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 (Δ*H)*.

70 **1. Introduction**

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

76

77 Several factors affect the ability of cassava to satisfy new and increasing demands.
78 Cassava is generally grown in marginal environments that are often far from processing
79 centers and have poor roads. In addition, cassava roots are bulky, containing
80 approximately 65% water. They also have a very short shelf life because of a process
81 known as post-harvest physiological deterioration (PPD), which rapidly renders the roots
82 unpalatable and unmarketable (Reilly *et al*., 2003; 2007; Wheatley, 1982; Wheatley and
83 Gomez, 1985). Consequently, cassava roots need to be consumed soon after harvest
84 (van Oirschot *et al*., 2000). The processes involved in PPD resemble changes typically
85 associated with the plant's response to wounding and trigger a cascade of biochemical
86 reactions, in which reactive oxygen species are central. Specific genes involved in PPD
87 have been identified and characterized, and their expression evaluated (Reilly *et al*.,
88 2007). Several secondary metabolites, particularly hydroxycoumarins, accumulate in the
89 process (Bayoumi *et al*., 2010; Blagbrough *et al*., 2010; Gnonlonfin *et al*., 2012).

90

91 Several approaches have been developed to preserve cassava roots, such as
92 underground storage; storage in boxes with moist sawdust; storage in bags combined

93 with the use of fungicides; pruning plants before harvest; cold storage (2-4 ℃) for up to
94 two weeks; freezing or waxing the roots to prevent access to oxygen; and even chemical
95 treatments (Ravi *et al.*, 1996). However, these methods are too expensive or
96 complicated for handling large volumes of roots, and have been restricted mostly to
97 high-value product chains such as the consumption of fresh cassava roots.
98
99 Cassava is the second most important source of starch after maize, and cassava starch
100 is traded more in international markets than any other starch source (Stapleton, 2012).
101 New root quality traits that offer particular advantages for the starch industry are likely to
102 strengthen and widen the industrial applications of cassava in the near future (Rolland-
103 Sabaté *et al.*, 2012; Sánchez *et al.*, 2010). Genetic transformation is an important tool
104 for developing cassava cultivars with such traits (Liu *et al.*, 2011; Koehorst-van Putten *et*
105 *al.*, 2012; Zhao *et al.*, 2011).
106
107 The recent report of genetic variation for tolerance to PPD (Morante *et al.*, 2010) has
108 created a new opportunity for the starch sector. However, further analysis is required to
109 determine whether the properties of starch from roots not affected drastically by PPD
110 change during the storage period. Changes in the physicochemical and/or functional
111 properties of root and tuber starches in storage have been reported for potato (Ooraikul
112 and Moledina, 1981; Singh *et al.*, 2008; Golachowski, 1985; Kaur *et al.*, 2007), sweet
113 potato (Zhang *et al.*, 2002) and yams (Akissoe *et al.*, 2004; Aishat *et al.*, 2007). Idowu
114 and Akindele (1994) reported qualitative changes in cassava gari and fufu after storage
115 of roots for up to four days. Ihedioha *et al.* (1996) and Akingbala *et al.* (1989) reported

116 that properties of stored cassava roots change long before PPD can actually be
117 observed. However, with the exception of studies by Osunsami *et al.* (1989), little is
118 known about changes in cassava starch functional properties occurring during root
119 storage as a result of limitations imposed by PPD.
120
121 The short shelf life of cassava roots severely limits marketing options by increasing
122 losses and overall marketing costs. Vlaar *et al*., (2007) estimated that the development
123 of a cassava variety whose roots could be stored for up to 45 days would generate
124 benefits valued at about US$35 million per year for Thai cassava farmers and factory
125 owners.
126
127 The objective of this study was to monitor PPD, changes in the weight and biochemical
128 properties of stored roots, and in the functional properties of the starches extracted from
129 them. Roots from two contrasting genotypes (PPD tolerant or susceptible) were stored
130 for up to 14 d. Such a study was not possible previously, because PPD prevented
131 storage of roots beyond a few days after harvest.
132

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 ℃ with a maximum value of 32.1 ℃. The average minimum temperature (night) was 18.9 ℃ with a minimum value of 16.5 ℃. 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.

156 ***2.1 PPD score***

157 Scoring the reaction to PPD is a destructive process developed initially by Booth *et al.*
158 (1976) and based on the storage of intact roots (also Booth 1976; 1977). A new method
159 for quantifying PPD was described by Marriott *et al.* in 1978 and 1979 and later modified
160 by Weathley in 1982. With this method, the proximal and distal ends of the root are
161 removed to accelerate the process and avoid microbial contamination, which occurs
162 during long storage periods. The distal open section of the root is covered with cling film
163 to prevent further flow of oxygen. Roots are then stored for 3 d. To score for PPD
164 reaction, seven transversal slices are cut along the root, starting at the proximal end. A
165 score ranging from 1 to 10 is assigned to each slice, corresponding to the percentage of
166 the cut surface showing discoloration (1=10%, 2=20%, etc). The mean PPD score for
167 each root is calculated by averaging the scores for the seven transversal sections.
168 Roots showing symptoms of microbial rotting (very different from those related to PPD)
169 or affected by insects were discarded. In this study, roots were left intact following the
170 methodology used by Booth, but PPD assessment was done on seven root slides as
171 suggested by Wheatley in 1982. The results obtained resemble more closely the
172 conditions of the roots in storage at a starch factory.

