Hydrogen Peroxide and Flavan-3-ols in Storage Roots of Cassava (*Manihot esculenta* Crantz) during Postharvest Deterioration

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Cassava storage roots are an important staple food throughout the lowland humid tropics. However, cassava suffers from a poorly understood storage disorder, known as postharvest physiological deterioration (PPD), which constrains its exploitation. In an attempt to broaden the understanding of PPD, nine different cassava cultivars were analyzed for specific compounds accumulating during the process. The production of hydrogen peroxide (H_2O_2) is involved in the early stages of PPD in cassava roots. H_2O_2 was quantified and localized histochemically at the tissue and cell level in deteriorating roots. This reactive oxygen species accumulated during the first 24 h after harvest, especially in the inner parenchymatic tissue. Three flavan-3-ols, (+)-catechin, (+)-catechin gallate, and (+)-gallocatechin, accumulated during the storage of cassava roots. However, these potential antioxidants cannot be related to early storage disorders or wound responses because they start to accumulate only after 4−6 days.

Keywords: *Antioxidants; cassava; catechins; flavan-3-ols; hydrogen peroxide; Manihot esculenta; postharvest deterioration; wound response*

INTRODUCTION

Cassava (*Manihot esculenta* Crantz, Euphorbiaceae) is one of the most important root crops in the world. With a production of >150 million tons per year, it is the staple food for >500 million people, especially in the lowland tropics. Its high yield in carbohydrate, low susceptibility to pathogens, and low demands on water supply and soil quality make it very attractive to farmers, processors, and consumers. However, these benefits are often unrealized due to its short shelf life after harvest. The cassava root rapidly shows signs of a deterioration process that occurs within 2−3 days of harvest at the latest. This deterioration process can be differentiated into two phases. In the first phase, primary deterioration starts from the central vascular bundles of the root. Its first visible signs are a black-blue to black discoloration, or vascular streaking, beginning at the broken or cut surfaces. Subsequently, the deterioration spreads to the adjacent storage parenchyma and the stored starch undergoes structural changes (Plumbley and Rickard, 1991). This initial deterioration process is a physiological process that does not involve microorganisms (Averre, 1967; Noon and Booth, 1977). These changes, known as postharvest physiological deterioration (PPD), of which vascular streaking is the first visible symptom, render the roots unpalatable and unmarketable. The secondary deterioration is due to infection with microorganisms leading to fermentation and softening of the root tissue (Plum-

bley and Rickard, 1991; Wenham, 1995). PPD is much more significant economically than the secondary microbial deterioration. The visible coloration of the root tissue is used as an indication of its culinary value and taste and makes the crop difficult to sell. For this reason, it is essential to understand these processes in order to identify potential means by which PPD may be controlled. Comparative evaluation of the visual symptoms of PPD in various cultivars of cassava revealed that there are differences in susceptibility to deterioration (Iglesias et al., 1996). These differences can provide breeders and biologists with the opportunity to use the genetic variability of cassava to improve the crop.

PPD is due to endogenous oxidative processes, as it can be delayed by the exclusion of oxygen, for example, by storing the roots in polyethylene bags or in a water bath or by coating them with wax (Rickard and Coursey, 1981; Best, 1990; Plumbley and Rickard, 1991). As during wound responses, fruit ripening, and senescence, the phytohormone ethylene accumulates shortly before the onset of tissue discoloration (Hirose et al., 1984a; Hirose, 1986; Plumbley et al., 1991; Uritani, 1998). This may be an indication of ethylene playing a coordinating role in PPD. However, the pruning of cassava plants before harvest, which inhibits the rapid PPD response, does not inhibit the production of ethylene in the roots after harvest (Hirose et al., 1984b).

In addition, Wheatley and Schwabe (1985) suggested a possible correlation of PPD with an increase in phenolic compounds (e.g., scopoletin) and H_2O_2. H_2O_2 is a reactive oxygen species (ROS) that can be synthesized actively by the plant as a response to stress or as a component of defense against pathogen attack. This stress-related synthesis can be very rapid and is part of a process called "oxidative burst". In these defensive reactions H_2O_2 is involved in at least three different ways: (a) during the process of lignification [e.g.,

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Halliwell (1978)]; (b) for binding phenolic compounds and proteins with pectins and hemicelluloses [e.g., Fry and Miller (1989)]; and (c) as an internal chemical mediator involved in the onset of signal transduction pathways [e.g.. Doke et al. (1991) and Hippeli et al. (1999)]. H_2O_2 may also develop during wounding and tissue decay as a result of loss of compartmentation within plant cells. Because of their high reactivity with biomolecules, ROS can damage the plant cell itself. To regulate and detoxify these oxygen species, plants have developed various strategies involving enzymatic processes (e.g., superoxide dismutases, catalases, peroxidases, glutathione transferases) or low molecular weight molecules (e.g., ascorbic acid, carotenoids, flavonoids) that function as radical quenchers or scavengers. The production and function of ROS as well as numerous routes of their detoxification have been described in detail for other plant systems as reactions to wounding, pathogen attack (Thompson et al., 1986; Baron and Zambryski, 1995), and natural and postharvest senescence (Hodges and Forney, 2000) but have never been investigated in detail in cassava.

