Changes in extended shelf life of cassava roots during storage in ambient conditions.

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Abstract
Cassava roots have a short shelf life due to a process known as post-harvest physiological deterioration (PPD). Within 2-3 d undesirable vascular streaking in the root develops. Tolerance to PPD was recently reported in different cassava genotypes, opening up new opportunities to analyze biochemical changes in stored roots and in the functional properties of their starches. Roots from PPD-susceptible (HMC-1) and tolerant (AM 206-5) clones were harvested and stored for up to 14 d in ambient tropical conditions. AM 206-5 is also characterized by amylose-free starch. Roots and starch were analyzed each day. PPD levels differed significantly between the two clones (35% and 8% at day 14) and showed a relation to scopoletin synthesis, which reached maximum levels around day 3 or 4 of storage. Roots lost weight consistently during storage (≈10% in two weeks). Starch loss per day of root storage was estimated at about 1%. This could be the result of consistent increases in total sugars and respiration of root tissue. Important changes in starch properties were observed. Gel clarity decreased gradually during storage, with more pronounced changes occurring in starches from HMC-1. Swelling power decreased only in the case of AM 206-5. Gel viscosity increased in both genotypes. Improved tolerance to PPD could significantly reduce the economic impact of the short shelf life of ordinary cassava root processing. It remains to be seen, however, whether changes in stored roots positively or negatively affect the quality of the final product.
Key words:
amylose-free starch; post-harvest physiological deterioration; scopoletin; starch functional properties; starch loss;

Abbreviations:
Post-harvest physiological deterioration (PPD); dry matter content (DMC); pasting temperature (PT); peak viscosity (PV); hot paste viscosity (HPV); cool paste viscosity (CPV); final viscosity (FV); Differential scanning calorimeter (DSC); gelatinization enthalpy ($\Delta H$).
1. Introduction

Cassava contributes vitally to global food security and is likely to play an even more significant role in the near future (Rosenthal and Ort, 2012), as demand grows for cassava roots to produce starch, food, animal feed and ethanol (Balagopalan, 2002; Buitrago 1990; Chauynarong et al., 2009; Moorthy, 2004; Srooth et al., 2010) as well as to make bread (Pasqualone et al., 2010) and snacks (Vitrac et al., 2002).

Several factors affect the ability of cassava to satisfy new and increasing demands. Cassava is generally grown in marginal environments that are often far from processing centers and have poor roads. In addition, cassava roots are bulky, containing approximately 65% water. They also have a very short shelf life because of a process known as post-harvest physiological deterioration (PPD), which rapidly renders the roots unpalatable and unmarketable (Reilly et al., 2003; 2007; Wheatley, 1982; Wheatley and Gomez, 1985). Consequently, cassava roots need to be consumed soon after harvest (van Oirschot et al., 2000). The processes involved in PPD resemble changes typically associated with the plant’s response to wounding and trigger a cascade of biochemical reactions, in which reactive oxygen species are central. Specific genes involved in PPD have been identified and characterized, and their expression evaluated (Reilly et al., 2007). Several secondary metabolites, particularly hydroxycoumarins, accumulate in the process (Bayoumi et al., 2010; Blagbrough et al., 2010; Gnonlonfin et al., 2012).

Several approaches have been developed to preserve cassava roots, such as underground storage; storage in boxes with moist sawdust; storage in bags combined
with the use of fungicides; pruning plants before harvest; cold storage (2-4 °C) for up to
two weeks; freezing or waxing the roots to prevent access to oxygen; and even chemical
treatments (Ravi et al., 1996). However, these methods are too expensive or
complicated for handling large volumes of roots, and have been restricted mostly to
high-value product chains such as the consumption of fresh cassava roots.

Cassava is the second most important source of starch after maize, and cassava starch
is traded more in international markets than any other starch source (Stapleton, 2012).
New root quality traits that offer particular advantages for the starch industry are likely to
strengthen and widen the industrial applications of cassava in the near future (Rolland-
Sabaté et al., 2012; Sánchez et al., 2010). Genetic transformation is an important tool
for developing cassava cultivars with such traits (Liu et al., 2011; Koehorst-van Putten et
al., 2012; Zhao et al., 2011).

The recent report of genetic variation for tolerance to PPD (Morante et al., 2010) has
created a new opportunity for the starch sector. However, further analysis is required to
determine whether the properties of starch from roots not affected drastically by PPD
change during the storage period. Changes in the physicochemical and/or functional
properties of root and tuber starches in storage have been reported for potato (Ooraikul
and Moledina, 1981; Singh et al., 2008; Golachowski, 1985; Kaur et al., 2007), sweet
potato (Zhang et al., 2002) and yams (Akissoe et al., 2004; Aishat et al., 2007). Idowu
and Akindele (1994) reported qualitative changes in cassava gari and fufu after storage
of roots for up to four days. Ihedioha et al. (1996) and Akingbala et al. (1989) reported
that properties of stored cassava roots change long before PPD can actually be observed. However, with the exception of studies by Osunsami et al. (1989), little is known about changes in cassava starch functional properties occurring during root storage as a result of limitations imposed by PPD.

