to adverse soil-climatic conditions and high carbohydrate content, which makes it a source of energy (Lobell et al., 2008; Howeler et al., 2013; Silva et al., 2017). These characteristics directly influence the global production of cassava, which reached approximately 277 million tons in 2018. Of this total, Brazil accounted for 17.6 million tons, ranking fifth among all producing countries (FAOSTAT, 2018).

One of the major obstacles to the sale of cassava roots is their high perishability, especially under ambient temperature conditions (Waddington et al., 2010; Campo et al., 2011). Cassava shows symptoms of physiological deterioration 24 to 72 h after being harvested, with the appearance of vascular streaks and browning of the plant tissue that limit its market potential (Salcedo; Siritunga, 2011; Vanderschuren et al., 2014). Minimal processing emerged as a response to the demand for more convenient, easily prepared products (Artés & Allende, 2014). Additionally, it is a non-thermal technique that induces synthesis and accumulation of nutraceutical compounds (Jacobo-Velazquez & Cisneros-Zevallos et al., 2017). However, phenolic compounds induced by cutting can cause browning in minimally processed cassava (Brito et al., 2017).

The post-harvest physiological deterioration (PPD) disorder has been studied for many years worldwide. In a recent review, “Cassava PPD: from triggers to symptoms”, Zaiuniddin et al. (2018) highlighted the various factors that influence the disorder. Cultivation practices such as pruning (Wheatley; Schawabe, 1985; Van Oirschot et al., 2000), harvest age and population density seem to modulate PPD in cassava (Andrade et al., 2017; Coelho et al., 2019). As regards fertilization, there are still many gaps in its relationship to PPD. However, phosphate fertilization can markedly influence the modulation of browning in roots, as recent studies using cut carrots as a model suggest that ATP and reactive oxygen species (ROS) act as primary and secondary signaling

molecules, respectively, for synthesis and accumulation of phenolic compounds (Jacobo Velázquez et al., 2011). Reactive oxygen species modulated by antioxidant enzymatic mechanisms, such as the enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), trigger a sequence of reactions, activating PAL, a precursor to the phenol pathway (Jacobo Velázquez et al., 2011). In their research, Jacobo Velázquez et al. (2011) proposed the production of bioactive compounds, since some have anti-cancer properties, such as chlorogenic acid (Santana-Gálvez et al., 2020). Nevertheless, the accumulation of phenolic compounds in minimally processed cassava can lead to enzymatic browning, as mediated by the enzymes polyphenoloxidase (PPO) and peroxidase (POD) (Fonseca et al., 2018).

Thus, it is believed that there is a fine line between beneficial phenolic compounds acting as bioactive compounds and the triggering of browning when the tissue is oxidized by PPO (Saltveit et al., 1997). The present study proposes to determine whether the incorporation of phosphorus (P) into the soil, once absorbed and assimilated by the root, can alter the quality of minimally processed root, based on browning. Therefore, this study aims to relate the increase in P supply in the soil, via phosphate fertilization, to oxidative damage and protection, phenylpropanoid metabolism and enzymatic browning in minimally processed cassava roots.

2 MATERIAL AND METHODS

Chemical products: sulfuric acid, phosphomolybdic acid, catechol, anhydrous monobasic potassium phosphate (AR grade), dibasic potassium phosphate, guaiacol, hydrogen peroxide, boric acid, β-mercaptoethanol, polyvinylpyrrolidone (PVPP), L-phenylalanine, nitro blue tetrazolium chloride (NBT), riboflavin, ascorbic acid, 3,3’ diaminobenzidine tetrahydrochloride (DAB), sodium hypochlorite, methanol, Folin-Ciocalteu reagent, anhydrous sodium carbonate, gallic acid, caffeic acid, methanol (HPLC grade), acetic acid, 2,2-diphenyl-1-picryl-hydrazil, sodium acetate, ferric chloride, 2, 4, 6-tris (2-pyridyl)-s-triazine (TPTZ), ferrous sulfate.

2.1 Characterization and management of the experimental area

The plants were grown at the Experimental Unit of the Rafael Fernandes Farm, which belongs to the Federal Rural University of the Semi-arid (UFERSA). The study area is located in the district of Alagoinha, in Mossoró - RN, Brazil (5°03'31.00" S, 37°23'47.57" W and 80 m asl). According to the Köppen classification, the

characteristic climate of the region is semi-arid, of the BSh type (dry and very hot) (Alvares et al., 2013), with the rainy season occurring between February and May. Annual average precipitation in the region is 670 mm, relative humidity is 68.9% and the dry period is from June to January (Carmo Filho et al., 1991). Meteorological data were collected throughout the experiment by the weather station installed on the Experimental Farm. The soil of the experimental area is classified as a typic-dystric Red Oxisol (Rêgo et al., 2016). Soil samples at the depths of 0-0.20 m and 0.20-0.40 m were collected for chemical (Silva, 2009) and physical (Donagema et al., 2011) characterization (Table 1).

Table 1. Chemical properties of the typic-dystric Red Oxisol with sandy texture on the Rafael Fernandes Experimental Farm, in Mossoró - RN, Brazil.

Profundity	pH	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	(H+Al)	SB	CTC	V
-----cm----		--mg dm^{-3}---			----------Cmolc dm^{-3}----------						%
0 – 20	6.1	6.3	53.9	2.2	1.1	0.5	0	0.75	1.8	2.5	70
20 - 40	5.6	1.23	52.3	1.2	0.7	0.3	0.05	0.99	1.2	2.1	53.5

pH - hydrogen potential; P - phosphor; K^+ - potassium; Na^+ - sodium; Ca^+ - calcium; Mg^{2+} - magnesium; Al^{3+} - aluminum; H + Al - acidity potential; SB - sum of bases; CTC - cation exchange capacity; V% - base saturation.

Vegetative propagules of cassava (*Manihot esculenta* Crantz) cv. Recife (nine-month cycle) were used in the cultivation. These were obtained from a multiplication field established on the Rafael Fernandes Experimental Farm.

Fertilization was carried out as recommended by Silva; Gomes (2008), based on soil analysis (Table 1), except for P, which was applied as single superphosphate (P_2O_5) at the levels (treatments) of 0, 60 and 120 kg ha^{-1} from July 2018 to April 2019. The phosphate fertilizer was applied entirely at planting. Urea (45% N) and potassium chloride (60% K_2O) were used as nitrogen and potassium sources, respectively. Half of the recommended nitrogen dose was applied 30 days after plant emergence (DAE), together with the total potassium dose. The second half of nitrogen fertilization was applied 60 DAE. Nitrogen and K were applied via irrigation, using a pressure differential system (fertilization tank).

Irrigation was provided by a drip system. The irrigation volume was determined according to the needs of the crop and following the crop coefficient (Kc) for each phenological stage of cassava. Emitters were spaced 0.30 m apart, the flow rate was 1.6 L h^{-1} and the average daily irrigation volume was 4.8 mm.

2.2 Harvesting, minimal processing and storage

The roots were harvested and transported to the Center for Graduate Studies in Plant Production (PGPV) at the Academic Unit of Serra Talhada, Federal Rural University of Pernambuco (UAST/UFRPE), located in the municipality of Serra Talhada - PE, Brazil. For minimal processing, the roots were cut into mini-setts, following the process flowchart proposed by Freire et al. (2014). The mini-setts were then packed in polypropylene bags (150 × 150 × 0.0005 mm) and stored for 12 days at 5 ± 2 °C and 90 ± 5% relative humidity. Each experimental unit consisted of approximately 140 g of mini-setts, with three replications used per treatment (0, 60 and 120 kg ha^{-1} P_2O_5).

Analyses were carried out at 0, 2, 4, 6, 8, 10 and 12 days. On each day of analysis, samples with a surface tissue ± 2 mm thick were collected, frozen in liquid nitrogen and stored in an ultra-freezer (-80 °C).

2.2.1 Commercial and agro-industrial yields and phosphorus content

Commercial yield (t ha^{-1}) was estimated by weighing the marketable roots per planted area. Marketable roots were considered those over 10 cm in length and over 2 cm in diameter.

