Chemical and histological changes in stored cassava roots of cultivars Catarina Amarela and Catarina Branca

Alterações químicas e histológicas em mandiocas armazenadas das cultivares Catarina Amarela e Catarina Branca

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Abstract

The research aimed evaluate and compare chemical and histological changes occurring after harvesting of cassava roots (*Manihot esculenta* Crantz) of cultivars Catarina Amarela and Catarina Branca. The cassava plants were grown at Instituto Agronômico do Paraná - Londrina-BR, plants were trimmed 30 days before harvest, and roots were collected at 10 months of age. The roots were stored for 5 days at room conditions and submitted to evaluate the degree of physiological deterioration, moisture content, polyphenoloxidase and peroxidase enzymes activities, phenolic compounds content, hydrogen peroxide detection, content of carotenoids, cellulose, lignin and, parenchyma microscopy. After storage the cultivars presented physiological deterioration and changes in moisture content, peroxidase activity, content of phenolic compounds and lignin. Both cultivars showed no polyphenoloxidase activity. Histological alterations were observed, which are more evident in Catarina Amarela. The cultivars Catarina Amarela and Catarina Branca present chemical and histological changes after storage, and Catarina Branca is the most resistant to post-harvest changes.

Key words: Alteration after harvesting, chemical composition, *Manihot esculenta*.

Resumo

A pesquisa teve por objetivo avaliar e comparar as alterações químicas e histológicas ocorridas após o armazenamento das raízes de mandioca (*Manihot esculenta* Crantz) das cultivares Catarina Amarela e Catarina Branca. A parte aérea da planta foi podada 30 dias antes da colheita e as raízes, cultivadas no Instituto Agronômico do Paraná, foram colhidas aos 10 meses de idade, armazenadas por 5 dias e submetidas à avaliação da deterioração fisiológica, teor de umidade, atividades de polifenoloxidase e peroxidase, teor de compostos fenólicos totais, detecção de peróxido de hidrogênio, teor de carotenóides totais, celulose, lignina, além de avaliações histológicas. Após o armazenamento as cultivares sofreram deterioração fisiológica e apresentaram alterações no teor de umidade, atividade de peroxidase, compostos fenólicos totais e lignina. Ambas cultivares não apresentaram atividade de polifenoloxidase. Foram observadas alterações histológicas, sendo esta mais pronunciada na cultivar Catarina Amarela. Deste modo, as cultivares Catarina Amarela e

Catarina Branca sofrem alterações químicas e histológicas após o armazenamento, sendo a Catarina Branca a mais resistente às alterações pós-colheita.

Palabras chave: Alterações pós-colheita, composição química, *Manihot esculenta*.

Introduction

In the world, the cassava crop (*Manihot esculenta* Crantz) has an annual production over 200 millions of tons ad Brazil is the third largest producer with a production close to 25 millions of tons by 2012 (FAO, 2011; IBGE, 2012). Specific traits like easy propagation, high efficiency in carbohydrate production, good production in low fertility soils, give the advantage of a flexible harvest, making the cassava an attractive crop (Cagnon *et al.*, 2002). However, two disadvantages of this crop are, first, presence of cyanogenic glycosides in all the parts of the plant and, second, the fact that the tuber roots suffer rapid deterioration caused by physiological processes that start between 24 and 48 h after harvesting (Buschmann *et al.*, 2000a). This deterioration is known as post-harvest physiological deterioration (PPD) and consists on the development of dark stretch marks on the storing parenchyma which affect the visual characteristics and cause a bad smell and taste (Reilly *et al.*, 2007).

The development of PPD is highly associated with damages caused during the harvesting and as an evidence of this is the lack of knowledge on the biochemical processes of PPD, because the changes resemble the typical plant changes in response to injuries that causes a chain of oxidative biochemical reactions (Morante *et al.*, 2010). Among these changes during PPD it has been observed the increase of some secondary metabolites like phenolic compounds and diterpenes (Buschmann *et al.*, 2000a, b), increase on the activity of enzymes like peroxidases and polyphenol oxidases (Campos e Carvalho, 1990), besides picks in reactive oxygen species like hydrogen peroxide (H_2O_2) (Reilly *et al.*, 2003).