There is no direct proof for ROS being involved in PPD, but there are many indirect indications, such as the increasing activities of peroxidases and polyphenol oxidases (Padmaja and Balagopal, 1985; Campos and de Carvalho, 1990). The decrease of preformed and the de novo synthesis of antioxidants have been described in the literature. Gloria and Uritani (1984) showed a decrease in β-carotene in the root tissues of cassava during PPD. In addition to this, there are findings that cassava cultivars with a high content of carotenoids in their roots are less susceptible to PPD than those with a low content (Adewusi and Bradbury, 1993; Iglesias et al., 1995). Unfortunately, consumers in many parts of the tropics prefer roots from "white" cassava cultivars and do not accept "yellow" ones, high in content of carotenoids. De novo synthesis or accumulation of other potential antioxidants derived from the phenylpropanoid pathway during PPD has been investigated in the past. These compounds have been identified as the hydroxycoumarins scopolin, scopoletin, and esculin (Uritani et al., 1984a,b; Tanaka et al., 1983) and the flavan-3-ols (+)-catechin and (+)-gallocatechin (Uritani et al., 1984b; Rickard, 1985). However, most of these identifications were based on retention times in chromatographic techniques such as high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) and were not based on spectroscopic procedures. In addition, there have been no detailed quantification of flavan-3-ols during PPD and no comparison of cassava cultivars with different susceptibilities toward deterioration.

Recently, flavan-3-ols have been investigated in other plant systems because of their biological properties and pharmacological potential [e.g., Matsuda et al. (1986) and Plumb et al. (1998)]. As monomers, they have a very high potential in quenching ROS (Rice-Evans et al., 1997) as well as antifungal properties (Li et al., 1999), whereas in polymeric form, as condensed tannins or proanthocyanidins, they can function either as antioxidants or as antifeedants for herbivores (Hagerman and Butler, 1991).

This study describes the synthesis of H_2O_2 in the very early stages of PPD of cassava storage roots as well as the localization of this accumulation at the tissue and cell levels. Three flavan-3-ols were identified and quantified in cassava root extracts, and their role as antioxidants is discussed. Moreover, this study provides a comparison of various economically important cassava cultivars that show differences in their susceptibility toward PPD.

MATERIALS AND METHODS

Plant Materials. Storage roots of at least three different plants per cultivar and nine different cassava cultivars (CM 7033-3, CMC 2177-2, MBRA 337, MCOL 22, MDOM 5, MNGA 1, MNGA 2, MVEN 77, and SM 985-9) were harvested and analyzed in the years 1998 and 1999 at CIAT, Cali, Colombia. Root material (cultivars MCOL 22 and MVEN 77) for H_2O_2 quantification was obtained from greenhouse-grown plants, University of Bath, Bath, U.K. The cultivars MCOL 22 and SM 985-9 are highly susceptible, MNGA 1 and MNGA 2 intermediately susceptible, and CM 7033-3, CMC 2177-2, MBRA 337, MDOM 5, and MVEN 77 slightly susceptible to PPD (M. Bonierbale, personal communication).

Storage and Extraction of Roots. All storage roots were cut transversely into 2 cm thick slices and stored for 7 days under controlled conditions (dark, 29 °C, 80−90% relative humidity) as described by Sakai and Nakagawa (1988). For extraction and chemical analysis, one slice per day from each cultivar was homogenized in absolute ethanol (Rathburn, U.K.) by means of a blender. The extracts were filtered, evaporated to a final volume of 3 mL, and stored at −20 °C until used. Roots for H_2O_2 detection using 3,3-diaminobenzidine tetrahydrochloride (DAB) were harvested and stored under field conditions at CIAT, Cali, Colombia. Immediately after harvest, the proximal and distal ends of the storage roots were removed and the distal end was covered with plastic film. Samples were taken daily over a 5-day time course from the proximal end of the root. For H_2O_2 quantification, root tubers were harvested and stored at ambient temperature. Samples were taken at daily intervals.

Light and Fluorescence Microscopy. Hand-cut cross sections of cassava roots taken during different stages of storage were transferred and mounted in glycerol/water (1:1, v/v). The samples were examined with an Aristoplan microscope (Leitz) using either bright-field illumination, fluorescence excitation at 340−380 nm (Leitz filter combination A), or excitation at 355−424 nm (Leitz filter combination D). For the induction of secondary fluorescence for the detection of flavonoids, Naturstoffreagenz A (diphenyl boric acid 2-aminoethyl ester) was used according to the method of Hutzler et al. (1998).

H_2O_2 Localization, Detection, and Quantification. H_2O_2 was detected histochemically by means of light microscopy and the staining technique described by Olson and Varner (1993) and Repka (1999). In this assay iodide is oxidized by H_2O_2 to iodine, which then forms a colored product after complexing with starch. For localization of H_2O_2 in tissues, the (DAB) vacuum infiltration method of Vallélian-Bindschedler et al. (1998) was used with root slices over a 5-day storage period. As a control, root tissue slices were infiltrated in the same way but with additional 1 mM ascorbate.