Agro-industrial yield was determined in percentage terms, following Freire et al. (2014), using the following formula:

$$RA = \left(\frac{Mf}{Mi}\right) * 100 \quad (1)$$

Where, RA = Agroindustrial yield (%); Mf = Final mass (g); Mi = Initial mass (g).

To measure the P levels (g kg^{-1}) in the vegetative parts (leaf, stem and root), 0.4-g samples were digested with sulfuric acid (EMBRAPA, 1997). Phosphorus was

determined by colorimetry, using the method of phosphomolybdic complex in reducing medium (EMBRAPA, 1997).

2.2.2 Visual analysis

Visual quality was assessed by six trained evaluators to determine the presence or absence of dark spots, streaks and discoloration on the surface tissue, in addition to the presence of odor, proliferation of *Pseudomonas* spp. and whitening in the samples. Scores from 5 to 1 were assigned subjectively, as described by Coelho et al. (2017).

2.2.3 Fresh weight loss and total soluble solids

The relative loss of fresh weight, expressed in percentage terms, was calculated by difference relative to the initial fresh weight, according to the following formula:

$$PMF = \left[\frac{(Mfi - Mff)}{Mfi}\right] * 100 \quad (2)$$

Where, Mfi is the initial fresh mass, weighed right after processing (g); and Mff is the final fresh mass, that is, weighed at intervals of two days. Samples were weighed on a semi-analytical balance (ARD 110, OHAUS Adventurer, Parsippany-USA).

To determine the total soluble solids content, samples of cassava roots weighing approximately 30 g were macerated using a porcelain mortar and pestle. The extract obtained from the plant tissue was deposited on the prism of a portable refractometer (model RTD-95) and results were expressed in BRIX°.

2.2.4 *In situ* detection of hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) was detected histochemically by microscopy and the staining technique described by Olson and Varner (1993) and Repka (1999). Fractions of the surface region of the cassava tissue (2 × 2 × 5 mm) were infiltrated *in vacuo* with 3,3'-diaminobenzidine-tetrahydrochloride (DAB) in the dark, at -25 Ba, for 5 h, in an adaptation of the method proposed by Vallélian-Bindschedler et al. (1998). Then, they were washed with sodium hypochlorite for 1 min. Control samples were infiltrated with ascorbic acid (1 mM). All images of the samples were recorded using a semi-professional digital camera (Nykon D3100) (14.2 megapixels) and recorded using a

stereomicroscope (Labomed Luxeo 4D Zoom Stereo Digital Binocular) (stereo zoom microscope under 10X magnification).

2.2.5 Enzyme extractions and assays

2.2.5.1 Superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX)

The extract was obtained by homogenizing 0.12 g of tissue from the surface region in 2 mL potassium phosphate buffer (0.1 M) (pH 7.0). Afterwards, the extract was centrifuged at 7,960 x *g* for 23 min at 4 ºC.

The SOD activity was determined as described by Giannopolitis and Ries (1977), with adaptations. Aliquots of 100 µL of supernatant were added to 1,660 mL of potassium phosphate buffer (50 mM) (pH 7.8) containing (1 µM EDTA and 13 mM methionine), 200 µL nitro blue tetrazolium chloride (NBT) (750 µM) and 40 µL riboflavin (1 mM). The reaction was conducted in a light chamber, composed of 18-W fluorescent lamps, for 15 min. After this period, readings were taken with a spectrophotometer (Biochrom, Libra S8, Cambridge, England) at 560 nm. The activity was determined based on the inhibition of NBT reduction, with one unit of activity defined as the amount of enzyme needed to inhibit 50% of photoreduction (Beauchamp and Fridovich, 1971). Results were expressed in U g^{-1} FW.

The CAT assay was determined according to Havir and Mchale (1987), with modifications. Aliquots of 300 µL of the supernatant were added to 2.7 mL potassium phosphate buffer (50 mM, pH 7.0) containing H_2O_2 (20 mM). The reaction was performed at 30 °C and was accompanied by a decline in absorbance at 240 nm for 2 min, with successive readings performed every 30 s. The catalase activity was calculated based on the molar extinction coefficient of 36 M^{-1} cm^{-1} for H_2O_2, and expressed in µmol H_2O_2 min^{-1} g^{-1} FW.

The APX activity was determined as proposed by Nakano and Asada (1981), with adaptations. Aliquots of 100 µL of the supernatant were added to a reaction medium composed of 2,700 µL potassium phosphate buffer (50 mM, pH 6.0), containing ascorbic acid (AsA; 0.5 mM). The reaction was initiated by the addition of 200 µL H_2O_2 (30 mM) to the reaction medium and monitored based on the decline in absorbance with a spectrophotometer at 290 nm, for 2 min, with successive readings

performed at 30-s intervals. The activity was calculated based on the molar extinction coefficient of 2.8 mM^{-1} cm^{-1} and expressed in µmol H_2O_2 min^{-1} g^{-1} FW.

2.2.5.2 Phenylalanine ammonia-lyase (PAL)

The PAL activity was determined by following the methodology described by Ke and Saltveit (1986), with adaptations. Samples of 0.5 g of plant tissue were collected and homogenized in a mortar with 3 mL sodium borate buffer (0.1 M, pH 8.8), composed of β-mercaptoethanol (5 mM), EDTA (2 mM) and 1% insoluble polyvinylpyrrolidone (PVPP) (w/v). Subsequently, the enzyme extract was filtered and centrifuged at 25,000 *g* for 20 min at 4 ºC.

For the assay, 1.5 mL L-phenylalanine (60 mM) were added to borate buffer (0.1 M, pH 8.8), which was maintained at 40 ºC for 15 min. Then, 0.5 mL of the enzyme extract was added. After 20 min of incubation at 40 ºC, absorbance was measured at 290 nm with a spectrophotometer (Biochrom, Libra S8, Cambridge, England). The PAL enzyme activity was expressed as ΔAbs h^{-1} g^{-1} FW.

2.2.5.3 Polyphenol oxidase (PPO) and peroxidase (POD)

The PPO and POD enzymes were extracted and their activity assayed according to Freire et al. (2015), with adaptations. Samples of 0.25 g of the surface region of the tissue were macerated and homogenized in 1.5 mL 0.2 M potassium phosphate buffer (pH 6.0). The extract was then centrifuged at 10,000 x *g* for 21 min at 4 ºC.

The PPO assay was conducted by adding 100 µL of supernatant to a reaction medium containing 1.5 mL potassium phosphate buffer (0.2 M, pH 6.0) and 1.3 mL catechol (0.2 M) that was previously kept at 25 ºC in a dry bath. Readings were taken at 30-s intervals, for 2 min, with a spectrophotometer (Biochrom, Libra S8, Cambridge, England) at 425 nm, at a temperature of 25 ºC. The PPO activity was calculated based on the molar extinction coefficient of 3,400 M cm^{-1} for catechol, and was expressed in µmol catechol min^{-1} g^{-1} FW.

The POD assay was performed by adding 100 µL of supernatant to a reaction medium containing 1 mL potassium phosphate buffer (0.2 M, pH 6.0), 100 µL guaiacol (40 mM) and 100 µL hydrogen peroxide (23 mM) that was previously maintained at a temperature of 25 ºC in a dry bath. Absorbances were read at 30-s intervals, for 2 min, using a spectrophotometer (Biochrom, Libra S8, Cambridge, England) at 470 nm. The

POD activity was calculated based on the molar extinction coefficient for guaiacol of 26.6 mM^{-1} cm^{-1} and expressed in µmol guaiacol min^{-1} g^{-1} FW.

2.2.6 Determination of total soluble phenols, total soluble quinones, gallic acid and caffeic acid

Total soluble phenols were determined according to Reyes et al. (2007), with adaptations. Samples of 0.3 g of surface regions of the tissue (0-5 mm) were macerated and homogenized with 1.5 mL of methanol (AR grade). Then, the samples were left to stand in the dark for 24 h, at 4 ºC. After that period, they were centrifuged at 10,000 × *g* at 2 ºC for 21 min. For the assay, 150 µL of the extract, 150 µL of the Folin-Ciocauteu reagent (0.25 N) and 2,400 µL distilled water were pipetted into a tube. The mixture was then homogenized for 3 min with a tube shaker. Next, 300 µL of sodium carbonate (1 M) were added and the solution was kept in the dark for 2 h. The blank was obtained with 150 µL methanol to replace the supernatant. Readings were taken with a spectrophotometer (Libra S8, Biochrom Cambridge, England) at 725 nm, and results were expressed in mg of gallic acid equivalents 100 g^{-1} FW, which were quantified based on a standard curve of gallic acid.