The short shelf life of cassava roots severely limits marketing options by increasing losses and overall marketing costs. Vlaar et al., (2007) estimated that the development of a cassava variety whose roots could be stored for up to 45 days would generate benefits valued at about US$35 million per year for Thai cassava farmers and factory owners.

The objective of this study was to monitor PPD, changes in the weight and biochemical properties of stored roots, and in the functional properties of the starches extracted from them. Roots from two contrasting genotypes (PPD tolerant or susceptible) were stored for up to 14 d. Such a study was not possible previously, because PPD prevented storage of roots beyond a few days after harvest.
2. Materials and methods.

Roots from two different cassava genotypes (AM206-5 and HMC-1) were harvested for this study. AM 206-5 has been reported to be tolerant to PPD (Morante et al., 2010), whereas HMC-1, a commercial variety grown in the mid-altitude valleys of Colombia, is susceptible. AM 206-5 is also the source of a spontaneous mutation for amylose-free (waxy) starch (Ceballos et al., 2007). Root samples were obtained from plants grown at the CIAT Experimental Farm in Palmira, Colombia, which is approximately 1000 meters above sea level and where cassava is harvested 11 months after planting. The two genotypes were grown under standard cultural practices, with fertilizer and irrigation provided as required.

Commercial-size roots were harvested and weighed individually. On harvest day, eight roots from each clone were processed for biochemical characterization. Starch and flour were extracted from them as explained below. Remaining roots were stored on shelves under a roof but without walls. Air, therefore, circulated freely through the shelves. During the experiment, the average maximum temperature (day) was 29.6 °C with a maximum value of 32.1 °C. The average minimum temperature (night) was 18.9 °C with a minimum value of 16.5 °C. Relative humidity was 94.7% at 7:00 AM; 61.3% at 1:00 PM and 76.7% at 7:00 PM. Every day four roots from each genotype were randomly selected, weighed again and scored for PPD. Roots were then processed for biochemical characterization and starch and flour extraction. The study continued through the day 14 of storage. Roots were not sampled, however, on days 5, 11 and 13.
2.1 PPD score

Scoring the reaction to PPD is a destructive process developed initially by Booth et al. (1976) and based on the storage of intact roots (also Booth 1976; 1977). A new method for quantifying PPD was described by Marriott et al. in 1978 and 1979 and later modified by Weathley in 1982. With this method, the proximal and distal ends of the root are removed to accelerate the process and avoid microbial contamination, which occurs during long storage periods. The distal open section of the root is covered with cling film to prevent further flow of oxygen. Roots are then stored for 3 d. To score for PPD reaction, seven transversal slices are cut along the root, starting at the proximal end. A score ranging from 1 to 10 is assigned to each slice, corresponding to the percentage of the cut surface showing discoloration (1=10%, 2=20%, etc). The mean PPD score for each root is calculated by averaging the scores for the seven transversal sections. Roots showing symptoms of microbial rotting (very different from those related to PPD) or affected by insects were discarded. In this study, roots were left intact following the methodology used by Booth, but PPD assessment was done on seven root slides as suggested by Wheatley in 1982. The results obtained resemble more closely the conditions of the roots in storage at a starch factory.

2.2 Root processing

After the roots were weighed and the PPD score taken, the seven root slices from each root were peeled and chopped into small pieces. The four roots from each treatment (genotype x duration of storage period) were randomly paired in two replications (with each replication made up of two roots combined). The two roots from each replication
were ground together using a food processor with stainless steel tools into a uniform mash (SKYMSEN Food Processor MODEL PA-7SE), from which sub-samples were taken for dry matter content measurement and flour production.

2.3 Dry matter content
Two independent samples ($5 \times 10^{-2}$ Kg aliquots) were taken from the homogenous paste of each replication for quantification of dry matter content (DMC) after measurement of PPD. For this purpose, the samples were dried in an oven (Thelco Oven Model 28, Precision Scientific Subsidiary of GCA Corporation. Chicago, USA) at 105°C for 24 h. Dry matter was expressed as the percentage of dry weight relative to fresh weight.

2.4 Flour production
Another sample (approximately 0.1 kg) was taken from the ground roots for flour production. Samples were dried for 2 d at 40°C and ground with a Glen Creston cross beater mill (Stanmore, England). There was a single flour sample per replication for each treatment (genotype x duration of storage period). Flour analyses were done twice.

2.5 Cassava starch isolation.
The homogeneous mash of root tissue from each replication (left after samples were taken for dry matter quantification and flour production) was further crushed in a 4 L capacity Waring Commercial blender (New Hartford, CT, USA). The slurry was filtered through a market grade 100 mesh ($0.149 \times 10^{-3}$ m) sieve. The starch was allowed to
settle and the supernatant decanted off. Solids were washed with distilled water twice
and centrifuged at 133.3 s$^{-1}$ for 600 s (Aristizábal and Sánchez, 2007). The sample was
then dried in an oven with fan-forced ventilation at 40 °C for 2 d (Thelco Oven Model 28,
Precision Scientific Subsidiary of GCA Corporation. Chicago, USA). All cassava
starches were obtained following the same extraction protocol. As in the case of flours,
there was a single starch sample (from two roots) for each of the two replications for
each treatment (genotype x duration of storage period). Quantities of starch used in the
different tests described below are on a dry weight basis.