Quantification of H_2O_2 in cassava root extracts was obtained using the method of Warm and Laties (1982) with some modifications. For removal of colored components, root extracts [0.05 g of fresh weight (FW) homogenized in 1.5 mL of 5% metaphosphoric acid] were passed twice through a Dowex basic anion-exchange resin (1 × 8−400, Sigma; 0.5 mL of resin in 1 mL of 5% metaphosphoric acid) by batch chromatography. To use a luminometer (Micro Lumat Plus, E and G Berthold), the original method needed to be scaled down to microplate volumes of 195 μL of Tris-HCl buffer (0.2 M, pH 8.5), 25 μL of luminol (0.1 mM), 25 μL of potassium ferricyanide (5 mM), and 5 μL of plant extract. A standard curve was obtained by measuring appropriate volumes of 10 μM H_2O_2 in metaphosphoric acid.

Chromatographic Analysis and Spectroscopy. For TLC, the aliquots (corresponding 0.1 g of FW) of ethanolic extracts were spotted onto HPTLC plates (silica gel 60 F_{254},

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20 × 20, Merck) and developed in a liquid phase of chloroform/ethyl acetate/methanol (2:2:1). The separated compounds were detected under a UV lamp (Camag) at 254 and 366 nm. For the detection of compounds with antioxidative properties, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method described by Takao et al. (1994) was used for the crude extracts as well as for isolated compounds (by HPLC) and reference compounds. This assay was used on HPTLC because of the speed of the method. The separation of the several components of cassava extracts was good, and the DPPH assay on the HPTLC plates sensitive enough to use the advantage of the rapidity offered by this method.

For HPLC, a system (Gilson) combined with a diode array detector (Hewlett-Packard) and an analytical reversed phase column (Techsphere ODS-BDS, 250 × 4.6 mm; 5 μm; HPLC Technology, U.K.) was used. The best separation of compounds was accomplished by using aqueous H_3PO_4 (pH 2.6) in a linear gradient of 2−100% of acetonitrile (Rathburn, U.K.) over 55 min and under a constant flow of 1.3 mL/min. Compounds were detected simultaneously at 215, 280, and 350 nm. The compounds were identified by their retention times, by co-chromatography with identical references [(+)-catechin and (+)-catechin gallate from Sigma; (+)-gallocatechin from Leuven Bioproducts, Belgium], and by UV and mass spectroscopy. For the quantification of catechins, the reference compounds were used as external standards in HPLC. LC-MS experiments were performed on a Finnigan TSQ 700 under atmosphere pressure chemical ionization (APCI) as described elsewhere (Vogler et al., 1998; Renukappa et al., 1999).

Identification of Flavan-3-ols. For rapid information on the nature and identity of compounds in cassava root extracts, spectroscopic methods (UV, MS) directly combined with liquid chromatography (LC) were used. Recently, these techniques proved to be very useful for the rapid identification of plant secondary metabolites (Vogler et al., 1998; Renukappa et al., 1999).

The analysis of components of the cassava root extracts resulted in the detection of three flavan-3-ols in all of the investigated cassava cultivars. These compounds, (+)-gallocatechin, (+)-catechin, and (+)-catechin gallate (Figure 3), were identified by their retention times as well as by UV spectroscopy and LC mass spectroscopy. The spectroscopic data are given below.

(+)-Gallocatechin: $C_{15}H_{14}O_7$; APCI + Q1MS; gradient 2−65% CH_3CN in 55 min (relative intensity) 306 $[M + H]^+$ (100), 289 (30), 139 (20); UV_{max} (50% CH_3CN) 204, 228, 269.

(+)-Catechin: $C_{15}H_{14}O_6$; APCI + Q1MS; gradient 2−65% CH_3CN in 55 min (relative intensity) 291 $[M + H]^+$ (100), 272 (20); UV_{max} (50% CH_3CN) 202, 226, 276.

(+)-Catechin gallate: $C_{22}H_{18}O_{10}$; APCI + Q1MS; gradient 2−65% CH_3CN in 55 min (relative intensity) 460 $[M + H]^+$ (70), 118 (100); UV_{max} (50% CH_3CN) 200, 218, 274.

Besides these flavan-3-ols, four hydroxycoumarins were identified as described by Buschmann et al. (2000).

Total Phenol Content. For the determination of the total phenol content in cassava root slices, the method of Cliffe et al. (1994) was used. The ethanolic extracts were diluted 100-fold with H_2O before measurement.

Statistical Analysis. The data sets were processed using regression analysis by means of the software package Minitab (Minitab Inc., 1998).