The soluble quinone content was determined by the methodology described by Gao et al. (2017), with modifications. Samples of 0.4 g of cassava were macerated and homogenized in 2 mL methanol AR and subsequently kept at rest for 24 h. Next, they were centrifuged for 5 min at 12,000 × g, at 4 ºC. For the assay, 60 µL of supernatant and 100 µL of Folin-phenol reagent were pipetted and this mixture was then placed in the dark for 5 min. Subsequently, 300 µL sodium carbonate solution (0.1 mol/L) and 500 µL distilled water were added to the mixture, followed by homogenization. After 1 h of reaction in the dark, absorbance was measured at 437 nm, using a spectrophotometer (Libra S8, Biochrom Cambridge, England).

Gallic and caffeic acid were identified and quantified using a Thermo Scientific Ultimate 3000 HPLC controlled by Chromeleon Chromatography Management System software, with a C18 column (250 mm × 4.6 mm; 5 µm). The isocratic method was used, whereby the mobile phase consisted of acidified water, 2% acetic acid (phase A) and pure methanol (phase B), at a flow rate of 0.800 mL/min, wavelength (λ) of 270 nm, injection volume of 20 µL and run time of 30 min. The standards used in the standard curves were gallic and caffeic acid at the concentrations of 0.05, 0.10, 0.15, 0.20 and 0.25 mg/mL. The same conditions were followed.

2.2.7 Extraction and *in vitro* testing of antioxidant activity (DPPH and FRAP)

To obtain methanolic extract, 0.4 g of fresh samples were macerated in 2 mL of methanol AR and left to stand for 24 h. After this period, the extracts were centrifuged at 9,000 × *g*, for 21 min, at 5 °C.

2.2.7.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

Antioxidant capacity by the DPPH method was determined as proposed by Brand-Williams et al. (1995), with adaptations. For the assay, 840 µL of the DPPH solution (0.1 mM) and 60 µL of the supernatant were added. The control assay was prepared by adding 840 µL of the DPPH solution and 60 µL of the supernatant. After 30 min of reaction, readings were taken with a spectrophotometer (Biochrom, Libra S8, Cambridge, England) at 517 nm, at 25 ºC. The decline in absorbance of the samples resulted in the percentage of free radical scavenging (% FRS), which was calculated by the following equation:

$$\%\ inibição = \frac{(\text{Abs controle DPPH} - \text{Abs amostra})\text{x } 100}{\text{Abs controle DPPH}} \qquad (3)$$

2.2.7.2 FRAP (Ferric Reducing Antioxidant Power Assay)

Antioxidant activity, as evaluated by the iron reduction power, was measured according to the methodology proposed by Benzie & Strain (1996), with adaptations. For the assay, a 900-µL aliquot of the FRAP reagent [25 mL acetate buffer (0.3 M; pH 3.6), 2.5 mL TPTZ solution (10 mM) and 2.5 mL aqueous ferric chloride solution (20 mM)] was mixed with 90 µL distilled water and 30 µL of the supernatant. The solution was then homogenized in a tube shaker and kept at rest in the dark for 30 min, at 37 °C. After incubation, readings were performed with a spectrophotometer (Biochrom, Libra S8, Cambridge, England) at 594 nm, at 25 ºC. For the blank, 900 µL of the FRAP reagent, 90 µL distilled water and 30 µL of the extractor (methanol AR) were used.

The antioxidant potential of the extracts was determined based on a calibration curve, which was drawn using ferrous sulfate ($FeSO_4.7H_2O$) at concentrations ranging from 0 to 1,500 µM. Results were expressed in mmol Fe^{2+} kg^{-1}.

2.2.8 Experimental design and statistical analysis

The experiment was laid out in a completely randomized design with a 3 × 7 factorial arrangement represented by three treatments (phosphate fertilization levels: 0, 60 and 120 kg ha^{-1} P_2O_5) and seven days of evaluation (0, 2, 4, 6, 8, 10, 12 days), with three replications. Each experimental unit was represented by a package containing approximately 140 g of minimally processed cassava, in the form of 'mini-setts'. The H_2O_2 detection analysis was conducted only on days 0, 6 and 12 of storage. Data were subjected to normality (Shapiro-Wilk) and homoscedasticity (Levene) tests, analysis of variance and Tukey's test at the 5% probability, using SAS software, and graphs were created using Sigma Plot software version 10.

3 RESULTS

3.1. Effect of phosphate fertilization on commercial and agro-industrial yields and P content in the organs

Table 2 shows the commercial yield, agro-industrial yield and partition of the P content between the organs of the plants of cassava cv. Recife. The increasing phosphate fertilization levels did not result in significant increases in root yield (t ha^{-1}) ($p = 0.7871$). In percentage terms, yield was less than 50% for all fertilized treatments. Fertilization with 120 kg ha^{-1} P_2O_5 significantly increased the P content in the roots and stems. The P content in the leaves did not differ significantly between the fertilization levels (Table 2).

Table 2. Commercial yield (t ha^{-1}), agricultural yield (%) and phosphorus content (g kg^{-1}) of roots of cassava cv. Recife subjected to different phosphate fertilization levels (0, 60 and 120 kg ha^{-1} P_2O_5).

Fertilizations (Kg ha^{-1} P_2O_5)	**Commercial yield (t ha^{-1})**	**Agricultural yield (%)**	**Phosphorus content (g kg^{-1})**		
			Root	**Stem**	**Leaf**
0	55,56±8,69a	32,49	0,98±0,17b	0,87±0,12b	2,91±0,23a
60	56,96±11,73a	32,49	1,22±0,11ab	0,92±0,06b	3,14±0,16a
120	61,19±3,63a	36,73	1,41±0,18a	1,39±0,19[a]	3,18±0,16a
p -value	0,7871	-	0,0166	0,0141	0,3376

Means followed by the same lower case letters in the column do not differ statistically by the Tukey test at 5% probability.

3.2 Effect of phosphate fertilization on oxidative damage and protection

The pieces that were stained with DAB and ascorbate (AsA) showed less browning (Figure 1). On the other hand, those stained only with DAB exhibited significant browning in all fertilized treatments, mainly at the beginning of storage (day zero).