173

174 ***2.2 Root processing***

175 After the roots were weighed and the PPD score taken, the seven root slices from each
176 root were peeled and chopped into small pieces. The four roots from each treatment
177 (genotype x duration of storage period) were randomly paired in two replications (with
178 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 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℃ 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℃ 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 10^{-3} m) sieve. The starch was allowed to

202 settle and the supernatant decanted off. Solids were washed with distilled water twice
203 and centrifuged at 133.3 s^{-1} for 600 s (Aristizábal and Sánchez, 2007). The sample was
204 then dried in an oven with fan-forced ventilation at 40 °C for 2 d (Thelco Oven Model 28,
205 Precision Scientific Subsidiary of GCA Corporation. Chicago, USA). All cassava
206 starches were obtained following the same extraction protocol. As in the case of flours,
207 there was a single starch sample (from two roots) for each of the two replications for
208 each treatment (genotype x duration of storage period). Quantities of starch used in the
209 different tests described below are on a dry weight basis.
210
211 ***2.6 HPLC Soluble sugar and organic acid determination***
212 Analyses on sugars and organic acids were made on flour as the standard approach
213 used for this type of non-volatile compounds (Holloway *et al.*, 1989). Every
214 measurement was made on a dry weight basis. Analyses from flours offer the
215 advantage that conditions for HPLC quantification were the same for each of these
216 compounds. As mentioned above, two determinations (aliquots) were made for each
217 flour sample; 7 10^{-4} Kg were eluted with 10^{-2} L of 5 10^{-3} mol L^{-1} sulphuric acid (mobile
218 phase). The solution was homogenized in a vortex and then mixed slowly for 1800 s at
219 25°C. The suspension was centrifuged for 600 s at 25 °C, at 3000 10^{-3} Kg The
220 supernatant was filtered through a 0.22 10^{-6} m disposable syringe membrane filter.
221 Sugars and organic acids were analyzed by HPLC using a column Biorad, Aminex HPX
222 87H, equipped with a UV detector (MWD G 1365D for organic acids) set to 210 10^{-12} m
223 and connected in series with a refractive index detector (RID G 1362A for sugars) and
224 an injection valve fitted with a 15 10^{-6} L loop. The samples were separated isocratically

225 at 10^{-5} L s^{-1}, at 30 ℃. Retention times and standard curves were prepared for the
226 following sugars – glucose (SIGMA-ALDRICH G7528), fructose (SIGMA-ALDRICH
227 F2543) and saccharose (SIGMA-ALDRICH (≥99.5%) S7903) – and for the following
228 organic acids – citric (SIGMA-ALDRICH CO759), malic (SIGMA-ALDRICH (99+%)
229 240179), succinic (SIGMA-ALDRICH (≥ 99%) S3674) and fumaric (SUPEL-Co
230 Analytical R412205).
231
232 ***2.7 HPLC determination of scopoletin***
233 The methodology described by Buschmann and co-workers (2000) was used with slight
234 modification in the solvent gradient profile in the HPLC column. One gram of the
235 homogenous mash obtained with the food processor (as described above) was placed
236 in 5 10^{-2} L falcon tubes; 10^{-2} L 98% ethanol (J.T. Baker, 9000-03, USA) were added and
237 homogenized with an ultraturrax for 30 s. Extract was filtered first with Whatman # 1
238 paper and then through a 0.22 10^{-6} m membrane and finally placed in glass tubes and
239 evaporated (NEvap 112, Organomation Associates, Berlin, MA, USA) through a
240 nitrogen flux at 80℃. Dried samples were next dissolved in 250 µL 98% ethanol and
241 vortex agitated for 60 s at 83.3 s^{-1}. Samples were transferred to 1.5 10^{-3} L vials for
242 HPLC (Agilent Technologies 1200 series, Waldbronn, Germany) quantification.
243
244 To isolate and quantify scopoletin, an inverse phase column (Techsphere BDS C_{18}, 250
245 x 4.6 10^{-3} m, 5 10^{-6} m, HPLC Technology, UK) was used for an HPLC system (Agilent
246 Technologies 1200 series, Waldbronn, Germany) with a diode array detector (No.
247 DE64257792). The column was kept at 25 ℃. Acetonitrile and 0.5% phosphoric acid in
248 aqueous solution gradients were used. The gradient profile was 60-1% for 1800 s with a