2.6 HPLC Soluble sugar and organic acid determination

Analyses on sugars and organic acids were made on flour as the standard approach
used for this type of non-volatile compounds (Holloway et al., 1989). Every
measurement was made on a dry weight basis. Analyses from flours offer the
advantage that conditions for HPLC quantification were the same for each of these
compounds. As mentioned above, two determinations (aliquots) were made for each
flour sample; 7 $10^{-4}$ Kg were eluted with $10^{-2}$ L of $5\times10^{-3}$ mol L$^{-1}$ sulphuric acid (mobile
phase). The solution was homogenized in a vortex and then mixed slowly for 1800 s at
25°C. The suspension was centrifuged for 600 s at 25 °C, at 3000 $10^{-3}$ Kg. The
supernatant was filtered through a 0.22 $10^{-6}$ m disposable syringe membrane filter.
Sugars and organic acids were analyzed by HPLC using a column Biorad, Aminex HPX
87H, equipped with a UV detector (MWD G 1365D for organic acids) set to 210 $10^{-12}$ m
and connected in series with a refractive index detector (RID G 1362A for sugars) and
an injection valve fitted with a 15 $10^{-6}$ L loop. The samples were separated isocratically
at $10^5$ L s$^{-1}$, at 30 °C. Retention times and standard curves were prepared for the following sugars – glucose (SIGMA-ALDRICH G7528), fructose (SIGMA-ALDRICH F2543) and saccharose (SIGMA-ALDRICH (≥99.5%) S7903) – and for the following organic acids – citric (SIGMA-ALDRICH CO759), malic (SIGMA-ALDRICH (99+% 240179), succinic (SIGMA-ALDRICH (≥ 99%) S3674) and fumaric (SUPEL-Co Analytical R412205).

2.7 HPLC determination of scopoletin

The methodology described by Buschmann and co-workers (2000) was used with slight modification in the solvent gradient profile in the HPLC column. One gram of the homogenous mash obtained with the food processor (as described above) was placed in 5 $10^{-2}$ L falcon tubes; $10^{-2}$ L 98% ethanol (J.T. Baker, 9000-03, USA) were added and homogenized with an ultraturrax for 30 s. Extract was filtered first with Whatman # 1 paper and then through a 0.22 $10^{-6}$ m membrane and finally placed in glass tubes and evaporated (NEvap 112, Organomation Associates, Berlin, MA, USA) through a nitrogen flux at 80 °C. Dried samples were next dissolved in 250 µL 98% ethanol and vortex agitated for 60 s at 83.3 s$^{-1}$. Samples were transferred to 1.5 $10^{-3}$ L vials for HPLC (Agilent Technologies 1200 series, Waldbronn, Germany) quantification.

To isolate and quantify scopoletin, an inverse phase column (Techsphere BDS C$_{18}$, 250 x 4.6 $10^{-3}$ m, 5 $10^{-6}$ m, HPLC Technology, UK) was used for an HPLC system (Agilent Technologies 1200 series, Waldbronn, Germany) with a diode array detector (No. DE64257792). The column was kept at 25 °C. Acetonitrile and 0.5% phosphoric acid in aqueous solution gradients were used. The gradient profile was 60-1% for 1800 s with a
78 $10^{-3}$ L s$^{-1}$ flow and a 50 µL injection volume. Scopoletin was simultaneously detected at 215, 280 and 350 $10^{-12}$ m, for which purpose time of retention and the standard spectral analysis of pure scopoletin (Sigma-Aldrich: Scopoletin ≥ 99%— No. S2500) were used. Scopoletin quantification was determined through a calibration standard curve. The correlation coefficient was 0.9993 for six concentration levels (1, 5, 10, 25, 50 and $75 \times 10^{-3}$ g L$^{-1}$) and three replications. Quantifications were made on a fresh weight basis.

2.8 Paste clarity.

The methodology suggested by Craig et al. (1989) was used for this purpose. A 1% aqueous dispersion of starch was boiled at 97 °C (1000m above sea level) and shaken thoroughly every 30 s for 1800 s. Transmittance was measured after cooling to room temperature at 650 $10^{-12}$ m. Two different quantifications per starch sample were made, and mean values were then calculated. Two independent analyses (aliquots) were made for each starch sample. Therefore, four clarity measurements (two aliquots x two replications) were available for roots from a given clone with a given storage period.