RESULTS

Visible and Microscopic Observations. Evaluation of PPD development in cassava roots over a storage period of 7 days in daylight and under UV (366 nm) confirmed the onset of vascular streaking of the root slices ∼24−48 h after harvest, depending on the cassava cultivar. The roots of different cassava cultivars showed differences in their susceptibility toward PPD, allowing classification into highly, intermediately, and slightly susceptible cultivars. Visible signs of PPD occurred rapidly in the cultivars CM 7033-3, MCOL 22, MNGA 1, and SM 985-9, whereas cultivars CMC 2177-2 and

MNGA 2 showed intermediate reactions. Cultivars MDOM 5 and MVEN 77 proved to be slightly susceptible cultivars. The results for CMC 2177-2, MNGA1, MNGA 2, and SM 985-9 were different from previous observations made at CIAT (M. Bonierbale, personal communication). However, great variation in susceptibility toward PPD was observed in the individual roots, which reflects results from more extensive field trials at CIAT (M. Fregene, personal communication).

Light microscopic observations of the root tissue over a storage period of 7 days revealed the formation of tyloses and brownish occlusions, which were first visible after 24 h in the xylem vessels. Blue fluorescence around the vessels and spreading into the apoplast of the storage parenchyma were detectable with fluorescence microscopy as described elsewhere (Buschmann et al., 2000). After a storage time of ≥5 days and after staining with Naturstofreagenz A, this fluorescence of the xylem and some parts of the parenchyma changed to yellow, indicating the accumulation of flavonoid compounds. After 7 days of storage, this yellow fluorescence spread over all of the storage parenchyma (results not shown).

Detection, Localization, and Quantification of H_2O_2. The vacuum infiltration of DAB into root slices of different cultivars and at different storage times revealed differences in the rate of H_2O_2 accumulation as well as its localization (Figure 1). In the intermediately and slightly susceptible cultivars (NGA 2, MDOM 5, and CMC 2177-2), significant color reactions were visible after 24 h. However, in the root slices of the highly susceptible cultivar, MCOL 22, this dramatic coloration occurred after 3 days. In the cultivars MDOM 5 and CMC 2177-2, the DAB reaction started in the cortical parenchyma and then spread into the storage parenchyma, whereas in MCOL 22 and NGA 2 the color change was first visible in the storage parenchyma near the cambium. These results indicate that the cortical parenchyma of these latter cultivars does not accumulate H_2O_2 at the same rate and to the same extent as the former cultivars.

Microscopic investigation of cassava root slices after staining with KI−starch−potassium permanganate revealed that H_2O_2 first accumulates (after 24 h) in the parenchymatic parts of the xylem. After 48 h, H_2O_2 accumulation was detected in the storage parenchyma and, depending on the cultivar, in the cortical parenchyma. In all cases, the H_2O_2 was detected in the apoplast of the cells, especially in the area of the middle lamella.

The quantification of H_2O_2 in the root extracts of two selected cultivars (MCOL 22 and MVEN 77) revealed rapid accumulation to a maximum concentration of 5.3 μmol/g of FW during the first 24 h of storage (Figure 2). During the following days of storage, but especially after 4−5 days, this concentration declined steadily, reaching values close to those observed directly after harvest by day 5. In contrast to other quantitative analyses, these data did not show great variation between the roots nor differences between the slightly and highly susceptible cultivars.

Quantification of Flavan-3-ols and Comparison of Cassava Cultivars. A typical quantitative development of (+)-catechin, (+)-catechin gallate, and (+)-gallocatechin during 7 days of storage of cassava root slices (cultivar CM 7033-3) is summarized in Figure 4. Even though these results show high individual quantitative differences between the roots, which reflects the