249 78 10^{-3} L s^{-1} flow and a 50 µL injection volume. Scopoletin was simultaneously detected
250 at 215, 280 and 350 10^{-12} m, for which purpose time of retention and the standard
251 spectral analysis of pure scopoletin (Sigma-Aldrich: Scopoletin ≥ 99%— No. S2500)
252 were used. Scopoletin quantification was determined through a calibration standard
253 curve. The correlation coefficient was 0.9993 for six concentration levels (1, 5, 10, 25,
254 50 and 75 10^{-3} g L^{-1}) and three replications. Quantifications were made on a fresh
255 weight basis.
256
257 ***2.8 Paste clarity.***
258 The methodology suggested by Craig *et al.* (1989) was used for this purpose. A 1%
259 aqueous dispersion of starch was boiled at 97 ºC (1000m above sea level) and shaken
260 thoroughly every 30 s for 1800 s. Transmittance was measured after cooling to room
261 temperature at 650 10^{-12} m. Two different quantifications per starch sample were made,
262 and mean values were then calculated. Two independent analyses (aliquots) were
263 made for each starch sample. Therefore, four clarity measurements (two aliquots x two
264 replications) were available for roots from a given clone with a given storage period.
265
266 ***2.9 Swelling power, solubility and dispersed volume fraction measurements.***
267 Swelling power (SW) and solubility patterns (SO) (Mestres *et al.*, 1997) were
268 determined using 1% (w/w) starch dispersions (0.28 10^{-3} Kg dispersed in 27.72 10^{-3} Kg
269 of distilled water) at 60, 75 and 90 ºC. Every measurement was made and reported on a
270 dry weight basis. Two independent analyses (aliquots) were made for each starch
271 sample. The low concentration used in the study was chosen to obtain optimal
272 separation between the pellet and supernatant phases after centrifugation. Paste was

prepared in RVA starting at 35 ℃ for 60 s, with temperature increasing at a rate of 0.1 ℃ s^{-1}. Three different and independent analyses were made with final temperatures held at 60, 75 or 90 ℃ 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 ℃, were collected and weighed (Wsu and Wse, respectively), then dried at 100 ℃ 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:

Solubility (%) = 100 * Dsu /0.28

Swelling Power (Kg_{water}/Kg_{Starch}) = (Wse – Dse)/Dse

(Φ) = (27.91 – (Wsu – 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 + (0.28 / 1.5) 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,

296 falling within the range of concentrations frequently reported in the literature. Viscosity
297 was recorded using the temperature profile: holding at 50 °C for 60s, heating from 50 °C
298 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
299 °C s^{-1}. The gel was then maintained for 120 s at 50 °C with continuous stirring at 2.67 s^{-1}
300 . Five parameters were measured: pasting temperature (PT), peak viscosity (PV), hot
301 paste viscosity at the end of the plateau at 90 °C (HPV),cool paste viscosity (CPV) at 50
302 °C (1160 s analysis), and final viscosity (FV). Three other parameters calculated were:
303 breakdown (BD), estimated as PV-HPV; setback (SB), estimated as CPV-PV; and
304 consistency (CS), estimated as CPV-HPV. One RVA analysis per starch replication was
305 made (no aliquots available).
306
307 ***2.11 Differential scanning calorimeter measurements (DSC)***
308 Starch samples (≈ 4 10^{-6} Kg) with ~12 10^{-6} L distilled water were hermetically sealed in
309 stainless-steel DSC pans and kept at room temperature for 2–3 hours. Next, samples
310 were scanned against a blank (empty pan), using a Perkin Elmer Pyris 6 DSC (Perkin-
311 Elmer Co., Norwalk, CT) from 15 to 120 °C at a scanning rate of 0.167 °C s^{-1}. Each
312 DSC endotherm was characterized by the onset temperature (*T*o) and gelatinization
313 enthalpy (Δ*H).* Data were obtained only for 0, 1, 2, 3, 7 and 14 d after harvest. One
314 measurement per starch replication was made.
315
316 **2.12 Data analysis.**
317 Most of the analysis was based on regressions of different variables on storage time.
318 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

342 have resulted in a gradual increase in DMC. These results are consistent with those
343 reported in the literature (Booth, 1977; Booth *et al.*, 1976; Osunsami et al., 1989),
344 indicating that dry matter content remains more or less constant during storage periods
345 of different lengths; the proportion of starch extracted from fresh roots is considerably
346 higher than in roots stored for several days; and there is a sharp increase in the
347 proportion of simple sugars. Ihedioha and co-workers reported in 1995, however, that
348 dry matter content increased from about 30% on the day of harvest to 35% after 4 d of
349 storage (average for four clones).
350
351 Total sugar content increased consistently throughout the storage period (Figure 1b,
352 Table 1) as reported in the literature (Akingbala *et al.*, 2005; Booth *et al.*, 1976; Lin *et al.*,
353 2011: Osunsami *et al.*, 1989). Total sugars increased at a faster rate for HMC-1 than for
354 AM 206-5, but the latter began with higher levels of sugars. In addition, total sugar
355 content in the roots from AM 206-5 oscillated more widely than those for HMC-1. Total
356 sugars in AM 206-5 remained more or less constant through the day 4 (≈ 0.9 g kg^{-1}) of
357 storage, then abruptly rose to 1.4 g kg^{-1} and remained at that level. Increases in HMC-1
358 were gradual and more consistent over time (Figure 1b). Glucose and fructose
359 increases were consistent and similar in both genotypes (Table 1). From day 0 to 14,
360 glucose increased from 0.063 to 0.404 g kg^{-1}, while fructose increased from 0.091 to
361 0.429 g kg^{-1}, based on average values across the two genotypes. Saccharose, on the
362 other hand, did not change significantly in AM 206-5 (from 0.620 to 0.554 g kg^{-1}),
363 whereas for HMC-1 it showed a statistically significant increase from 0.368 to 0.421 g