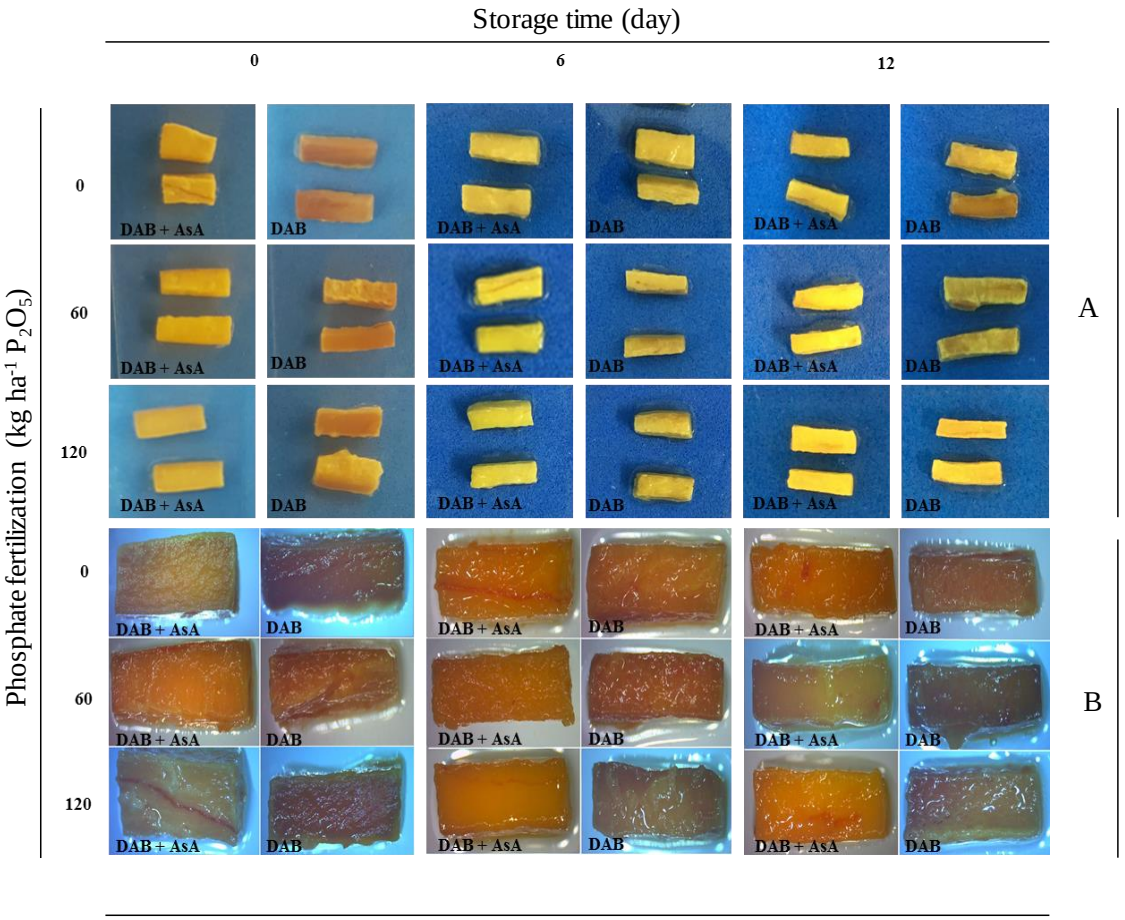

Figure 1. Detection of hydrogen peroxide (H_2O_2) at days 0, 6 and 12 of storage, by vacuum infiltration with diaminobenzidine tetrahydrochloride (DAB) + ascorbate (control) and only DAB, in segments (0-5 mm) of roots of cassava cv. Recife grown at different levels of phosphate fertilization (0, 60 and 120 kg ha^{-1} P_2O_5), minimally processed into 'mini-setts' and stored for 12 days at 5 ± 2 °C and 90 ± 5% RH. A- Images of the samples were recorded using a semi-professional digital camera (Nykon D3100) (14.2 megapixels). B- Images of the samples were recorded using a stereomicroscope (Labomed Luxeo 4D Zoom Stereo Digital Binocular) (stereo zoom microscope under 10X magnification).

At the beginning of storage, the SOD and CAT enzyme activities increased in the roots fertilized with 60 and 120 kg ha^{-1} P_2O_5 (Figures 2A and 2B). The SOD activity decreased throughout the preservation period, whereas the CAT activity increased (Figure 2A and 2B). Significantly higher enzyme activities occurred in the cassava mini-setts subjected to fertilization with 120 kg ha^{-1} P_2O_5 (Figure 2A and 2B).

Although there was no significant interaction effect for APX activity ($p > 0.05$), this variable showed significant differences as influenced by the factors of preservation time and fertilization level separately (Figure 2C and 2D, respectively). Thus, like SOD and CAT, the APX enzyme activity also exhibited a more significant increase in the roots fertilized with 120 kg ha^{-1} P_2O_5, followed by 60 and 0 kg ha^{-1} P_2O_5, in this order (Figure 2D). Moreover, during storage, the changes were also significant, with the greatest activity observed on the 10th day (Figure 2C).

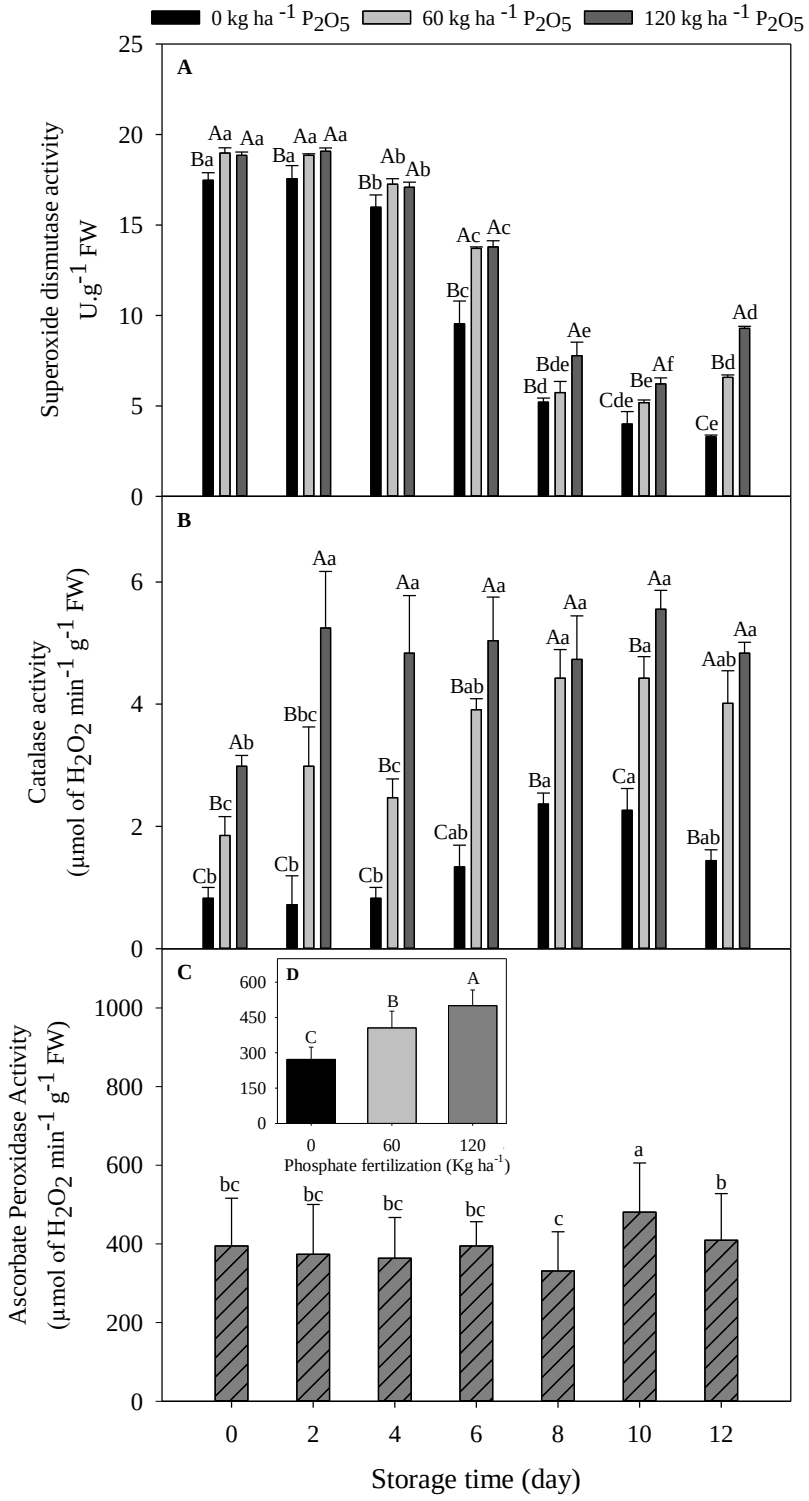

Figure 2. Superoxide dismutase activity - SOD (U g^{-1} FW) (A); Catalase activity - CAT (µmol of H_2O_2 min^{-1} g^{-1} FW) (B); and Ascorbate peroxidase activity - APX (µmol H_2O_2 min^{-1} g^{-1} FW) (C and D) in roots of cassava cv. Recife grown at different levels of phosphate fertilization (0, 60 and 120 kg ha^{-1} P_2O_5), minimally processed into 'mini-setts' and stored for 12 days at 5 ± 2 °C and 90 ± 5% RH. Bars represent the standard deviation of the mean. Letters represent significant differences by Tukey's test at 5%

probability. Uppercase letters compare the different phosphate fertilization levels and lowercase letters compare storage days.