Comparative studies in the development of PPD in several cultivars showed that there are differences in their susceptibility. Therefore, knowing more cultivars and their susceptibility is important, because they may offer to producers and biologist the opportunity to use the genetic variability to improve the crop (Buschmann *et al.*, 2000a; Wenham, 1995). According to Miranda (2000) among the cassava cultivars with wide adaptation and distribution in the Estado do Paraná are Catarina Amarela and Catarina Branca, which is due to the good performance in productivity and processing of the tubers of these cultivars. Knowing the performance of these cultivars and the lack of research on PPD resistance, this study has as objective to evaluate and compare the chemical and histological alterations happening after storage tubers of the Catarina Amarela and Catarina Branca cultivars.

Materials and methods

The table cassava cultivars Catarina Amarela and Catarina Branca were grown in Londrina, PR - Brazil (23° 18' 37" S, 51° 09' O, and 576 MASL). Harvesting of the root tubers was done 10 months after sowing (august 2010) trimming the aerial part 30 days before harvesting. After harvesting, the tubers for the experiment were selected using intact roots of medium size between 20 to 40 cm, with outstanding ends and the distal part was covered with PVC (polyvinyl chloride) film as done by Buschmann *et al.* (2000a). after that, roots were stored in plastic containers at room conditions.

Initially and after 5 days of storage, five roots of each cultivar were randomly removed to be analyzed. Each root was transversally sectioned in three pieces being two the ends, proximal and distal, and one the middle part. Separately, each piece was subjected to: (1) evaluation of darkening of the parenchyma caused by PPD determined with a portable digital colorimeter, using the Hunter (CIELAB) system the luminosity (L*) values were taken according to the method described by Miranda (2000); (2) water content was determined according to the gravimetric method (Method 44-15) described by the AACC (1995); (3) activities of the polyphenoloxidase and peroxidase were determined according to the technique proposed by Nogueira and Silva (1989); to determine the polyphenol oxidase the cathecol 0.1 M colorimetric reaction was used and reading on a spectrophotometer at 425 nm; for the peroxidase was used guaiacol 0.5% and H_2O_2 0.08% and the reading was done at 470 nm; (4) total phenolic compounds were extracted according to the method described by Adom and Liu (2002) using ethanol 80% and quantifying by spectrometry at 760 nm using a standard curve prepared with gallic acid and the reagent Folin-Ciocalteau as described by Swain and Hillis (1959); (5) *in situ* H_2O_2 was detected with the method described by Thordal-Christensen *et al.* (1997) in which the solution 3.3 Diaminobenzidine (DAB) (2 mg/ml, pH 3.8) was infiltrated in the sample by vacuum during 3 h; (6) total carotenoids content was measured according to the method described by Rodriguez-Amaya and Kimura (2004), being the pigments extracted with cool acetone at 4 °C and then transferred to petroleum ether, quantification was performed by spectrometry at 453 nm; (7) contents of cellulose and lignin were determined by the micro ingestion method described by Mizubuti *et al.* (2009), in which a dry sample is previously digested on a solution of acid detergent.

For histological observations, transversal sections were obtained manually using fresh material of the region where the roots are inserted and were stained with a solution containing phloroglucin 1% and hydrochloric acid 25% (Johansen, 1940). The microscopy slides were observed on an optical microscope (Motic B1 – JVC TK-CI380) and the images were digitalized with the software Motic Images Plus 2.0 in the suitable optical conditions.

The statistical analysis followed was a factorial design 2 x 2 x 3 with five replicates in triplicates for each experiment, being the studied factors: cultivar (Catarina Amarela and Catarina Branca), storing time (zero and five days) and the regions of the root (insertion, middle and tip). The results were subjected to analysis of variance (Anova) and mean test (Tukey) when required, using the Statistica7 software at significance level of $P < 0.05$.

Results and discussion

Among the studied factors the differences between cultivars and storing time were significant ($P < 0.05$) for the darkening of the storing parenchyma. In Table 1 is shown that initially the color of the pulp of the Catarina Branca cultivar differed from the one of Catarina Amarela. This difference was expected since the parameter L* varies from black (0) to white (100) and Catherine White is a cultivar with white pulp, meanwhile Catarina Amarela has a pulp with a yellowish color. After 5 days of storage there was a reduction in the L* values indicating a darkening in both cultivars, being more accentuated and significant only for Catarina Amarela (Table 1). In this case, Catarina Branca shows that is more resistant to the typical darkening by PPD.