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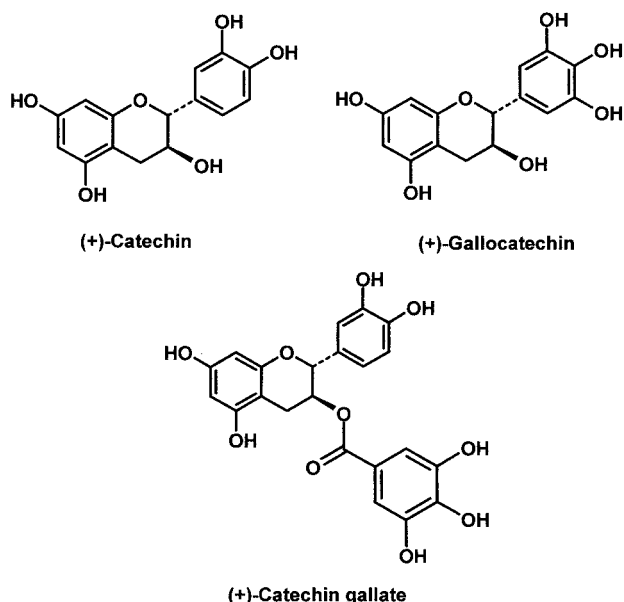*J. Agric. Food Chem.,* Vol. 48, No. 11, 2000 **5525**
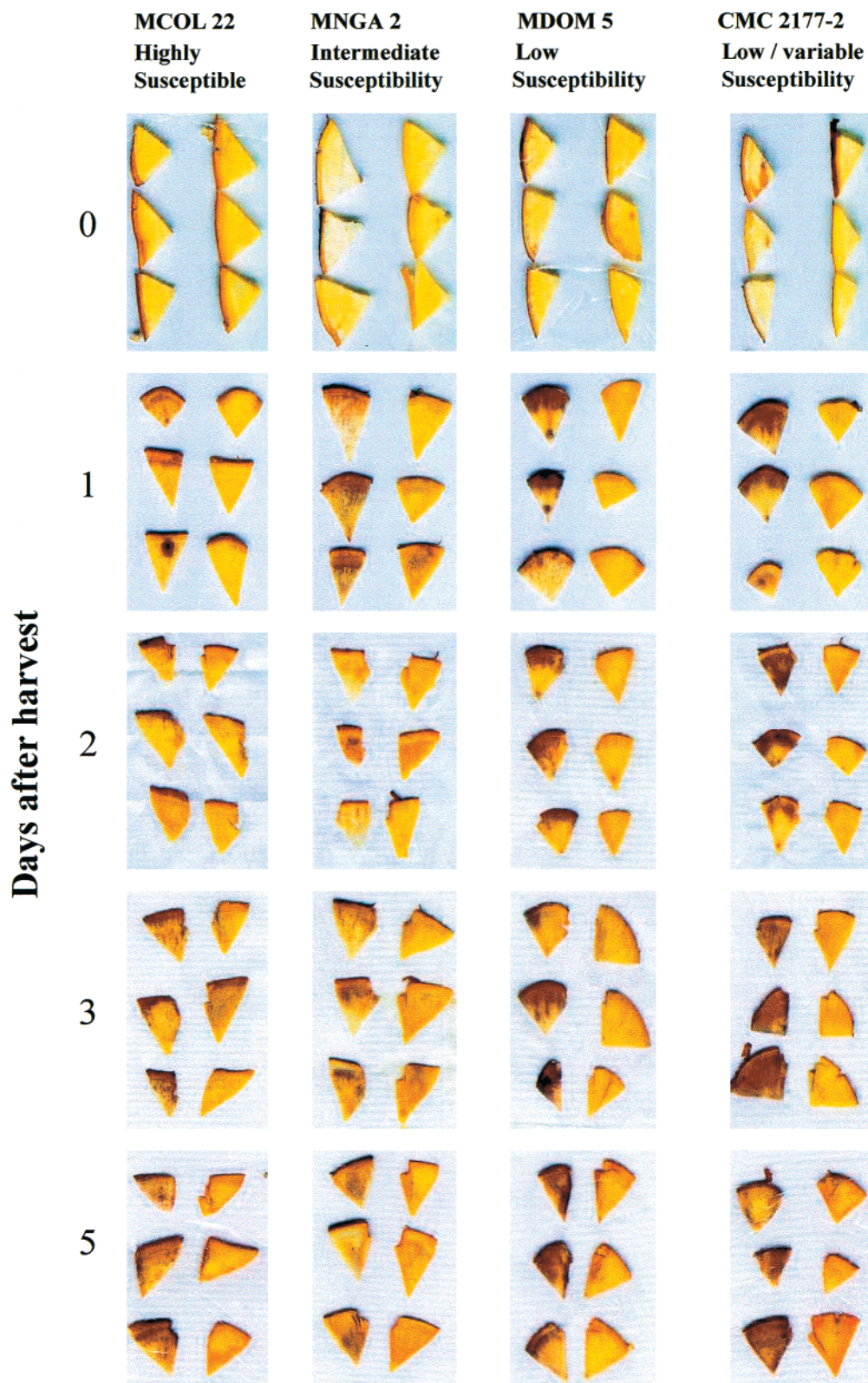
Figure 1. Detection of H_2O_2 in slices of cassava roots by means of vacuum infiltration with DAB. Each column represents a cassava cultivar, each row a different storage time of the roots. In each block the three slices on the left were infiltrated with DAB only, whereas the slices on the right were co-infiltrated with DAB and the antioxidant ascorbate.

other observations of PPD in cassava, these data reveal general trends. During the first 24−48 h, (+)-catechin, (+)-catechin gallate, and (+)-gallocatechin were just detectable (Figure 4; Table 1). After 2−3 days there was some accumulation of all three flavan-3-ols. The (+)-gallocatechin then accumulated rapidly at day 4, fol-lowed by (+)-catechin at day 5. After 7 days, there was a rapid decline of all flavan-3-ols and, after >10 days, these compounds were hardly detectable (<0.05 nmol/g of FW; data not shown). In all seven analyzed cultivars the three identified flavan-3-ols were not synthesized de novo but were detectable in low concentrations
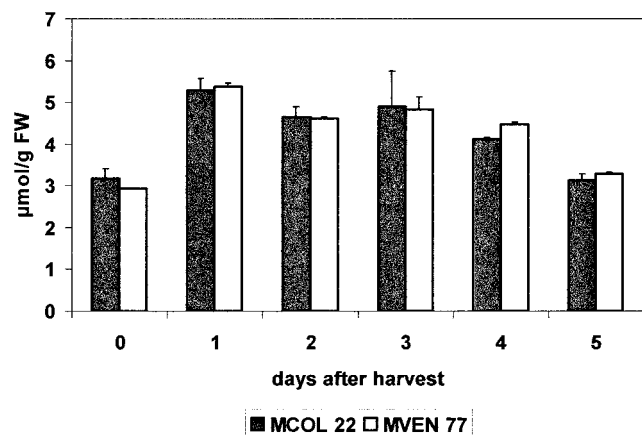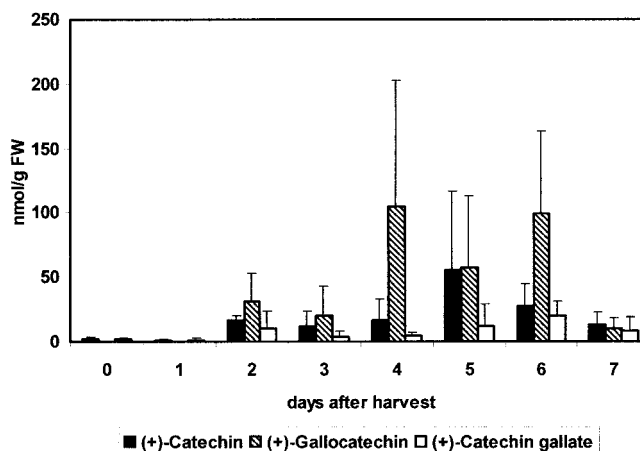
Figure 2. Quantification of free H_2O_2 in cassava root slices of two different cultivars [MCOL 22 (highly susceptible) and MVEN 77 (slightly susceptible)] over a storage period of 5 days. Each column represents the mean of three different roots per cultivar taken from different plants (mean ± SD).