364 kg^{-1}. Early reports indicated that saccharose tends to decline, while fructose and glucose
365 increase, although changes vary according to storage conditions (Booth *et al*., 1976).
366
367 Estimations of extractable starch loss during storage are provided in Figure 1c and
368 Table 1. Responses for the two clones differed significantly, with higher losses for HMC-
369 1 than AM 206-5. In general, each day of storage resulted in a starch reduction of
370 slightly less than 1%. Therefore, by the end of the experiment, roots had lost around
371 12% of starch. These losses were estimated indirectly, and the methodology involved
372 some assumptions. Dry matter content in both clones remained more or less constant
373 during storage despite clear root weight losses. It was assumed that weight loss as
374 water through transpiration must be similar to loss as CO_2 through respiration (Ravi and
375 Aked, 1996). For each clone, therefore, starch loss as CO_2 was estimated to be half of
376 its total weight loss (CO_2 loss). However, other starch losses, such as the gradual
377 hydrolysis of starch into simple sugars, are not reflected in weight loss. As indicated in
378 Figure 1b, soluble sugars increased constantly with storage time. These sugars would
379 be lost in the process of starch extraction. Since 1 Kg of starch produces 1.1 Kg of
380 sugars, total sugars/1.1 would be an appropriate estimation of starch loss into sugars
381 (sugar loss). The starch losses for each clone, presented in Figure 1c and Table 1, were
382 obtained for each day of storage by adding the CO_2 loss and sugar loss.
383
384 Organic acids, many of them related to the Krebs cycle, reflect metabolic activities
385 within the root. Citric acid was reduced over time in both clones, while the remaining
386 acids tended to increase (Table 1). Citric acid in AM 206-5 dropped from 0.088 to 0.043

387 g kg^{-1}, while in HMC-1 it declined from 0.073 to 0.031 g kg^{-1} between days 0 and 14. In
388 general, roots from HMC-1, accumulated significant amounts of malic, succinic and
389 fumaric acids, while roots from AM 206-5 showed positive but not significant changes
390 (except with malic acid, which increased but with a $P < 0.05$). The higher accumulation
391 of organic acids in roots from HMC-1 may reflect a more active metabolism, which
392 would explain its higher rate of root weight and starch loss and higher rate of sugar
393 production in comparison with roots from AM 206-5.
394
395 As expected, there were large differences between the two genotypes in the PPD levels
396 of their roots (Figure 2a, Table 1). Average PPD for AM 206-5 was 4%, compared to
397 20% for HMC-1. The possibility of storing roots for 14 d without much PPD prompted
398 this study to analyze biochemical changes in the roots and the starches extracted from
399 them. The consistent PPD tolerance of AM206-5 was confounded in this study by the
400 lower dry matter content of its roots (37%), compared with those of the susceptible
401 check HMC-1 (48%). This difference in dry matter content is not surprising, as HMC-1 is
402 a commercial variety, whereas AM 206-5 is a partially inbred clone selected only
403 because of its amylose-free starch. However, this study found a larger difference in
404 DMC than did previous studies involving AM 206-5 (Morante *et al*., 2010, Ceballos *et al*.,
405 2012). PPD tends to be lower in roots with low dry matter content (Sánchez *et al*., 2006).
406
407 The two clones evaluated showed large differences in scopoletin production during
408 storage (Figure 2b). Data presented in this figure are the averages of two independent
409 quantifications of scopoletin in different root samples from each of the two genotypes. In

410 general, the results are consistent with those reported earlier (Buschmann *et al.*, 2000).
411 In this study, the susceptible clone reached a maximum scopoletin concentration (28
412 µmol kg^{-1}) on the 3rd day, and levels remained relatively high for a couple of days
413 (Figure 2b). Roots from AM 206-5, on the other hand, reached a scopoletin peak on the
414 4th day (17 µmol kg^{-1}) and then decreased sharply thereafter. As expected, roots from
415 the PPD-tolerant clone showed a drastically lower and delayed accumulation of
416 scopoletin compared with the susceptible clone. Scopoletin contents after a week of
417 storage were negligible with a small peak, as also reported by Buschmann and co-
418 workers. However, PPD continued to increase over time (Figure 2a).
419
420 **3.2 Changes in starch properties.**
421 The clarity of gels was drastically reduced as the starch used to make them was
422 extracted from roots stored for longer periods (Figure 3a, Table 1). The tendency was
423 more pronounced with HMC-1 than AM 206-5. This is the first report of such a change
424 in cassava starch during root storage. A similar phenomenon was observed in potato
425 but only after six months of tuber storage (Singh *et al.*, 2008) and 7 d of gel storage.
426 These authors suggested that the changes could be attributed to decreases in granule
427 size and phosphorus content, which ultimately increase the concentration of granule
428 remnants, resulting in lower light transmittance. In addition, enzymatic hydrolysis of
429 starch granules, can be expected to modify the granule architecture (i.e., amorphous
430 versus crystalline, amylose/amylopectin ratio and chain length) and hence also
431 contribute to the physico-chemical properties of starch.
432