Antioxidant capacity, which was measured based on the DPPH radical scavenging activity and iron reduction (FRAP), was detected in all fertilization treatments (Figure 3). Mini-setts from roots fertilized with 120 kg ha^{-1} P_2O_5 showed significantly higher DPPH scavenging activity and FRAP as compared with the other fertilized treatments, at the beginning of storage (day zero). In contrast, the pieces from roots fertilized with 0 and 60 kg ha^{-1} P_2O_5 did not differ from each other, by the FRAP method (Figure 3B). During the preservation period, until the 10th day, the DPPH radical scavenging activity remained higher in the roots fertilized with 60 and 120 kg ha^{-1} P_2O_5 (Figure 3A). The FRAP method indicated a different result for antioxidant activity, with the pieces that were subjected to phosphate fertilization showing greater activity on the last day (day 12th) only (Figure 3B).

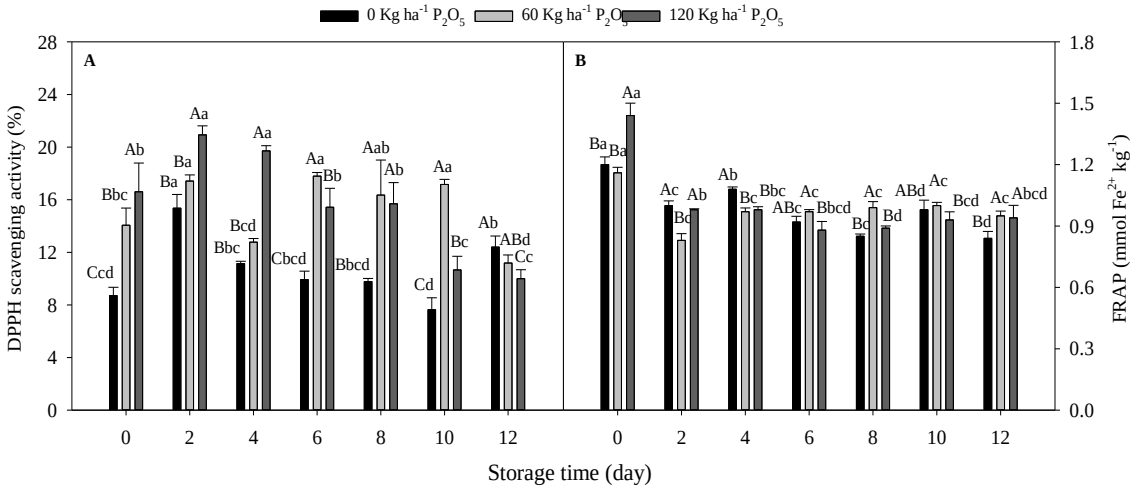

Figure 3. DPPH scavenging activity (%) (A); and FRAP (mmol Fe^{2+} kg^{-1}) (B) in roots of cassava cv. Recife grown at different levels of phosphate fertilization (0, 60 and 120 kg ha^{-1} P_2O_5), minimally processed into 'mini-setts' and stored for 12 days at 5 ± 2 °C and 90 ± 5% RH. Bars represent the standard deviation of the mean. Letters represent significant differences by Tukey's test at 5% probability. Uppercase letters compare the different phosphate fertilization levels and lowercase letters compare storage days.

3.3 Effect of phosphate fertilization on quality, phenolic compounds and enzymes associated with browning

The mini-setts showed minimal fresh weight loss (less than 1%) at all fertilization levels, especially in control treatment (0 kg ha^{-1} of P_2O_5) (Figure 4A). There

was no significant interaction effect for total soluble solid levels ($p > 0.05$), which were influenced only by the storage time (Figure 4B), decreasing at the end of the period.

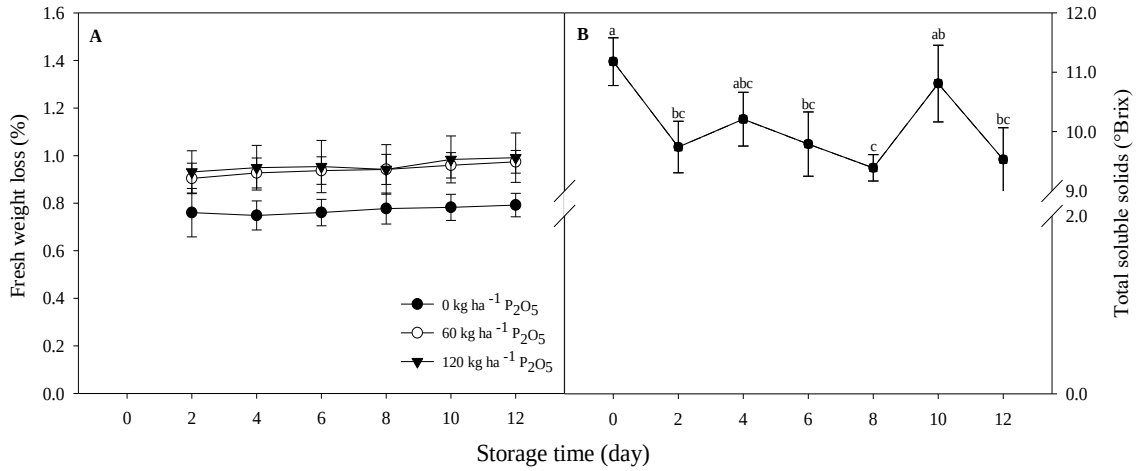

Figure 4. Fresh weight loss (%) (A) and total soluble solids (°Brix) (B) in roots of cassava cv. Recife grown at different levels of phosphate fertilization (0, 60 and 120 kg ha^{-1} P_2O_5), minimally processed into 'mini-setts' and stored for 12 days at 5 ± 2 °C and 90 ± 5% RH. Bars represent the standard deviation of the mean. Letters represent significant differences by Tukey's test at 5% probability. Lowercase letters compare storage days.

The scores referring to the visual scale decreased throughout the storage period for the roots subjected to all fertilization treatments (Figure 5A). The pieces from roots grown with 0, 60 and 120 kg ha^{-1} P_2O_5 did not show any difference on day zero ($p = 0.59$). However, they showed significant differences during the storage period ($p < 0.001$), which was observed from the sixth day onwards (Figure 5A). Although there was no significant difference, the sensory panel detected a slight visual difference between the fertilization levels, especially in the roots under 120 kg ha^{-1} P_2O_5, with the lowest scores being assigned on the 12th day of preservation (Figure 5A). Nevertheless, the root scores at all fertilization levels at the end of the storage period remained above the acceptable sale and consumption threshold, represented by score 3 (Figure 5A).