When the regions of the same cultivar are compared, there is no difference at the beginning. After storage only the insertion

Table 1. Mean values for the L* parameter, humidity, peroxidase activity (POD), content of total phenols, cellulose and lignin in cassava roots (*M. esculenta*) of the Catarina Amarela and Catarina Branca cultivars after harvesting and 5 days of storage.

Storage time	Cultivars		Mean
	Catarina Amarela	Catarina Branca	
	L* parameter		
Initial	88.60+0.56 ^{B}	90.88+0.91^{aA}	89.74^{a}
5 days	86.63+2.13^{bB}	89.79+0.95^{aA}	88.26^{b}
Mean	87.62B	90.34^{a}	
	Humidity (%)		
Initial	59.01+0.83aB	61.10+2.40aA	60.06a
5 days	56.93+1.74bB	58.42+1.72bA	57.68b
Mean	57.97^{B}	59.76^{a}	
	POD (Enzymatic units/ml/min)		
Initial	14.20+0.09^{bA}	11.82+0.31^{bA}	13.01^{b}
5 days	268.99+125.78^{aA}	162.84+112.12^{aB}	215.92^{a}
Mean	141.59A	87.33B	
	Phenols (mg eq. of gallic acid/100g)		
Initial	48.27+0.89^{aA}	49.66+1.03 ^{A}	48.97$_{a}$
5 days	44.15+3.37^{aA}	42.78+0.85^{bA}	43.47^{b}
Mean	46.21A	46.22^{A}	
	Cellulose (%)		
Inicial	2.87+0.09^{aA}	2.47+0.07^{aB}	2.67^{a}
5 days	2.50+0.21^{aA}	2.66+0.13^{aA}	2.58^{a}
Mean	2.69A	2.57A	
	Lignin (%)		
0 days	0.683+0.01^{aA}	0.447+0.01^{bB}	0.57^{b}
5 days	0.865+0.04^{aB}	1.29+0.07^{aA}	1.07^{a}
Mean	0.774^{A}	0.868^{A}	

* Means followed by the same lowercase letter in the vertical and the same uppercase letter in the horizontal do not have significant differences according to the Tukey´s test (P < 0.05)

of the roots of the Catarina Amarela cultivar differ from the middle part, presenting a reduction in the L* value, which means a stronger darkening (Table 2). A large variation in the development of PPD symptoms in the cassava roots was noticed because, although some presented different intensities after 5 days of storage others did not present any symptom, indicating that this process is not uniform in the same cultivar.

For water content, among the studied factors the differences among cultivars, storing time and regions were significant (P < 0.05). As shown in Table 1, initially, the water content was higher in the Catarina Branca cultivar and after storage there were reductions in both cultivars being the cultivar with higher water content the most resistant to PPD. That relationship was also seen in other cultivars in the studies of Campos and Carvalo (1990), van Oirschot *et al.* (2000), Chávez *et al.* (2005) and Morante *et al.* (2010) who indicate that the works to select more resistant cultivars to post-harvest loses should consider a high water content in the root tubers.

When comparing the regions, after storage the water content was lower in the insertion region of both cultivars, differing from the tip that was the region with higher water content (Table 2). This is possibly attributed to the fact that the tip was covered with PVC, which prevented further loss of moisture.

In this study was not detected polyphenol oxidase enzyme activity and, although Campos e Carvalho (1990) and Kato *et al.* (1991) suggest that there is a correlation between the polyphenol oxidase activity with the development of PPD, this opinion is not unanimous and the results are controversial, because Carvalho *et al.* (1985) studied the relationship between the phenolic compounds, peroxidase, polyphe-

Table 2. Mean values of the L* parameter, humidity and Peroxidase activity (POD) in 3 regions of the cassava (*M. esculenta*) roots in the Catarina Amarela and Catarina Branca cultivars after harvesting (initial) and 5 days of storage.