433 The evolution of solubility and swelling index in starch extracted from roots stored for
434 different periods is presented in Figures 3b and 3c. Whereas the solubility of starch from
435 AM206-5 increased over time (though with wide oscillation around the regression line), it
436 tended to decline for HMC-1. In both cases regression coefficients were statistically
437 significant at $P < 0.01$ (Table 1). Swelling power did not change much in starches from
438 HMC-1 but decreased over time in starches from AM206-5 (Figure 3c, Table 1). The
439 volume fraction of the dispersed phase (Φ) followed trends similar to those for the
440 swelling index, with values for AM 206-5 declining significantly over time, while
441 remaining more or less constant for starches from HMC-1 (Figure 3d, Table 1).
442
443 Results for different parameters measured with the RVA are summarized in Figures 4
444 and 5 and Table 1. Figures 4a and 4b present key amylograms (starch from roots stored
445 for 0, 7 and 14 d) to illustrate general trends for the two clones. PT did not change over
446 time (Table 1), but the values were considerably higher for AM 206-5 (65-66 ℃) than for
447 HMC-1 (63-64 ℃). AM 206-5 starch develops higher PV than non-waxy starches
448 (Figure 5a). These findings agree with previous reports (Ceballos *et al*., 2007; Sánchez
449 *et al*., 2010). In addition, PV in waxy starch from AM 206-5 tended to increase
450 throughout root storage (about 0.15 Pa s in 14 d). On the other hand, PV for starch from
451 HMC-1 remained approximately unchanged. The shapes of the amylograms were very
452 contrasting with an apparent shoulder before starches from HMC-1 reach their PV
453 (Figure 4b). As roots from HMC-1 were stored for longer periods their starch tended to
454 show a delayed PV (around 80 and 90 ℃ for days 0 and 14 of storage, respectively),

455 suggesting higher resistance to shearing and temperature. FV tended to be higher in
456 starch extracted during long storage periods (Figure 5b).
457
458 HPV was lower in AM 206-5 than in HMC-1 (Figure 5c), and both clones responded
459 similarly regarding the length of the storage period (Table 1). CPVs were considerably
460 higher in HMC-1 (≈ 0.90 Pa s) than in AM 206-5 (≈ 0.60 Pa s) and reacted similarly in
461 both genotypes in terms of their response to the duration of the storage period (Figure
462 5d, Table 1). BD, therefore, was considerably larger in AM 206-5 than HMC-1,
463 remaining more or less constant in the former, while decreasing considerably over
464 longer storage periods in the latter (Table 1). SB showed the opposite trend with
465 negative values for AM 206-5 and positive ones for HMC-1 (Table 1). Finally,
466 consistency showed a similar negative trend over storage time in both genotypes (Table
467 1).
468
469 Onset temperatures did not change much in relation to duration of the storage period
470 with AM 206-5 showing higher values (around 66.0 °C) than HMC-1 (63.5 °C). ΔH was
471 higher and relatively stable for starches from AM 206-5, while for HMC-1 values tended
472 to decrease over time (Table 1).
473
474 **4. Discussion (PPD, Starch losses, starch properties)**
475 Cassava roots obviously undergo considerable changes during storage. The two
476 genotypes included in this study showed similarity in some of these changes, whereas in
477 others drastic differences indicated the influence of genetic variation. Particularly

478 relevant was the rate of progress for PPD, with final values around 30% for roots from
479 HMC-1 and only 10% for those from AM 206-5. The limited occurrence of vascular
480 streaking in the roots from AM 206-5 makes it possible to evaluate alternatives for their
481 post-harvest handling. For example, would it be advisable to store roots for few days
482 when they are to be used for the production of sweeteners? There are certainly losses in
483 the total amount of energy stored in the roots, which are responsible in part for the
484 weight and starch losses illustrated in Figure 1c.
485
486 Many research groups have sought for a long time to develop PPD-tolerant cassava.
487 This is clearly a desirable aim, which would offer considerable advantages for certain
488 value chains. However, the loss of starch in cassava roots over time diminishes the
489 relevance of PPD tolerance, at least for the starch industry. Gradual changes in the
490 functional properties of starch extracted from stored roots are also a matter of concern,
491 though there may be alternatives for starch modification *in planta*.
492
493 As previous reports indicate (Buschmann *et al.*, 2000; Tanaka *et al.,* 1983), PPD is
494 associated with scopoletin concentration. This study revealed clear differences in
495 scopoletin concentration between a susceptible and tolerant clone as well as a delay in
496 reaching maximum values. Buschmann *et al.* (2000) sliced the roots to further
497 accelerate PPD and observed maximum concentrations of scopoletin after 2 d (up to 65
498 µmol kg^{-1}) in roots from the most PPD-susceptible clone (MCOL 22). PPD-tolerant
499 clones, on the other hand, showed a maximum peak considerably later (after 6 d). In
500 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