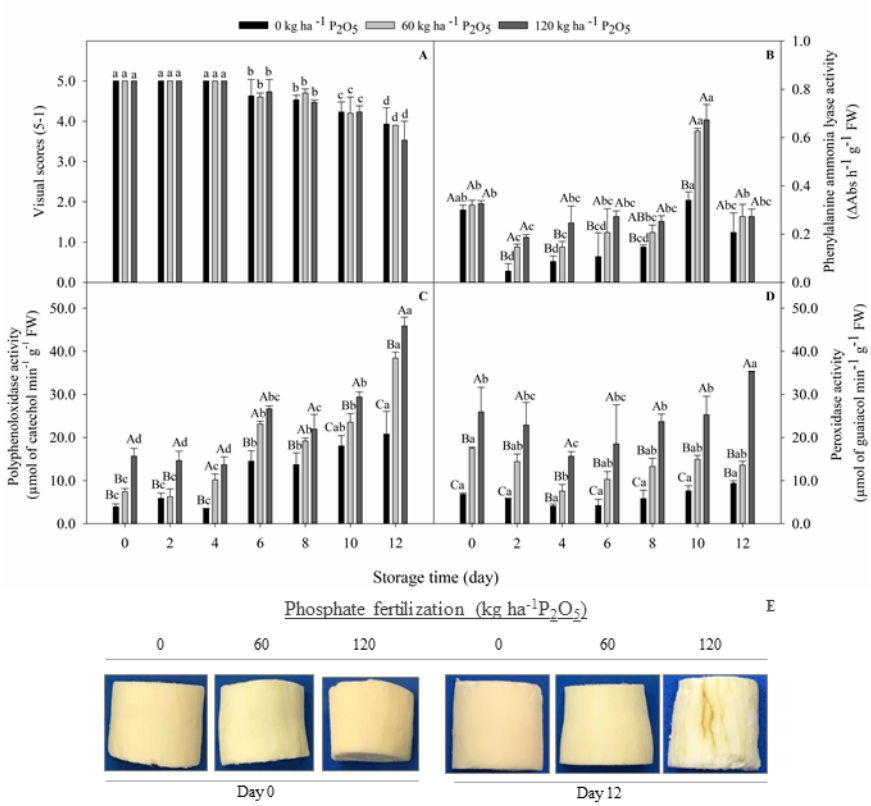

Figure 5. Visual scores (A); Phenylalanine ammonia lyase activity - PAL (ΔAbs h^{-1} g^{-1} FW) (B); Polyphenoloxidase activity - PPO (µmol of catechol min^{-1} g^{-1} FW) (C); Peroxidase activity - POD (µmol of guaiacol min^{-1} g^{-1} FW) (D); and visual appearance illustrations (E) in roots of cassava cv. Recife grown at different levels of phosphate fertilization (0, 60 and 120 kg ha^{-1} P_2O_5), minimally processed into 'mini-setts' and stored for 12 days at 5 ± 2 °C and 90 ± 5% RH. Bars represent the standard deviation of the mean. Letters represent significant differences by Tukey's test at 5% probability. Uppercase letters compare the different phosphate fertilization levels and lowercase letters compare storage days.

At the beginning of storage (day zero), regardless of the phosphate fertilization level, the PAL activity did not differ (Figure 5B). Conversely, fertilization with 120 kg ha^{-1} P_2O_5 resulted in the highest activities of the PPO and POD enzymes (Figure 5C and 5D). The PAL activity showed a parabolic response throughout storage, with its peak occurring at 10 days (Figure 5B). In addition, the pieces treated with the phosphate fertilizer always exhibited higher PAL activity than the control roots (Figure 5B). The PPO and POD activities increased during refrigerated storage, with the roots fertilized

with 120 kg ha^{-1} P_2O_5 showing significantly higher values in all evaluated periods (Figure 5C and 5D).

Phosphate fertilization significantly increased the levels of total phenolic compounds on the first day of storage (day zero) (Figure 6A). On the same day, the control samples (0 kg ha^{-1} of P_2O_5) had the highest gallic acid content, whereas the caffeic acid content was significantly higher when the roots were fertilized with 60 kg ha^{-1} P_2O_5, as compared with the other treatments (Figures 6D and 6E). During storage, total phenolic compounds, including caffeic acid, decreased. However, the roots fertilized with 60 and 120 kg ha^{-1} P_2O_5 showed the highest levels of total phenols, whereas the caffeic acid content was clearly influenced by fertilization with 60 kg ha^{-1} P_2O_5 (Figure 6A and 6C). Figure 7 shows the chromatograms for the identification of phenolic acids. Two peaks are observed: one at 3.23 and the other at 5.10 min (gallic acid and caffeic acid, respectively).

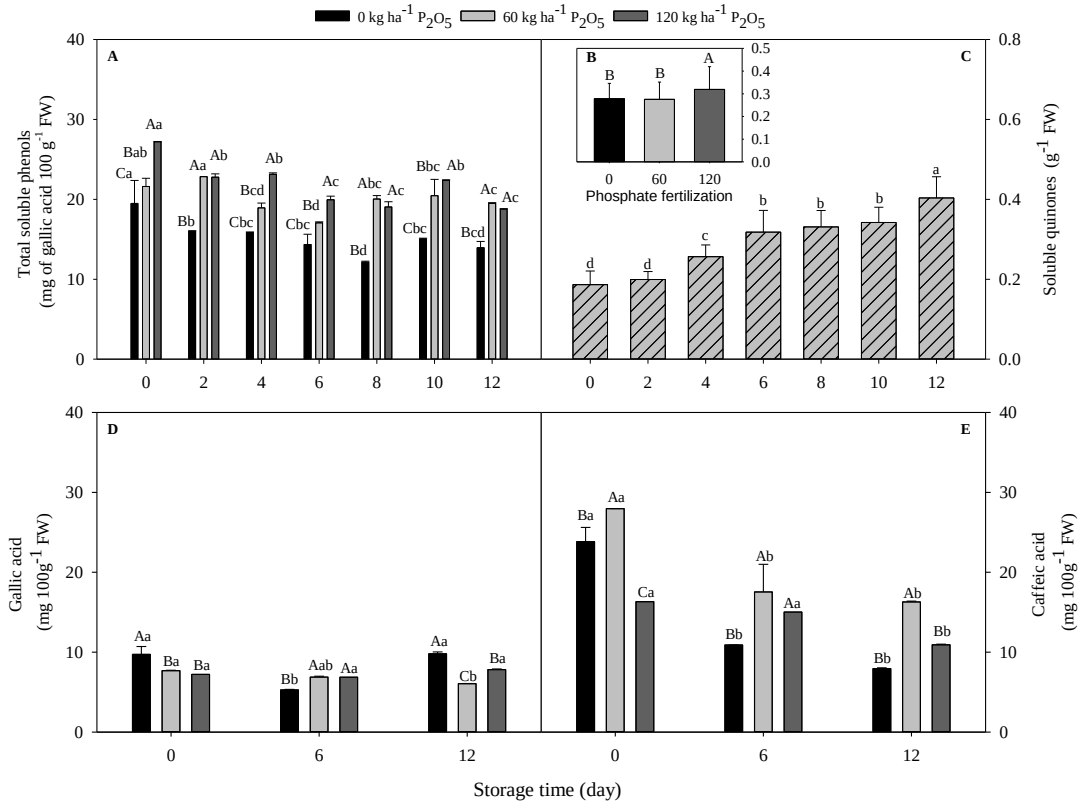

Figure 6. Total soluble phenols (mg of gallic acid 100 g^{-1} FW) (A); soluble quinones (g^{-1} FW) (B and C); gallic acid (mg 100 g^{-1} FW) (D); and caffeic acid (mg 100 g^{-1} FW) (E) in roots of cassava cv. Recife grown at different levels of phosphate fertilization (0, 60 and 120 kg ha^{-1} P_2O_5), minimally processed into 'mini-setts' and stored for 12 days at 5 ± 2 °C and 90 ± 5% RH. Bars represent the standard deviation of the mean. Letters

represent significant differences by Tukey's test at 5% probability. Uppercase letters compare the different phosphate fertilization levels and lowercase letters compare storage days.

There was no significant interaction effect for the soluble quinone content ($p > 0.05$). However, this variable was significantly affected by the factors of fertilization and storage time in isolation (Figure 6B and 6C, respectively). There was a significant accumulation of quinones in the pieces from roots that were fertilized with 120 kg ha^{-1} P_2O_5 (Figure 6B) and at the end of refrigerated storage (Figure 6C).