Root region	Storage time			
	Initial		5 days	
	Catarina Amarela	Catarina Branca	Catarina Amarela	Catarina Branca
	L* parameter			
Insertion	88.33+0.46k	90.98+0.20[a]	85.54+2.98[b]	89.53+0.69[a]
Middle	88.68+0.61k	91.22+0.70[a]	87.63+0.73[a]	90.01+1.16[a]
Tip	88.80+0.60k	90.45+1.40[a]	86.73+1.91[ab]	89.84+1.09[a]
	Humidity (%)			
Insertion	58.07+0.87k	58.60+1.44[b]	55.32+1.31[b]	56.74+1.36[b]
Middle	59.66+0.82k	61.30+2.24[a]	56.68+1.32[ab]	58.33+1.11[ab]
Tip	59.30+1.31k	63.39+1.30[a]	58.79+1.75[a]	60.18+2.77[a]
	POD (Enzymatic units/ml/min)			
Insertion	14.22+1.75k	11.87+1.47k	410.12+176.5[a]	290.67+96.8[a]
Middle	14.29+1.74k	12.12+0.93k	228.19+177.9[b]	116.75+81.5[b]
Tip	14.11+2.73k	11.48+2.22k	168.68+97.1[b]	81.12+89.1[b]

*Means followed by the same letter in vertical do not significant differ according to the Tukey´s test (P < 0.05)

nol oxidase activity and the PPD in cassava roots, and did not detect any polyphenol oxidase activity in roots of the cultivar IAC-1418.

In relation to the peroxidase activity, the interaction cultivar x time and region x time was significant (P < 0.05). Initially, there was no difference between cultivars but, after storage there was an increase in the activity, as well as a difference among the cultivars (Table 1). The Catarina Amarela showed the highest peroxidase activity after storage and it is also the most affected by PPD. This relation between the increase of the peroxidase activity and PPD was also evidenced by Campos and Carvalho (1990) when studying the process of post-harvest deterioration of cassava, they observed that the roots of the IAC 12829 cultivar (susceptible to PPD) after 7 days of storing presented higher activity (325 enzymatic units/min) and at the end of the experiment were totally darkened, since Guaxupé cultivar, a resistant cultivar to PPD, presented less activity (90.72 enzymatic units/min).

When comparing the regions among cultivars, there were no differences at the beginning but, after storage, the insertion sections presented higher activity of the peroxidase differing from the middle and the tip region (Table 2). This higher activity of the region of the insertion is in agreement with the observation of Carvalho *et al.* (1985) that detected a strong increment in the peroxidase activity after 8 days of storing for the insertion and tip regions and, explained that the increment is due to injuries happened during harvesting in these regions, which activate the peroxidase for the subsequent protection of the damage tissue, which also results in reaction that darken the parenchyma.

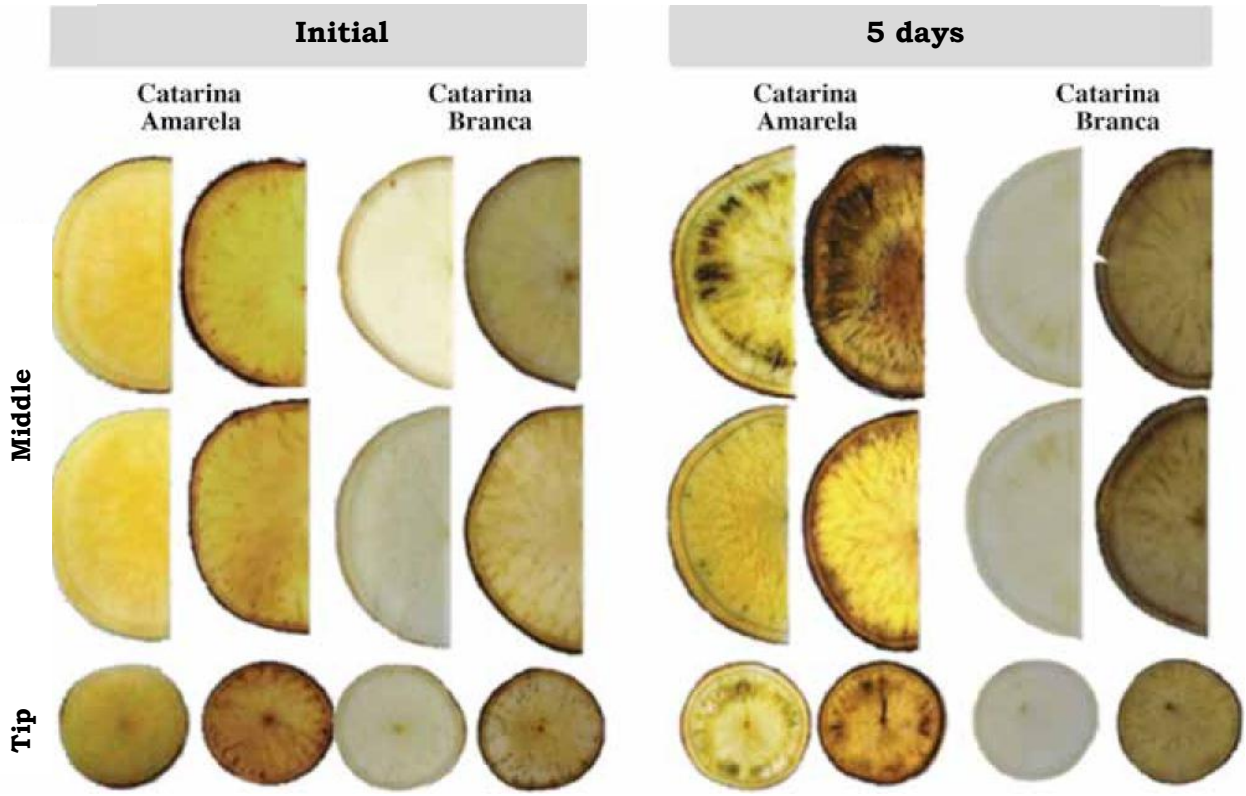
With relation to the content of total phenolic compounds, among the studied factors only the storing time was significant (P < 0.05). Thus, when comparing the total phenolic compounds content after storage with the initial one, there was a decreasing trend for both cultivars, which was significant only for Catarina Branca cultivar (Table 1). Carvalho *et al.* (1985) studied the PPD process in cassava roots of the cultivar