2.9 Swelling power, solubility and dispersed volume fraction measurements.

Swelling power (SW) and solubility patterns (SO) (Mestres et al., 1997) were determined using 1% (w/w) starch dispersions ($0.28 \times 10^{-3}$ Kg dispersed in $27.72 \times 10^{-3}$ Kg of distilled water) at 60, 75 and 90 °C. Every measurement was made and reported on a dry weight basis. Two independent analyses (aliquots) were made for each starch sample. The low concentration used in the study was chosen to obtain optimal separation between the pellet and supernatant phases after centrifugation. Paste was
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prepared in RVA starting at 35 °C for 60 s, with temperature increasing at a rate of 0.1 °C s⁻¹. Three different and independent analyses were made with final temperatures held at 60, 75 or 90 °C for 150 s. Stirring was maintained at 16 s⁻¹ for the first minute and then at 2.67 s⁻¹ during the remainder of the analysis. The paste was immediately transferred to a 5 × 10⁻² L centrifuge tube. The supernatant and sediment, after centrifugation for 600 s at 6000 g at 25 °C, were collected and weighed (Wsu and Wse, respectively), then dried at 100 °C for 86 400 s and 172 800 s, respectively, and weighed (Dsu and Dse, respectively). The values thus obtained were used to calculate three parameters: concentration of soluble material in the supernatant (solubility) and the swelling power and volume fraction of the dispersed phase (Φ), as follows:

\[
\text{Solubility (\%) } = 100 \times \frac{\text{Dsu}}{0.28}
\]

\[
\text{Swelling Power (Kg_{water}/Kg_{Starch}) } = \frac{\text{Wse}}{\text{Dse}}
\]

\[
(\Phi) = \frac{(27.91 - (\text{Wsu} - \text{Dsu}))}{27.91}
\]

Factor 27.91 is calculated as total volume (10⁻³ L) of the paste.

Starch specific density is 1.5 Kg L⁻¹

\[
27.91 = 27.72 + \left(\frac{0.28}{1.5}\right) 10⁻³ L
\]

2.10 Pasting properties.

Hot starch dispersion viscosity profiles were obtained with a Rapid Visco Analyzer model RVA-4 Series (Newport Scientific, Australia). Starch (1.25 10⁻³ Kg) was dispersed in distilled water (near 23 10⁻³ L) to 5% suspension. Starch concentration is critical for RVA results. The concentration used was adequate for comparing different starches,
falling within the range of concentrations frequently reported in the literature. Viscosity was recorded using the temperature profile: holding at 50 °C for 60s, heating from 50 °C to 90 °C at 0.1 °C s⁻¹, holding at 90 °C for 300 s, and then cooling down to 50 °C at 0.1 °C s⁻¹. The gel was then maintained for 120 s at 50 °C with continuous stirring at 2.67 s⁻¹. Five parameters were measured: pasting temperature (PT), peak viscosity (PV), hot paste viscosity at the end of the plateau at 90 °C (HPV), cool paste viscosity (CPV) at 50 °C (1160 s analysis), and final viscosity (FV). Three other parameters calculated were: breakdown (BD), estimated as PV-HPV; setback (SB), estimated as CPV-PV; and consistency (CS), estimated as CPV-HPV. One RVA analysis per starch replication was made (no aliquots available).

2.11 Differential scanning calorimeter measurements (DSC)

Starch samples (≈ 4 10⁻⁶ Kg) with ~12 10⁻⁶ L distilled water were hermetically sealed in stainless-steel DSC pans and kept at room temperature for 2–3 hours. Next, samples were scanned against a blank (empty pan), using a Perkin Elmer Pyris 6 DSC (Perkin-Elmer Co., Norwalk, CT) from 15 to 120 °C at a scanning rate of 0.167 °C s⁻¹. Each DSC endotherm was characterized by the onset temperature (T₀) and gelatinization enthalpy (ΔH). Data were obtained only for 0, 1, 2, 3, 7 and 14 d after harvest. One measurement per starch replication was made.

2.12 Data analysis.

Most of the analysis was based on regressions of different variables on storage time. Proc Reg from SAS (SAS, 2008) was used for the analysis.
3. Results

3.1 Changes in root quality.

Table 1 presents the regression coefficients and their respective standard errors for different variables during the storage period. Where regression coefficients for the PPD-tolerant and -susceptible clones were significantly different, regression analysis was done independently for each clone, and individual regression coefficients are presented. Where regression coefficients were not significantly different, a combined analysis with data from the two clones was made, and a single coefficient is presented.

As reported many years ago (Camargo Pacheco de, 1954; Rickard and Coursey, 1981; George and Browne, 1994), roots in this study lost weight gradually during the entire storage period. Weight loss and changes in dry matter content in stored cassava roots depend on storage conditions (Akingbala et al., 2005; Booth, 1977; Booth et al., 1976; Taye, 2000). Weight loss is presented in this study as a percentage of the original weight of individual roots (Figure 1a, Table 1). At the start of the experiment, roots from HMC-1 were heavier, on average, than those of AM 206-5 (0.460 versus 0.426 Kg, respectively). Roots from AM 206-5 had lost 23.4 \(10^{-3}\) Kg by the end of the experiment, whereas those from HMC-1 had lost 31.1 \(10^{-3}\) Kg. On a percentage basis, however, the differences tended to be lower.