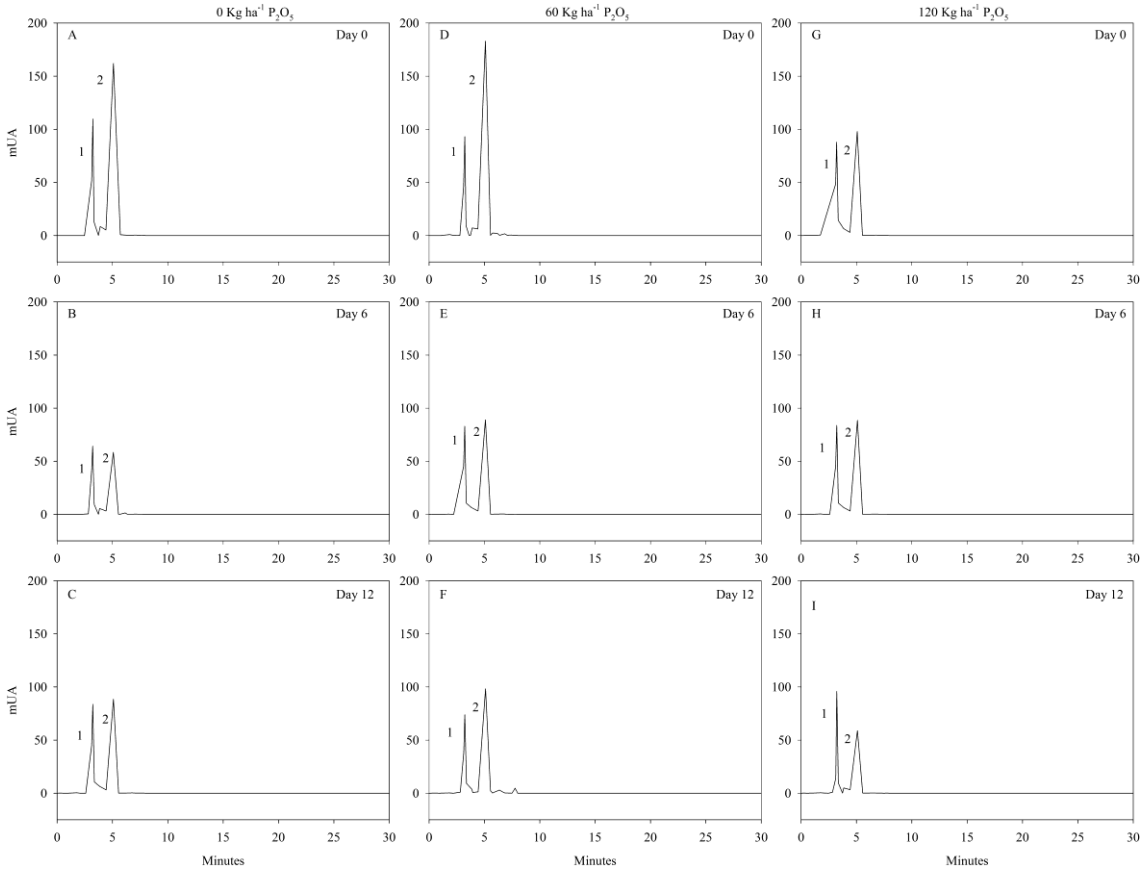

Figure 7. Chromatograms obtained by high performance liquid chromatography (HPLC) of phenolic compounds present in roots of cassava cv. Recife grown at 0, 60 and 120 kg ha^{-1} P_2O_5 and minimally processed into 'mini-setts', on days 0 (A, D and G), 6 (B, E and H) and 12 (C, F and I) of storage at 5 ± 2 °C and 90 ± 5% RH. (1) Gallic acid, (2) Caffeic acid, at the retention times of 3.10 and 5.10 min, respectively.

4 DISCUSSION

Strategies that lessen the effects of the postharvest physiological deterioration process in cassava roots are of great relevance, since there is a need to extend the

storage time of minimally processed products to meet the demand for food. Furthermore, this practice provides a food product of excellence to the consumer. Agronomic management practices and factors, e.g. harvest time, planting density, pruning and soil fertilization, can alter the phytochemical content of the tissues after harvest, causing deteriorating changes such as enzymatic browning (Van Oirschot et al., 2000; Andrade et al., 2017; Coelho et al., 2019).

Phosphorus is an essential nutrient, as it participates in several physiological processes vital for plants such as cell development, photosynthesis, respiration, energy storage and transfer, carbohydrate metabolism and constitution of enzymes and structures such as DNA, RNA, thereby influencing root development and plant growth (Buchanan et al., 2015). The different phosphate fertilization levels tested in this study did not significantly influence the yield (t ha^{-1}) of the roots of cassava cv. Recife (Table 2). This may indicate that the application of 120 kg ha^{-1} P_2O_5 would represent luxury fertilization and could thus be dispensable. This is in agreement with the Agronomic Institute of Pernambuco, which recommends 60 kg ha^{-1} P_2O_5 for this cultivar (Silva; Gomes, 2008). The P content present in the soil at the beginning of the study was sufficient, although its concentration (Table 1) is below the minimum critical point for the crop (10 mg dm^{-3}) (Silva; Gomes, 2008). In contrast, the roots fertilized with 120 kg ha^{-1} P_2O_5 showed a significant increase in P content (Table 2), meaning that even without an increase in yield, the cells of these roots absorbed more of the element. In the technique used to determine P in this study, the samples were digested with sulfuric acid (EMBRAPA, 1997). This method determines the total P, which is the sum of inorganic and organic P, but these were not discriminated in this study.

The present findings show that the P added to the soil (120 kg ha^{-1} P_2O_5) was absorbed by the roots (Table 2). However, its assimilation via oxidative phosphorylation and/or at the substrate level could not be confirmed, although this phenomenon happens in roots (Buchanan et al., 2015). This coincided with a greater browning of the pieces stored under refrigeration (Figure 5A). These results suggest that the P incorporated into the soil induced the activation of phenolic compounds (Figure 6A), which may be the result of assimilation into ATP and of ATP into cell signaling, as proposed by Jacobo-Velazquez et al. (2011), or even by a route independent of ATP (as proposed in Figure 8). In the former case, this would occur after the perception of receptor proteins, possibly one analogous to DORN1 in plants (Choi et al., 2014). As a result, there would be a transient increase in Ca^{2+} that activates the enzyme NADPH oxidase, which

catalyzes the conversion of oxygen (O_2) to a superoxide radical (O_2^-) (Song et. Al., 2006; Jacobo-Velazquez et al., 2011). This oxidative burst participates in an integrated and amplified signaling system, triggering cellular defense mechanisms of the plant (Lamb & Dixon, 1997) such as the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) (Quin et al., 2017).

As observed in the present study, phosphate fertilization induced a greater detection of H_2O_2 at the beginning of storage (day zero) and at 12 days (Figure 1), which coincided with the peak activity of SOD (Figure 2A), an enzyme that catalyzes the dismutation of superoxide into H_2O_2. Unlike SOD, the CAT and APX enzyme activities increased throughout the storage period, mainly in the mini-setts from the roots fertilized with P (Figure 2B and 2C).

The oxidative burst observed at the beginning of storage, by the H_2O_2 qualitative test, occurred with greater intensity in the pieces from roots fertilized with 120 kg ha^{-1} P_2O_5 (Figure 1). From the second day onwards, this was accompanied by a significant increase in PAL activity in the pieces from roots fertilized with 60 and 120 kg ha^{-1} P_2O_5, suggesting that these fertilization treatments were triggers for the activation of the phenylpropanoid pathway mediated by PAL (Figure 5B). Because PAL is the first enzyme involved in the metabolism of phenylpropanoids (Dixon; Paiva, 1995), phenolic compounds also increased (Figure 6A). This supports the hypothesis that this fertilization was an environmental trigger to modulate the synthesis and accumulation of phenolic compounds. On the 10th day of storage, the PAL activity also showed a significant increase, which was more pronounced in the pieces from roots treated with P_2O_5 (Figure 5B). In general, the PAL activity behaves in a downward parabolic fashion in leaves (Saltveit, 2000), in whole cassava roots (Tanaka et al., 1983) and in minimally processed carrot (Simões et al., 2010). This increased enzyme activity is very characteristic of the moment that precedes physical damage, and its subsequent sudden decline is linked to the *de novo* synthesis of a PAL inactivation factor known as PAL-IF (Ritenour & Saltveit, 1996). In the present study, the PAL activity showed significant values on day zero of storage, as compared with the second day (Figure 5B). Day zero refers to a few hours after minimal processing, suggesting that a parabolic peak might have occurred until the second day that was not evaluated in this study.