IAC-1418, and do not report large variations in the content of total phenolic compounds, with initial values of 48.0, 50.7 and 68.0 mg/100 g and after 4 days of storage 46.7, 58.0, 58.0 mg/100 g, respectively. In the same order Buschman *et al.* (2000b) did not find alterations in total phenols in the roots of three cultivars during 5 days of storage.

Both cultivars accumulated H_2O_2 during storage (Picture 1) and after 5 days of storage, the Catarina Amarela cultivar accumulated more when compared to Catarina Branca. Being this one the cultivar with higher peroxidase activity, enzyme that uses H_2O_2 as substrate and, is also the most affected by darkening. Some authors like Buschmann *et al.* (2000b) and Reilly *et al.* (2003) associated the accumulation of H2O2 with the PPD process stating that the synthesis of this reactive oxygen species occurs in response to a stress or as a defense against pathogens´ attacks that can affect the roots damaged during harvesting.

Among the studied factors, the total carotenoids content only has a difference among the cultivars ($P < 0.05$), having in the Catarina Amarela cultivar 10 times more total carotenoids (0,389 mg/100g) than Catarina Branca (0,34 mg/100g), and after storage there were no differences in relation to the initial content. That difference between cultivars is the responsible of the difference in the pulp color previously discussed on the L* parameter data, as it is known there is a correlation between the carotenoid content and the yellow color of the pulp (Chávez *et al.*, 2005).

Beeching (2001) states that cassava cultivars with high carotenoid content are less susceptible to the PPD process. Mean-