Figure 3. Flavan-3-ols identified from cassava root extracts.

Figure 4. Quantification of flavan-3-ols in cassava root slices (cultivar CM 7033-3) over a storage period of 7 days. The columns represent the mean of four different roots taken from different plants (mean ± SD).

directly after harvest (Table 1). The concentration of (+)-gallocatechin did not increase significantly in all roots investigated over the whole storage time. In contrast, (+)-catechin and (+)-gallocatechin accumulated after 2−6 days. In MBRA 337 and SM 985-9, (+)-catechin accumulated after 6 days to very high concentrations, whereas MCOL 22 and MNGA 1 did not show any significant increase. The (+)-gallocatechin accumulated in six cultivars (CM 7033-3, MBRA 337, MCOL 22, MDOM 5, MNGA 2, and SM 985-9) after 4−6 days. Cultivars MBRA 337 and MDOM 5 especially reached very high concentrations of this flavan-3-ol. Only cultivar MNGA 1 did not show a great accumulation.

Comparing all of the cassava cultivars examined, an obvious relationship of susceptibility toward PPD and the accumulation of total flavan-3-ols emerges. The most important flavan-3-ol seems to be (+)-gallocatechin. Here, the slightly susceptible cultivars accumulated very high amounts after 5−6 days, whereas the highly and intermediately susceptible cultivars showed significantly lower concentrations. In contrast to this, there was no obvious difference in the accumulation of (+)-catechin and (+)-catechin gallate among the cultivars.

The increases in flavan-3-ols (Table 1) was hardly reflected by the results on the total phenol content (TPC) (Figure 5). Over the storage period of 7 days there was nearly no change in TPC for all investigated cultivars. Only for cultivar CM 2177-2 was a parallel increase of catechins and TPC after 5 days of storage recognizable.

Antioxidant Properties of Phenolic Compounds in Roots of Storage Cassava. The major phenolic components of cassava roots were the hydroxycoumarins esculin, esculetin, scopolin, and scopoletin (Buschmann et al., 2000), the flavan-3-ols (+)-catechin, (+)-catechin gallate, and (+)-gallocatechin (described here), and the flavone 3-glycosides rutin and kaempferol 3-rutinoside (unpublished data). TLC of ethanolic cassava root extracts and these isolated components (in physiological concentrations) and subsequent staining with DPPH [according to the method of Takao et al. (1994)] revealed rapidly different hydrogen-donating activities of these compounds. The (+)-catechin, (+)-catechin gallate, (+)-gallocatechin, esculetin, and rutin reacted very quickly (after ~5 s) with the radical and proved to be very potent antioxidants. Scopoletin was much slower (5 min) and weaker in its reaction toward DPPH, and esculin, scopolin, and kaempferol 3-rutinoside did not react at all.

DISCUSSION

The involvement of H_2O_2 and antioxidants, such as flavan-3-ols, in wound responses has been described often in the literature for other plant systems. In general, wound responses in plants share many features with postharvest disorders. Most recently, it has been shown that the quantitative shifts of ROS and antioxidants play an important role in postharvest senescence in spinach leaves (Hodges and Forney, 2000).