One interesting and somewhat surprising finding is that dry matter content in the roots remained more or less constant throughout the entire storage period (data not presented). Therefore, weight loss cannot be explained only by water loss, as this would
have resulted in a gradual increase in DMC. These results are consistent with those reported in the literature (Booth, 1977; Booth et al., 1976; Osunsami et al., 1989), indicating that dry matter content remains more or less constant during storage periods of different lengths; the proportion of starch extracted from fresh roots is considerably higher than in roots stored for several days; and there is a sharp increase in the proportion of simple sugars. Ihedioha and co-workers reported in 1995, however, that dry matter content increased from about 30% on the day of harvest to 35% after 4 d of storage (average for four clones).

Total sugar content increased consistently throughout the storage period (Figure 1b, Table 1) as reported in the literature (Akingbala et al., 2005; Booth et al., 1976; Lin et al., 2011; Osunsami et al., 1989). Total sugars increased at a faster rate for HMC-1 than for AM 206-5, but the latter began with higher levels of sugars. In addition, total sugar content in the roots from AM 206-5 oscillated more widely than those for HMC-1. Total sugars in AM 206-5 remained more or less constant through the day 4 (= 0.9 g kg$^{-1}$) of storage, then abruptly rose to 1.4 g kg$^{-1}$ and remained at that level. Increases in HMC-1 were gradual and more consistent over time (Figure 1b). Glucose and fructose increases were consistent and similar in both genotypes (Table 1). From day 0 to 14, glucose increased from 0.063 to 0.404 g kg$^{-1}$, while fructose increased from 0.091 to 0.429 g kg$^{-1}$, based on average values across the two genotypes. Saccharose, on the other hand, did not change significantly in AM 206-5 (from 0.620 to 0.554 g kg$^{-1}$), whereas for HMC-1 it showed a statistically significant increase from 0.368 to 0.421 g.
kg\textsuperscript{-1}. Early reports indicated that saccharose tends to decline, while fructose and glucose increase, although changes vary according to storage conditions (Booth \textit{et al.}, 1976).

Estimations of extractable starch loss during storage are provided in Figure 1c and Table 1. Responses for the two clones differed significantly, with higher losses for HMC-1 than AM 206-5. In general, each day of storage resulted in a starch reduction of slightly less than 1%. Therefore, by the end of the experiment, roots had lost around 12% of starch. These losses were estimated indirectly, and the methodology involved some assumptions. Dry matter content in both clones remained more or less constant during storage despite clear root weight losses. It was assumed that weight loss as water through transpiration must be similar to loss as CO\textsubscript{2} through respiration (Ravi and Aked, 1996). For each clone, therefore, starch loss as CO\textsubscript{2} was estimated to be half of its total weight loss (CO\textsubscript{2} loss). However, other starch losses, such as the gradual hydrolysis of starch into simple sugars, are not reflected in weight loss. As indicated in Figure 1b, soluble sugars increased constantly with storage time. These sugars would be lost in the process of starch extraction. Since 1 Kg of starch produces 1.1 Kg of sugars, total sugars/1.1 would be an appropriate estimation of starch loss into sugars (sugar loss). The starch losses for each clone, presented in Figure 1c and Table 1, were obtained for each day of storage by adding the CO\textsubscript{2} loss and sugar loss.

Organic acids, many of them related to the Krebs cycle, reflect metabolic activities within the root. Citric acid was reduced over time in both clones, while the remaining acids tended to increase (Table 1). Citric acid in AM 206-5 dropped from 0.088 to 0.043
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In general, roots from HMC-1, accumulated significant amounts of malic, succinic and fumaric acids, while roots from AM 206-5 showed positive but not significant changes (except with malic acid, which increased but with a $P < 0.05$). The higher accumulation of organic acids in roots from HMC-1 may reflect a more active metabolism, which would explain its higher rate of root weight and starch loss and higher rate of sugar production in comparison with roots from AM 206-5.

As expected, there were large differences between the two genotypes in the PPD levels of their roots (Figure 2a, Table 1). Average PPD for AM 206-5 was 4%, compared to 20% for HMC-1. The possibility of storing roots for 14 d without much PPD prompted this study to analyze biochemical changes in the roots and the starches extracted from them. The consistent PPD tolerance of AM206-5 was confounded in this study by the lower dry matter content of its roots (37%), compared with those of the susceptible check HMC-1 (48%). This difference in dry matter content is not surprising, as HMC-1 is a commercial variety, whereas AM 206-5 is a partially inbred clone selected only because of its amylose-free starch. However, this study found a larger difference in DMC than did previous studies involving AM 206-5 (Morante et al., 2010, Ceballos et al., 2012). PPD tends to be lower in roots with low dry matter content (Sánchez et al., 2006).

The two clones evaluated showed large differences in scopoletin production during storage (Figure 2b). Data presented in this figure are the averages of two independent quantifications of scopoletin in different root samples from each of the two genotypes. In
In this study, the susceptible clone reached a maximum scopoletin concentration (28 µmol kg\(^{-1}\)) on the 3\(^{rd}\) day, and levels remained relatively high for a couple of days (Figure 2b). Roots from AM 206-5, on the other hand, reached a scopoletin peak on the 4th day (17 µmol kg\(^{-1}\)) and then decreased sharply thereafter. As expected, roots from the PPD-tolerant clone showed a drastically lower and delayed accumulation of scopoletin compared with the susceptible clone. Scopoletin contents after a week of storage were negligible with a small peak, as also reported by Buschmann and co-workers. However, PPD continued to increase over time (Figure 2a).