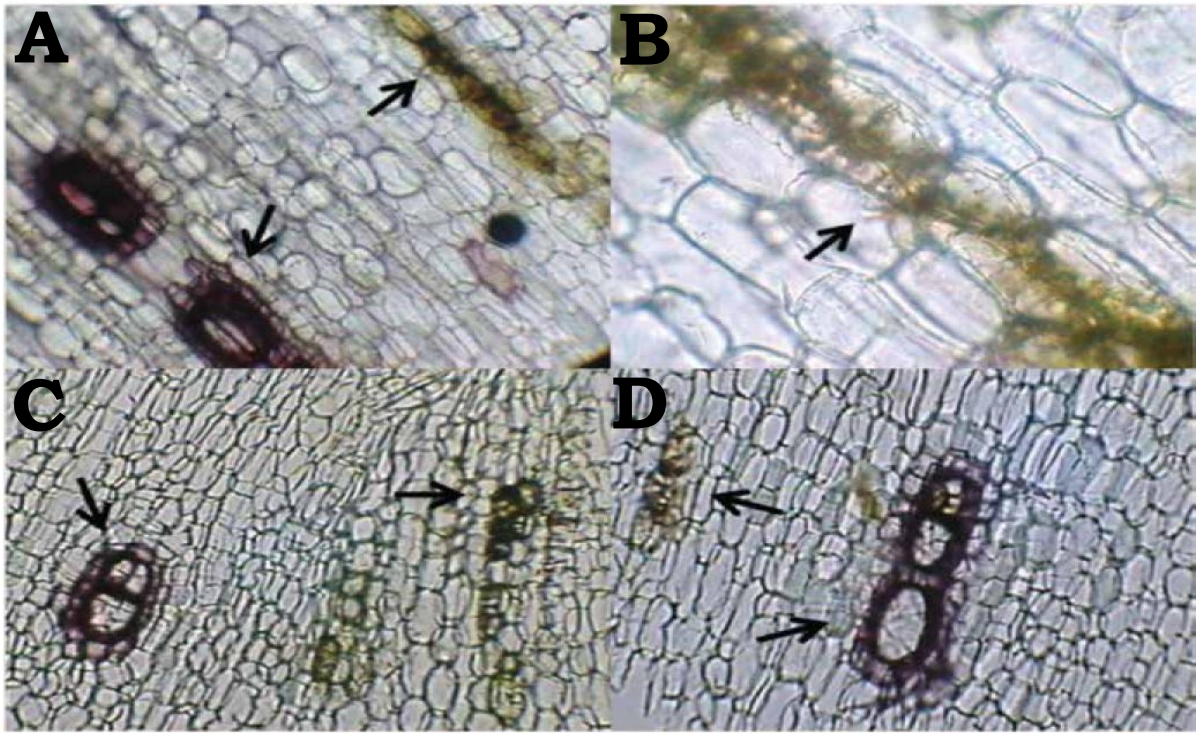


Picture 1. Detection of hydrogen peroxide (H_2O_2) in slices or cassava (*M. esculenta*) roots of the Catarina Amarela and Catarina Branca cultivars. Each column in each cultivar presents three analyzed regions, each block of the left side is before infiltration and right side is after infiltration.

while, Reilly *et al.* (2003) suggest that there should be an upper threshold of 5 mg/kg of fresh weight for carotenoids to be involved in the reduction of the PPD development. In this study none of the cultivars showed carotenoid levels above the threshold to be effective in the PPD control.

In relation to the cellulose and lignin contents, among the studied factors the interaction cultivar x time was significant ($P < 0.05$) for both and initially there was difference between the cultivars as shown in Table 1. After storage, there was no difference when compare to the initial content, as well as for cellulose among cultivars. Lignin content had an increasing trend for both cultivars studied but, it was only significant for the Catarina Branca cultivar (Table 1). When comparing the regions among cultivars, there were not differences for cellulose and lignin content initially and after storage.

Few studies have evaluated the alterations in lignin and cellulose content during storage of cassava roots. Carvalho *et al.* (1988) observed that during storage there was a decreasing trend, however, not significant when compared with the initial values for cellulose content. For lignin content there was also a decreasing trend after 7 days of storage only for the IAC 12829 cultivar which showed a significant alteration in lignin content from 0.12% to 0.16%.In relation to the microscopic observations, there were no differences detected in the parenchyma structures of the tuber roots of the cultivars. After 5 days of storage in both cultivars there was an evidence of a dark pigment with brown-greenish color between the storing parenchyma cells, which are precipitates that correspond to the regions where the PPD process is going on (Picture 2). The presence of dark pigments have been described in microscopic studies of the root storing paren-



Picture 2. Transversal section of storing parenchyma of cassava (*M. esculenta*) tuber roots after 5 days of storage. (A) Secondary xylem and precipitate between the storage parenchyma cells of the Catarina Amarela cultivar (100x) (B) precipitate between the storing parenchyma cells of the Catarina Amarela cultivar (400x) (D and C) Secondary xylem and precipitate between the storing parenchyma cells of the Catarina Branca cultivar (100x).

chyma under PPD processes, as described by Beeching *et al.* (1998) and Buschmann *et al.* (2000a). No alterations in the primary xylem of both cultivars after storage were observed, suggesting that the PPD process happens mainly in the periferic region of the root without affectng the vascular cambium (primary xylem).

Conclusions

- The Catarina Amarela and Catarina Branca cultivars suffer chemical and histological alterations after harvesting being the Catarina Branca cultivar the most resistant to these alterations.

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