523 decreased while that for HMC-1 remained more or less stable (Figure 3c), which is
524 unusual for amylose-free starch.
525
526 Morante *et al.* (2010) reported different sources of tolerance to PPD. The present article
527 concentrates on the source of PPD tolerance that was found in the amylose-free starch
528 mutation, a genotype of particular interest for the starch industry. The research therefore
529 concentrated on PPD, scopoletin, starch functional properties and starch losses. Similar
530 studies could focus on other sources of PPD tolerance, particularly those related to high
531 carotenoids, with the aim of determining the fate of carotenoid content and cooking
532 quality during storage, along with PPD and scopoletin content. Such studies have
533 already begun, but since key germplasm must first be multiplied to produce the number
534 of roots required, it will take another two years to obtain results.
535
536 **5. Conclusions**
537 This study provides further evidence of PPD tolerance in AM 206-5 as well as insight
538 into the implications of deploying PPD-tolerant cassava varieties. Changes in starch
539 functional properties during storage may offer an innovative way to induce starch
540 modification *in planta*, but this is possible only for PPD-tolerant cassava germplasm.
541 Realizing the potential of this strategy will require considerable additional research and
542 fine-tuning by the starch industry, once commercial cultivars with PPD tolerance are
543 released.
544

545 Unfortunately for the starch industry the gradual loss of starch (at a rate of about 1% d^{-1})
546 limits the extent to which roots from a PPD-tolerant clone can be stored, despite their
547 lack of vascular streaking. Thus, while PPD tolerance can reduce losses in transport by
548 extending shelf life for few days, the starch losses become too high after a week. This
549 finding is very relevant to important ongoing efforts to develop PPD-tolerant germplasm,
550 including through genetic transformation.
551
552 For ethnic uses of cassava roots, such as the preparation of gari, fufu and farinha, PPD-
553 tolerant clones could be advantageous, at least in terms of appearance, since the
554 products would not present the grayish blue color that is typical of products made from
555 deteriorated roots. In addition, such clones would give the final product a somewhat
556 sweeter taste. Finally, the digestibility of roots stored for a few days could increase,
557 creating new alternatives for the animal industry.
558
559 The availability of PPD tolerance makes possible further studies on metabolic changes
560 during root storage. In this study, metabolism in roots of the PPD-tolerant genotype
561 seemed slower than in the susceptible one. Respiration is likely different in the two types
562 of roots.
563
564
565
566 **Acknowledgments**

567 Editorial corrections by Nathan Russell greatly improved the quality of the manuscript by
568 enhanced clarity and readability. The sensible and useful comments made by the two
569 reviewers and the guidance by the Editor of the journal are also acknowledged and
570 appreciated.
571

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

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

726 * Significant at 5%; ** Significant at 1%

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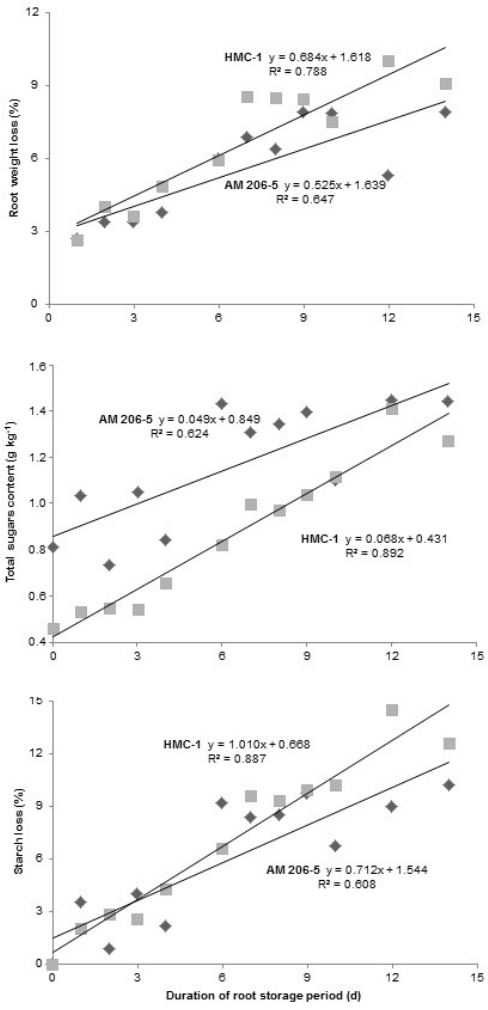

731

732 **Figure 1**. Changes in cassava roots from two clones after storage under ambience
733 conditions through 14 days. **1a.** Weight loss expressed as percentage of the original
734 root weight; **1b**. Evolution of total sugars content (g kg^{-1}); and **1c.** Estimated losses of
735 starch (%).