### 3.2 Changes in starch properties.

The clarity of gels was drastically reduced as the starch used to make them was extracted from roots stored for longer periods (Figure 3a, Table 1). The tendency was more pronounced with HMC-1 than AM 206-5. This is the first report of such a change in cassava starch during root storage. A similar phenomenon was observed in potato but only after six months of tuber storage (Singh et al., 2008) and 7 d of gel storage. These authors suggested that the changes could be attributed to decreases in granule size and phosphorus content, which ultimately increase the concentration of granule remnants, resulting in lower light transmittance. In addition, enzymatic hydrolysis of starch granules, can be expected to modify the granule architecture (i.e., amorphous versus crystalline, amylose/amylopectin ratio and chain length) and hence also contribute to the physico-chemical properties of starch.
The evolution of solubility and swelling index in starch extracted from roots stored for different periods is presented in Figures 3b and 3c. Whereas the solubility of starch from AM206-5 increased over time (though with wide oscillation around the regression line), it tended to decline for HMC-1. In both cases regression coefficients were statistically significant at $P < 0.01$ (Table 1). Swelling power did not change much in starches from HMC-1 but decreased over time in starches from AM206-5 (Figure 3c, Table 1). The volume fraction of the dispersed phase ($\Phi$) followed trends similar to those for the swelling index, with values for AM206-5 declining significantly over time, while remaining more or less constant for starches from HMC-1 (Figure 3d, Table 1).

Results for different parameters measured with the RVA are summarized in Figures 4 and 5 and Table 1. Figures 4a and 4b present key amylograms (starch from roots stored for 0, 7 and 14 d) to illustrate general trends for the two clones. PT did not change over time (Table 1), but the values were considerably higher for AM206-5 (65-66 °C) than for HMC-1 (63-64 °C). AM206-5 starch develops higher PV than non-waxy starches (Figure 5a). These findings agree with previous reports (Ceballos et al., 2007; Sánchez et al., 2010). In addition, PV in waxy starch from AM206-5 tended to increase throughout root storage (about 0.15 Pa s in 14 d). On the other hand, PV for starch from HMC-1 remained approximately unchanged. The shapes of the amylograms were very contrasting with an apparent shoulder before starches from HMC-1 reach their PV (Figure 4b). As roots from HMC-1 were stored for longer periods their starch tended to show a delayed PV (around 80 and 90 °C for days 0 and 14 of storage, respectively),
suggesting higher resistance to shearing and temperature. FV tended to be higher in starch extracted during long storage periods (Figure 5b).

HPV was lower in AM 206-5 than in HMC-1 (Figure 5c), and both clones responded similarly regarding the length of the storage period (Table 1). CPVs were considerably higher in HMC-1 (≈ 0.90 Pa s) than in AM 206-5 (≈ 0.60 Pa s) and reacted similarly in both genotypes in terms of their response to the duration of the storage period (Figure 5d, Table 1). BD, therefore, was considerably larger in AM 206-5 than HMC-1, remaining more or less constant in the former, while decreasing considerably over longer storage periods in the latter (Table 1). SB showed the opposite trend with negative values for AM 206-5 and positive ones for HMC-1 (Table 1). Finally, consistency showed a similar negative trend over storage time in both genotypes (Table 1).

Onset temperatures did not change much in relation to duration of the storage period with AM 206-5 showing higher values (around 66.0 °C) than HMC-1 (63.5 °C). ΔH was higher and relatively stable for starches from AM 206-5, while for HMC-1 values tended to decrease over time (Table 1).

4. Discussion (PPD, Starch losses, starch properties)

Cassava roots obviously undergo considerable changes during storage. The two genotypes included in this study showed similarity in some of these changes, whereas in others drastic differences indicated the influence of genetic variation. Particularly
relevant was the rate of progress for PPD, with final values around 30% for roots from HMC-1 and only 10% for those from AM 206-5. The limited occurrence of vascular streaking in the roots from AM 206-5 makes it possible to evaluate alternatives for their post-harvest handling. For example, would it be advisable to store roots for few days when they are to be used for the production of sweeteners? There are certainly losses in the total amount of energy stored in the roots, which are responsible in part for the weight and starch losses illustrated in Figure 1c.

Many research groups have sought for a long time to develop PPD-tolerant cassava. This is clearly a desirable aim, which would offer considerable advantages for certain value chains. However, the loss of starch in cassava roots over time diminishes the relevance of PPD tolerance, at least for the starch industry. Gradual changes in the functional properties of starch extracted from stored roots are also a matter of concern, though there may be alternatives for starch modification in planta.