The increase in phosphate fertilization resulted in increased antioxidant activity in the first 10 days of storage, by the DPPH method, which declined gradually throughout the storage period (Figure 3A). This may indicate greater oxidative

protection ability. This behavioral pattern was in line with the phenolic compound content (Figure 6A). The relationship between phenol content and antioxidant capacity—due to DPPH, mainly—has been frequently observed considering the additive and synergistic effects of all potential antioxidants against oxidative stress (Puchau et al., 2009; Floegel et al., 2011). Antioxidant activity by the FRAP method was higher in the roots fertilized with P only on the first and last days of evaluation (Figure 3 B). The present study revealed one of the roles of increasing phosphate fertilization rates in the oxidation of phenolic compounds and in increasing quinone levels, with a possible participation of H_2O_2. These responses are important, since phenols are largely part of a group of molecules termed "bioactive", which provide health benefits for people who consume them (Torres-Contreras et al., 2017). On the other hand, in tissues that brown quickly after cutting, such as white-fleshed cassava, these responses may make the product unacceptable in terms of sale and consumption due to depreciation of the product. These results show that there is a fine line between the beneficial aspects of management strategies that provide increases in phenolic compounds with bioactive properties and phenolic compounds that induce browning. Therefore, it is important that future studies identify phenylpropanoid metabolites of different metabolic deviations. In the present study, we quantified gallic and caffeic acid and their transient behaviors. Unlike gallic acid, the caffeic acid concentration was always higher in the pieces from roots that were fertilized (Figure 6 E and Figure 7). Hydroxamic acid derivatives are intermediates of important metabolic routes such as lignification and suberization, in responses to environmental stress (Wei et al., 2017). Additionally, they possess antibacterial properties (Bernards; Razem, 2001).

A trained panel conducted a sensory evaluation test to discriminate the acceptance of the cut pieces (Figure 5 A). Although the panel did not classify any samples as inappropriate for sale or consumption, the pieces from roots fertilized with 120 kg ha^{-1} P_2O_5 were assigned lower scores at the end of storage, due to browning (Figure 5 E). However, according to the panel, these pieces could still be marketed. On the other hand, some parameters related to quality, such as the aspect of dehydration, were not noticeable, and soluble solids did not change between the tested fertilization treatments (Figure 4A and 4B). The activities of classic enzymes and metabolites that participate in these disorders, such as PPO and POD (Freire et al., 2015; Andrade et al., 2017; Oms-Oliu et al., 2010) and quinones (Wang et al., 2019; García; Barret, 2002), increased with the increasing phosphate fertilization levels (Figures 5 C and D). This

also coincided with a greater accumulation of total phenolic compounds and quinones in the pieces from roots fertilized with 120 kg ha^{-1} P_2O_5 (Figure 6 A and B). This finding also revealed an important role of phosphate fertilization in the defense of cassava roots subjected to cutting.

The present study allowed the development of a hypothetical model with another role of P in the induction of synthesis of phenolic compounds in cut cassava (Figure 8). In this case, ATP or Pi (both overflowing from cutting) triggered the production of H_2O_2, as also described by Jacobo-Velazquez et al. (2011). This secondary signaling, mediated by ROS and antioxidant enzymes (SOD, CAT and APX), activates the phenylpropanoid metabolism initiated by PAL. The intermediate phenolic acids, detected as being gallic and caffeic acids, were transient, but contributed to non-enzymatic detoxification. This detoxification was nonetheless limited, as evidenced by the increase in antioxidant capacity as measured by the DPPH and FRAP methods. Additionally, fertilization with 120 kg ha^{-1} P_2O_5 promoted the increase in quinones, mediated by PPO and POD, which led to more pronounced browning. This information is important for defining limits on phosphate fertilization in roots that brown after being cut, such as cassava, which compromises their sale. On the other hand, in pigmented roots, where browning is not easily visible (e.g. carrots, colored sweet potatoes and cream-to-yellow-fleshed cassava), the limit on P fertilization could be more flexible, but further investigations are warranted.

The present study demonstrates the importance of care in the application of phosphorus, not only for the productive aspects, but also in view of the consequences of post-harvest phosphate fertilization in minimally processed roots. Further research is needed to determine the path of applied phosphorus, its incorporation into ATP and the signaling cascade in which it is involved. In addition, studies like the present one are of great relevance due to their applicability in the production chain of minimally processed products. This pre-harvest management constitutes an additional tool to help control enzymatic browning in roots that brown.

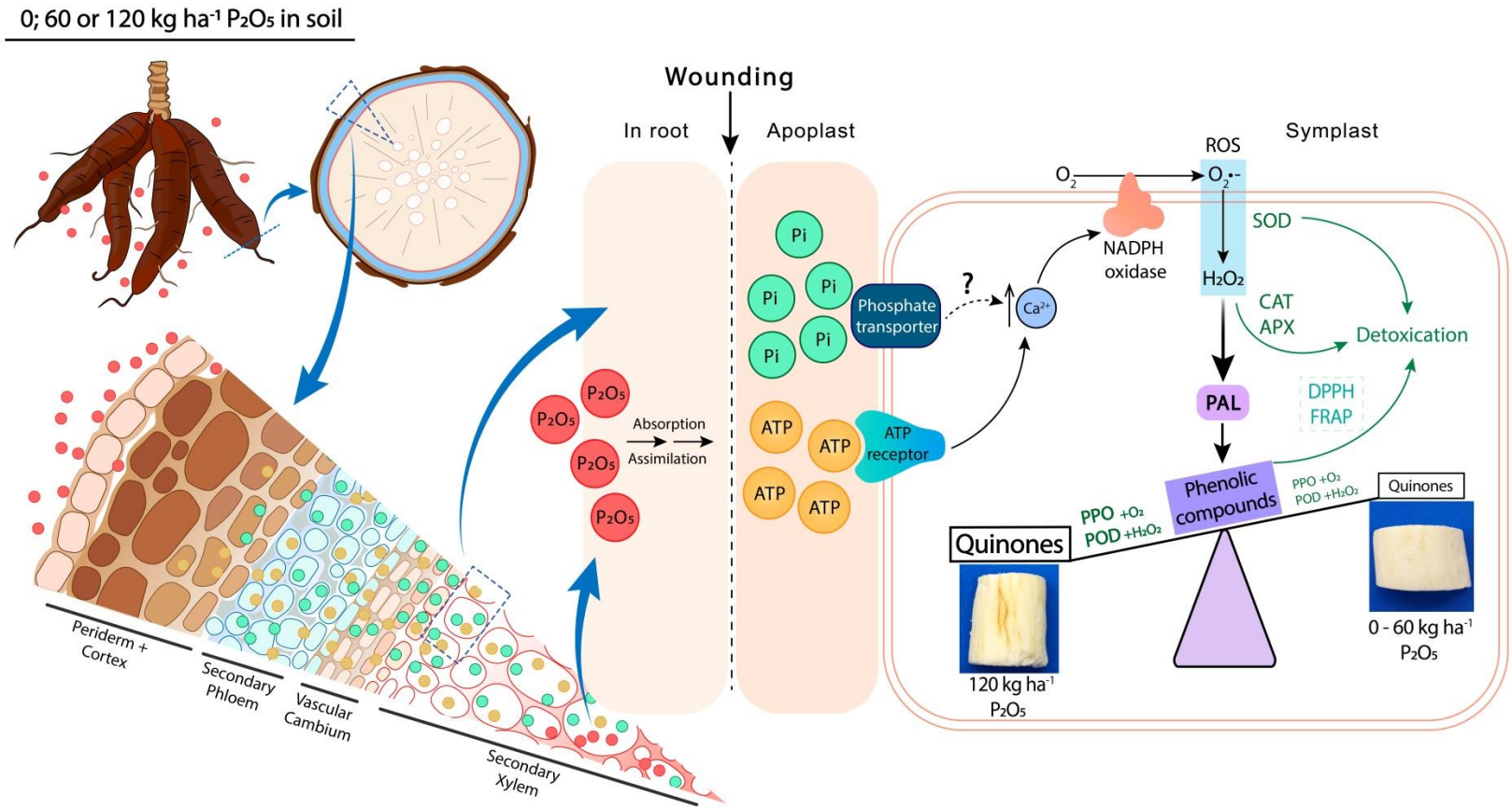

Figure 8. Proposed hypothetical model, adapted from Jacobo-Velazquez et al. (2011), highlighting another role of phosphorus, via phosphate fertilization, in the induction of the antioxidant system (enzymatic and non-enzymatic); in the synthesis, accumulation and oxidation of phenolic compounds, mediated by the enzymes PPO and POD; and in the browning of minimally processed cassava roots.

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