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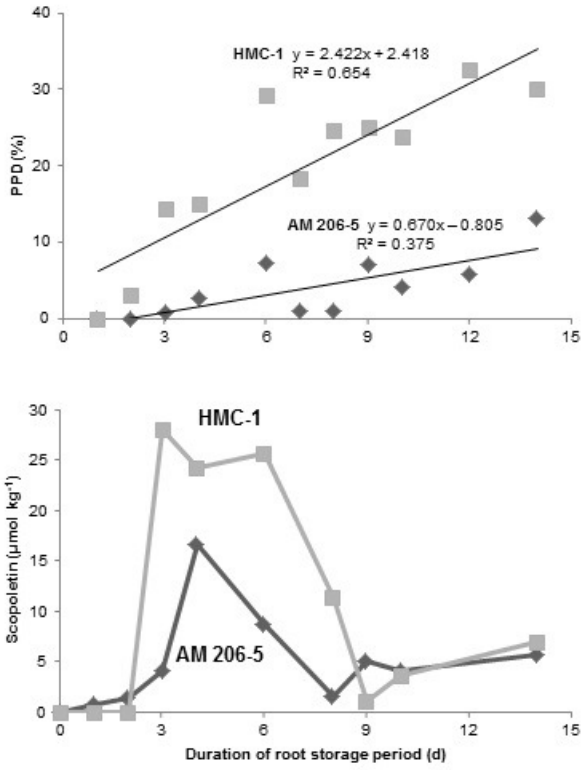

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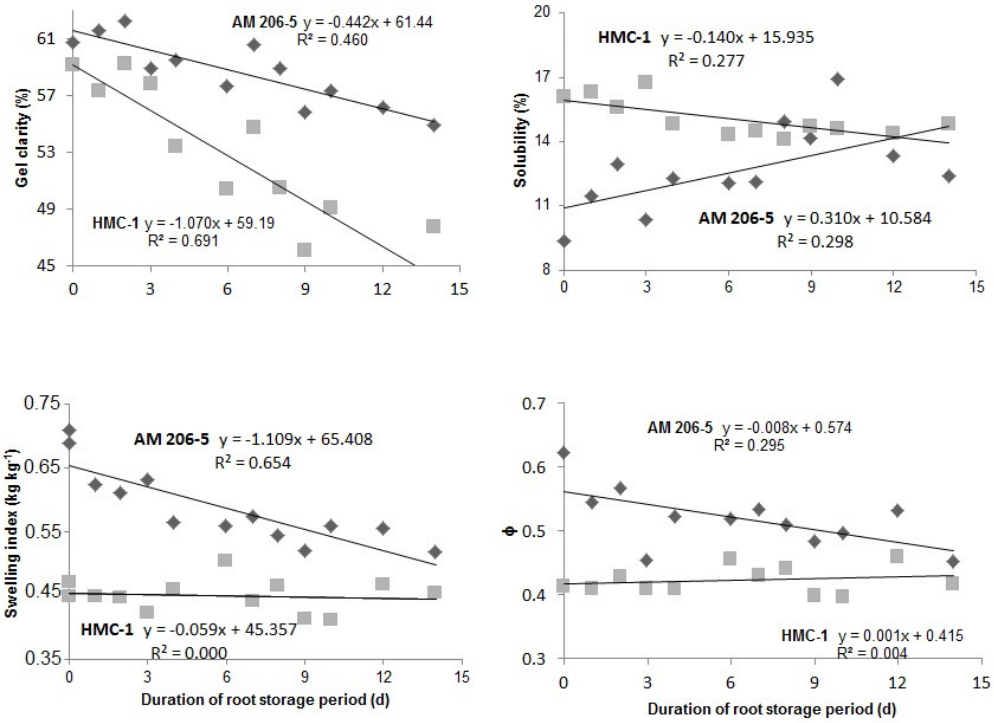
739 **Figure 2**. Changes in cassava roots from two clones after storage under ambience
740 conditions through 14 days. **2a.** Postharvest physiological deterioration (PPD) score
741 (%); and **2b** Scopoletin contents (µmol kg^{-1}).

742



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745 **Figure 3**. Starch physicochemical properties in starches extract from cassava roots
746 stored through 14 days. **3a.** Gel clarity (%); **3b.** Solubility (%); **3c.** Swelling index (Kg
747 Kg^{-1}); and **3d.** Volume fraction of the dispersed phase (Φ).