As previous reports indicate (Buschmann et al., 2000; Tanaka et al., 1983), PPD is associated with scopoletin concentration. This study revealed clear differences in scopoletin concentration between a susceptible and tolerant clone as well as a delay in reaching maximum values. Buschmann et al. (2000) sliced the roots to further accelerate PPD and observed maximum concentrations of scopoletin after 2 d (up to 65 µmol kg⁻¹) in roots from the most PPD-susceptible clone (MCOL 22). PPD-tolerant clones, on the other hand, showed a maximum peak considerably later (after 6 d). In this study, PPD and scopoletin accumulation was delayed, in comparison with the
findings of the earlier report, because roots were not sliced and AM 206-5 is clearly tolerant to PPD. Scopoletin should be quantified 2 d after harvest if roots are sliced or after 4 d of storage if the roots are kept intact.

The reduced levels of PPD in roots from AM 206-5 may be related to the higher sugar levels found in the roots of this clone from day 0. Van Oirschot et al. (2000) found that pruning cassava plants two weeks before harvest can increase shelf life. In that study, pruning resulted in the hydrolysis of starch into simpler sugars, showing a relationship between higher sugar levels and tolerance to PPD.

The quick changes observed in starch physical and functional properties can have a serious impact on the final quality of starch when it is produced on an industrial scale. The only reports on gel clarity changes related to storage of roots or tubers deal with potatoes stored for 4-6 months (Singh et al., 2007). In the present study, changes in gel clarity were considerably more pronounced; gel clarity was reduced by 10% in AM 206-5 and 25% in HMC-1 after two weeks of root storage. Since one advantage of cassava and potato starches is the outstanding clarity of their gels, the changes reported here would affect the final quality of cassava starch, particularly in cases such as that of HMC-1.

As illustrated in Figure 5b, final gel viscosity increased significantly along with the duration of the storage period of the roots, even though the swelling power for AM 206-5...
decreased while that for HMC-1 remained more or less stable (Figure 3c), which is unusual for amylose-free starch.

Morante et al. (2010) reported different sources of tolerance to PPD. The present article concentrates on the source of PPD tolerance that was found in the amylose-free starch mutation, a genotype of particular interest for the starch industry. The research therefore concentrated on PPD, scopoletin, starch functional properties and starch losses. Similar studies could focus on other sources of PPD tolerance, particularly those related to high carotenoids, with the aim of determining the fate of carotenoid content and cooking quality during storage, along with PPD and scopoletin content. Such studies have already begun, but since key germplasm must first be multiplied to produce the number of roots required, it will take another two years to obtain results.

5. Conclusions

This study provides further evidence of PPD tolerance in AM 206-5 as well as insight into the implications of deploying PPD-tolerant cassava varieties. Changes in starch functional properties during storage may offer an innovative way to induce starch modification in planta, but this is possible only for PPD-tolerant cassava germplasm. Realizing the potential of this strategy will require considerable additional research and fine-tuning by the starch industry, once commercial cultivars with PPD tolerance are released.
Unfortunately for the starch industry the gradual loss of starch (at a rate of about 1% d⁻¹) limits the extent to which roots from a PPD-tolerant clone can be stored, despite their lack of vascular streaking. Thus, while PPD tolerance can reduce losses in transport by extending shelf life for few days, the starch losses become too high after a week. This finding is very relevant to important ongoing efforts to develop PPD-tolerant germplasm, including through genetic transformation.

For ethnic uses of cassava roots, such as the preparation of gari, fufu and farinha, PPD-tolerant clones could be advantageous, at least in terms of appearance, since the products would not present the grayish blue color that is typical of products made from deteriorated roots. In addition, such clones would give the final product a somewhat sweeter taste. Finally, the digestibility of roots stored for a few days could increase, creating new alternatives for the animal industry.

The availability of PPD tolerance makes possible further studies on metabolic changes during root storage. In this study, metabolism in roots of the PPD-tolerant genotype seemed slower than in the susceptible one. Respiration is likely different in the two types of roots.

Acknowledgments
Editorial corrections by Nathan Russell greatly improved the quality of the manuscript by enhanced clarity and readability. The sensible and useful comments made by the two reviewers and the guidance by the Editor of the journal are also acknowledged and appreciated.
References


Golachowski, A. 1985. Properties of starch obtained from potato tubers influenced by various temperatures. Starch/Stärke 37, 263–266.


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with higher carotenoid content. Journal of the Science of Food and Agriculture 86, 634–639.