The involvement of endogenous oxidative processes in PPD has been suggested by various authors based either on the observation that excluding oxygen from the cassava root tubers slows PPD (Rickard and Coursey, 1981; Best, 1990; Plumbley and Rickard, 1991) or on the increase of activities of specific enzymes that use H_2O_2 as a substrate, such as peroxidases (Padmaja and Balagopal, 1985; Campos and de Carvalho, 1990). Furthermore, Wheatley and Schwabe (1985) presented evidence that PPD was correlated with the oxidation processes of the hydroxycoumarin scopoletin. The data presented here show for the first time that there is a

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Table 1. Quantification of Flavan-3-ols (Nanomoles per Gram of Fresh Weight) in Cassava Roots over a Storage Time of 6 Days[a]

compound	DAH	S: Cv:	high: CM 7033-3	low: MBRA 337	high: MCOL 22	low: MDOM 5	high: MNGA 1	high: MNGA 2	high: SM 985-9
(+)-catechin	0		1.62 ± 2.22	0.98 ± 0.65	0.39 ± 0.39	3.62 ± 2.84	7.81 ± 5.36	2.88 ± 3.42	4.63 ± 7.78
	2		16.16 ± 3.85	1.45 ± 1.16	2.77 ± 0.83	19.42 ± 15.51	7.75 ± 5.23	2.22 ± 1.23	2.81 ± 1.47
	4		16.04 ± 17.09	36.36 ± 30.18	0.37 ± 0.14	9.01 ± 4.29	5.48 ± 4.28	6.06 ± 1.66	7.99 ± 3.24
	6		27.00 ± 17.92	100.06 ± 49.67	0.10 ± 0.07	39.72 ± 20.08	1.65 ± 0.08	41.88 ± 12.53	87.38 ± 38.74
(+)-catechin gallate	0		1.74 ± 1.28	1.11 ± 0.66	1.97 ± 2.19	2.08 ± 1.58	7.84 ± 4.79	0.90 ± 0.56	1.07 ± 0.81
	2		9.84 ± 13.61	3.41 ± 4.76	4.54 ± 1.44	3.06 ± 0.19	2.85 ± 0.44	0.57 ± 0.24	0.81 ± 0.16
	4		4.33 ± 3.36	15.01 ± 5.32	6.25 ± 3.39	5.61 ± 3.18	3.85 ± 1.47	1.82 ± 1.66	1.34 ± 0.84
	6		20.13 ± 10.94	10.06 ± 6.43	8.70 ± 7.00	9.13 ± 6.37	2.26 ± 1.56	3.90 ± 3.15	2.83 ± 0.91
(+)-gallo- catechin	0		0.06 ± 0.06	0.02 ± 0.01	0.03 ± 0.03	0.65 ± 0.57	0.07 ± 0.07	0.89 ± 1.46	6.55 ± 5.57
	2		31.16 ± 22.16	1.33 ± 1.16	0.15 ± 0.17	48.84 ± 68.84	4.63 ± 3.10	0.04 ± 0.03	6.42 ± 1.78
	4		105.15 ± 97.84	91.44 ± 40.18	8.81 ± 2.16	48.99 ± 51.54	20.81 ± 15.19	10.41 ± 2.63	5.25 ± 5.70
	6		99.74 ± 63.52	280.62 ± 197.76	81.78 ± 86.99	358.56 ± 146.27	10.00 ± 1.18	53.03 ± 17.50	63.64 ± 28.49

[a] Cv, cassava cultivar; DAH, days after harvest; S, susceptibility to PPD; mean ± SD; $n = 4$.
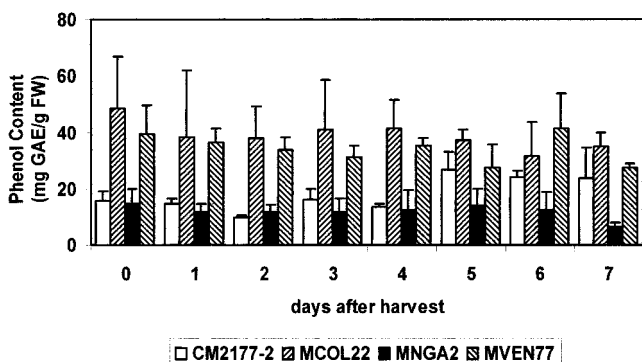
Figure 5. Quantification of the soluble total phenol content in cassava root extracts [cultivar CM 7033-3 (intermediately susceptible), MCOL 22 (highly susceptible), MNGA 2 (intermediately susceptible), and MVEN 77 (slightly susceptible)] over a storage period of 7 days [expressed in gallic acid equivalent (GAE)]. The columns represent the mean of three different roots taken from different plants (mean ± SD).

rapid increase of H_2O_2 in cassava roots over the first 24 h of storage. This concurs with the data from wound responses in other plant systems (Thompson et al., 1987; Schopfer, 1994).

The accumulation of H_2O_2 does not show significant differences among the cassava cultivars investigated, which reveals that there are other factors involved in the process that determine the susceptibility of the cultivar toward PPD. Linear regression analysis of these H_2O_2 data (cultivar MCOL 22) with other biochemical data revealed the highest correlation toward peroxidase activities ($R^2 > 52\%$) and scopoletin ($R^2 > 49\%$) accumulation. The slight decrease of H_2O_2 after 4–5 days can be explained by general biochemical oxidative processes.