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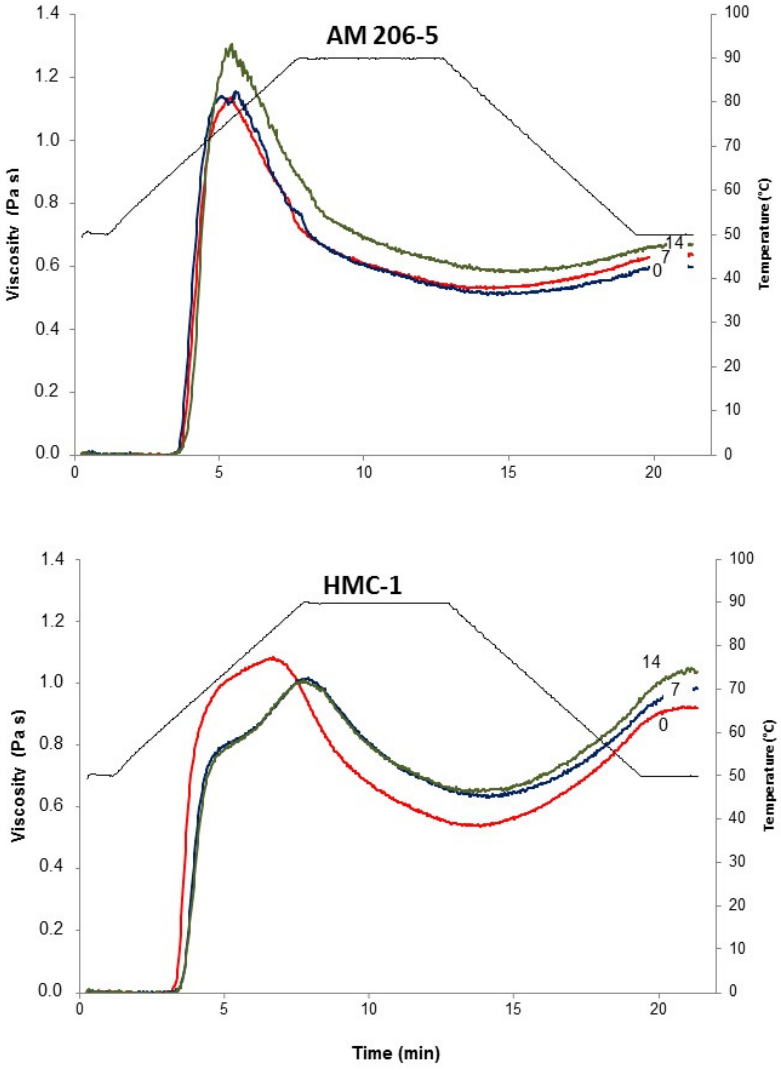

Figure 4. RVA amylograms from starches extracted from cassava roots stored for 0, 7, and 14 days (Pa s). **4a.** Amylograms from AM 206-5 starches; and **4b.** Amylograms from HMC-1 starches.

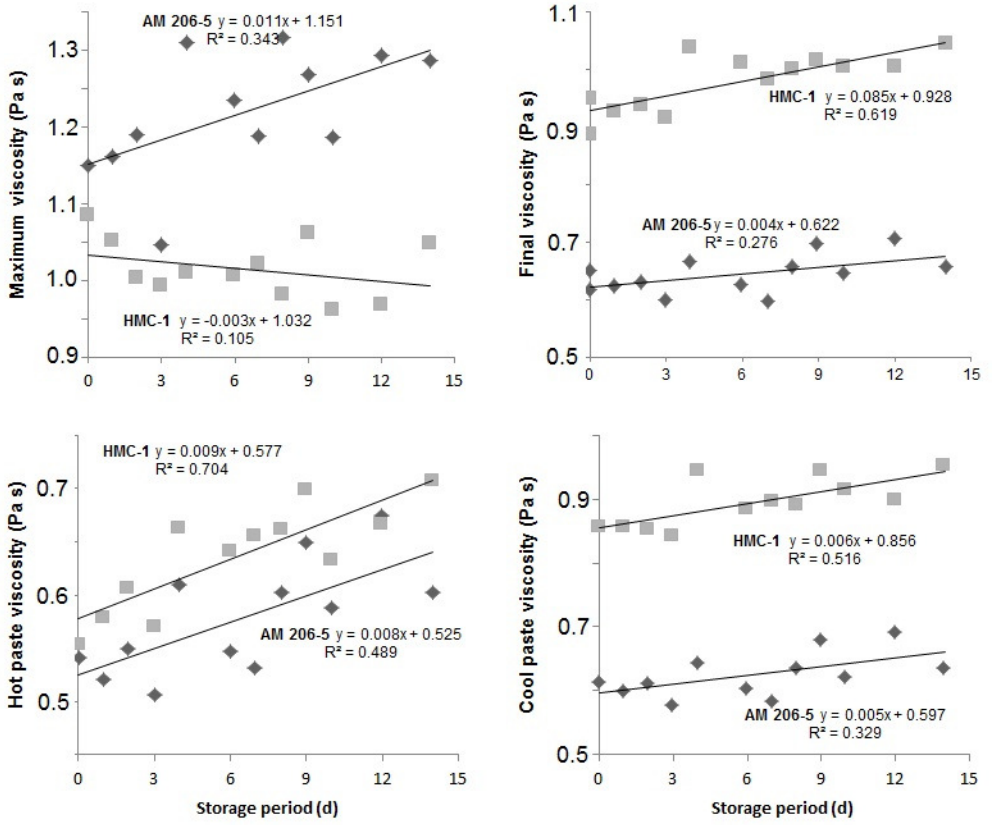

Figure 5. Selected RVA parameters in starches extracted from cassava roots stored through 14 days. **5a.** Maximum viscosity (Pa s); **5b.** Final viscosity (Pa s); **5c.** Hot paste viscosity (Pa s); **5d.** Cool paste viscosity.

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