Table 1. Results of linear regression analyses made for different starch or root quality traits on duration of storage periods for two cassava genotypes. When the regressions in the two clones were similar, results from a combined analysis is presented and standard error values are presented within parenthesis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AM 206-5</th>
<th></th>
<th>HMC-1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>St. Error</td>
<td>Coefficient</td>
<td>St. Error</td>
</tr>
<tr>
<td>ROOTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight loss (%)</td>
<td>0.525</td>
<td>0.054**</td>
<td>0.684</td>
<td>0.049**</td>
</tr>
<tr>
<td>Starch loss (%)</td>
<td>0.712</td>
<td>0.083</td>
<td>1.010</td>
<td>0.052</td>
</tr>
<tr>
<td>PPD (%)</td>
<td>0.700</td>
<td>0.124**</td>
<td>2.422</td>
<td>0.246**</td>
</tr>
<tr>
<td>FLOUR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccharose (g kg⁻¹)</td>
<td>0.011</td>
<td>0.007</td>
<td>0.022</td>
<td>0.005**</td>
</tr>
<tr>
<td>Glucose (g kg⁻¹)</td>
<td></td>
<td></td>
<td>0.021 (0.002**)</td>
<td></td>
</tr>
<tr>
<td>Fructose (g kg⁻¹)</td>
<td></td>
<td></td>
<td>0.021 (0.003**)</td>
<td></td>
</tr>
<tr>
<td>Total sugars (g kg⁻¹)</td>
<td>0.049</td>
<td>0.007**</td>
<td>0.068</td>
<td>0.003**</td>
</tr>
<tr>
<td>Citric acid (g kg⁻¹)</td>
<td>-0.003</td>
<td>0.001**</td>
<td>-0.004</td>
<td>0.001**</td>
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<tr>
<td>Malic acid (g kg⁻¹)</td>
<td>0.003</td>
<td>0.001*</td>
<td>0.006</td>
<td>0.001**</td>
</tr>
<tr>
<td>Succinic acid (g kg⁻¹)</td>
<td>0.000</td>
<td>0.001</td>
<td>0.002</td>
<td>0.001**</td>
</tr>
<tr>
<td>Fumaric acid (g kg⁻¹)</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.000</td>
<td>0.000**</td>
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<tr>
<td>STARCH</td>
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<tr>
<td>Gel Clarity (%)</td>
<td>-0.442</td>
<td>0.066**</td>
<td>-1.070</td>
<td>0.100**</td>
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<tr>
<td>Solubility (%)</td>
<td>0.310</td>
<td>0.066**</td>
<td>-0.140</td>
<td>0.031**</td>
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<tr>
<td>Swelling power (kg kg⁻¹)</td>
<td>-0.011</td>
<td>0.001**</td>
<td>-0.001</td>
<td>0.001</td>
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<tr>
<td>Fraction volume (φ)</td>
<td>-0.008</td>
<td>0.002**</td>
<td>0.001</td>
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<tr>
<td>Pasting temperature (°C)</td>
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<td>-0.012 (0.037)</td>
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<td></td>
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<tr>
<td>Maximum viscosity (Pa s)</td>
<td>0.011</td>
<td>0.003**</td>
<td>-0.004</td>
<td>0.003</td>
</tr>
<tr>
<td>Final viscosity (Pa s)</td>
<td>0.004</td>
<td>0.002*</td>
<td>0.008</td>
<td>0.002**</td>
</tr>
<tr>
<td>Hot paste viscosity (Pa s)</td>
<td></td>
<td>0.009 (0.001**)</td>
<td></td>
<td></td>
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<tr>
<td>Cool paste viscosity (Pa s)</td>
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<td>0.005 (0.004)</td>
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<td></td>
</tr>
<tr>
<td>Breakdown (Pa s)</td>
<td>0.003</td>
<td>0.003</td>
<td>-0.014</td>
<td>0.002**</td>
</tr>
<tr>
<td>Setback (Pa s)</td>
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<td>0.003*</td>
<td>0.010</td>
<td>0.002**</td>
</tr>
<tr>
<td>Consistency (Pa s)</td>
<td></td>
<td>-0.004 (0.003)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset temperature (°C)</td>
<td></td>
<td>-0.008 (0.060)</td>
<td></td>
<td></td>
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<tr>
<td>Gelatinization enthalpy (kJ kg⁻¹)</td>
<td>0.011</td>
<td>0.033</td>
<td>-0.051</td>
<td>0.050</td>
</tr>
</tbody>
</table>

* Significant at 5%; ** Significant at 1%
Figure 1. Changes in cassava roots from two clones after storage under ambience conditions through 14 days. 1a. Weight loss expressed as percentage of the original root weight; 1b. Evolution of total sugars content (g kg$^{-1}$); and 1c. Estimated losses of starch (%).
Figure 2. Changes in cassava roots from two clones after storage under ambience conditions through 14 days. 2a. Postharvest physiological deterioration (PPD) score (%); and 2b Scopoletin contents (µmol kg⁻¹).
Figure 3. Starch physicochemical properties in starches extract from cassava roots stored through 14 days. 3a. Gel clarity (%); 3b. Solubility (%); 3c. Swelling index (Kg Kg$^{-1}$); and 3d. Volume fraction of the dispersed phase (Φ).
Figure 4. RVA amylograms from starches extracted from cassava roots stored for 0, 7, and 14 days (Pa s). 4a. Amylograms from AM 206-5 starches; and 4b. Amylograms from HMC-1 starches.

Changes in extended shelf life cassava roots during storage at ambient conditions.
Figure 5. Selected RVA parameters in starches extracted from cassava roots stored through 14 days. 5a. Maximum viscosity (Pa s); 5b. Final viscosity (Pa s); 5c. Hot paste viscosity (Pa s); 5d. Cool paste viscosity.
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