The localization of H_2O_2 at the tissue level revealed that the major site of accumulation, beginning after 24 h, is the parenchyma. To our knowledge this is the first report on H_2O_2 localization in storage roots, but the results correspond with the findings of Schopfer (1994), who described a rapid H_2O_2 accumulation in the storage parenchyma of potato stem tubers as a reaction to wounding. The distribution of H_2O_2 in the roots of different cassava cultivars is interesting. Some accumulate H_2O_2 over the whole parenchymatic tissue (MDOM 5 and CMC 2177-2), whereas other cultivars accumulate H_2O_2 first in the storage parenchyma (MCOL 22 and MNGA 2) and then after some time in the cortical parenchyma.

At a cellular level, the histochemical localization of H_2O_2 in cassava roots showed an accumulation in the apoplast of the parenchymatic cells. This site of accumulation has been reported previously (Bestwick et al., 1997, 1998) for other plant systems, especially during the hypersensitive reaction to microbial infections. The origin of this rapid H_2O_2 accumulation has still to be identified. It may be due to the breakdown of lipids as a response to harvesting and wounding, which may generate ROS as described for peas by Dörnenburg and Davies (1999), or general enzyme activities that are related to wounding, such as NADH oxidase/peroxidase (Thompson et al., 1987; Sutherland, 1991) or lipoxygenases (Hildebrand, 1989; Siedow, 1991; Marci et al., 1994).

It is well-known from other plant systems that flavan-3-ols, such as (+)-catechin, accumulate after pathogen infection or wounding (Brignolas et al., 1995). Rickard (1985) and Uritani (1998) found that the flavan-3-ols (+)-catechin and (+)-gallocatechin accumulated after 4 days of storage of cassava roots and thus have little relationship to PPD itself, which starts earlier after harvesting. However, neither author gave any detailed data on quantification or structure elucidation. The data presented here show the occurrence of three flavan-3-ols, (+)-catechin, (+)-catechin gallate, and (+)-gallocatechin, and the quantitative comparison of different cassava cultivars over a long-term storage period. The conclusion that flavan-3-ols are not involved in PPD must be qualified by the finding that slight accumulations of (+)-catechin and (+)-gallocatechin occurred during the first 24–48 h of storage in cultivars CM 7033-3 and MDOM 5. The significance of this needs to be tested by the investigation of more cultivars and during more longer term field trials. Even if there is no relation to PPD, the data shown in this paper reveal differences in overall accumulation of flavan-3-ols among cultivars with different susceptibilities toward PPD.

These differences are not reflected in the quantification of soluble total phenols as presented in this paper. This content hardly differs over the storage period and seems to be dominated by phenolic components other than flavan-3-ols.

The rapid increase of flavan-3-ols in cassava roots is followed by a similarly rapid decrease 1 or 2 days later. This may be due to a turnover of these molecules by oxidative or metabolic processes. Evidence for an oxidation of the polyphenols may be found in the histochemical investigation of cassava roots and the increase in activity of peroxidases (Buschmann et al., 2000) and

polyphenol oxidases (Campos and de Carvalho, 1990) during this time. The localization of yellow fluorescent occlusions on the cell walls of xylem vessels shortly after harvest and the spreading of brown-yellow coloration into the parenchyma may be due to deposition and oxidation of flavonoids, such as flavan-3-ols. This has been described for various plant systems as a response to abiotic and biotic stresses (Dai et al., 1996; Bonsen and Kucera, 1990). On the other hand, there is evidence for the metabolism of flavan-3-ols into condensed tannins (or proanthocyanidins) in the data presented by Rickard (1985) and Rickard and Gahan (1983). The latter authors attribute lignin-like properties to the occlusions formed by condensed tannins. Other properties of condensed tannins are those of antioxidants (Plumb et al., 1998) and antifeedants to herbivores [summarized in Hagerman and Butler (1991)].

Flavan-3-ols and flavonoids, such as rutin, that were isolated from cassava storage roots have been shown by this investigation to be very potent hydrogen donors and may function as antioxidants. The capacity of a compound to scavenge the DPPH radical, however, does not necessarily mean that the compound is as potent in scavenging H_2O_2 [e.g., Lugasi et al. (1999)]. The flavonoids and coumarins identified from cassava root extracts are identical or similar to those antioxidants that were investigated more thoroughly by Plumb et al. (1998) and Rice-Evans et al. (1997) as to their capacity to scavenge ROS. However, as already stated, the production of the flavan-3ols is not related to the earlier more rapid development of PPD.

The evaluation of data from various biochemical and chemical analyses of deteriorating cassava roots showed that, because of their rapid production, H_2O_2 (this publication) and the hydroxycoumarins scopoletin and its glucoside scopolin (Buschmann et al., 2000) are more closely related to PPD than other components. The synthesis of catechins and their possible metabolism into condensed tannins occurs after a storage time of 5–6 days and for that reason can be interpreted only as a defense reaction during secondary deterioration or microbial decay but not to PPD.

However, there is still the need for further research on the origin, the compartmentation, and the regulation of H_2O_2 and other ROS, as well as on the biosynthetic pathways and the control mechanisms for secondary metabolites in deteriorating cassava roots. Only evaluation of all biochemical components involved in PPD and comparison of further biochemically distinct cassava cultivars may reveal basic insights into and understanding of the problem and thereby lead to possible solutions based on breeding or molecular approaches that may benefit small farmers in developing countries.

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