

# Changes in Free Phenolics Contents during Tuber Development, Dormancy and Sprouting in White Yam (*Dioscorea rotundata* Poir.)

Elsie I. Hamadina<sup>1,\*</sup>, Peter Q. Craufurd<sup>2</sup>

<sup>1</sup>Department of Crop & Soil Science, University of Port Harcourt, Port Harcourt, Nigeria

<sup>2</sup>Global Conservation Agriculture Program, CIMMYT, Nairobi, Kenya

**Abstract** The objectives of this study were 1) to determine the changes in free phenolics in yam tubers during development, dormancy and sprouting, and 2) evaluate the relationship between concentration of phenolics and, start and end of dormancy. Tubers were harvested at 127 DAP (Days after planting), while the plants were still green, and at 176 DAP (at vine senescence). During tuber storage, samples were collected at 246 DAP and 303 DAP (start of sprouting). Tuber tissue samples were collected from seven tubers at each of these harvest dates, freeze-dried and analysed for total free phenolics, using High Performance Liquid Chromatography (HPLC). Highest mean total free phenolics content occurred at 127 DAP (2.46 AU units/g dry wt.), while the lowest was recorded at vine senescence (1.79 AU units/g dry wt). Individual phenolic compounds did not have lower concentrations prior to vine senescence (i.e., 176 DAP). After vine senescence, the mean total free phenolics declined, but individual phenolic compounds did not exhibit any discernible decline in concentration over time. The decline in total free phenolics between harvests at 176 DAP and sprouting indicate that free phenolics may have a role in the release of *D. rotundata* tuber dormancy.

**Keywords** *Dioscorea rotundata*, Free Phenolics, Dormancy, Sprouting

## 1. Introduction

The cessation of yam tuber growth (bulking) occurs just before the start of vine senescence ([1], [2], [3]) and this is accompanied by the slowing down of physiological activity. The onset of these events is generally considered to mark the start of tuber dormancy, which coincides with the start of the dry season in yam growing areas ([4] [5], [6], [7], [8]). However, the control of the onset of dormancy and its release is still not clear [9].

Many plant growth inhibitions, including dormancy, are thought to be under the influence of endogenous plant growth regulators (PGRs) with growth inhibitory characteristics ([3], [10], [11]). Several of these compounds are phenolics, and belong mostly to the stilbenoids group of phenolics [12]; and they have been isolated in healthy *D. alata*, *D. esculenta*, *D. opposita* and *D. floribunda* tubers ([3], [10], [13], [14]). In a study, the growth inhibitors were not found in healthy *D. rotundata* [13]. Nevertheless, Coxon *et al.*, 1982 [15] suggested the need for further investigations as small amounts of batatasin 1 (a member of the phenanthrene

class of stilbenoids) might be present in even healthy tubers.

Since endogenous phenolic compounds are generally known to inhibit plant growth directly or indirectly ([15], [16], [17]), studies that measure changes in free phenolic compounds during dormancy may provide useful information on the role of phenolics in yam tuber dormancy. In addition, it is important to identify the phase(s) of yam tuber dormancy that may be under the control of phenolic growth inhibitors. In the study reported by Hashimoto *et al.*, 1972 [10] and Coxon *et al.*, 1982 [15] the period of dormancy that is inhibited by exogenously applied batatasins, is the progress towards sprouting, which may be related to the phases 2-3 of dormancy described by Ile *et al.*, 2006 [18]. Hashimoto *et al.*, 1972 [10] and Coxon *et al.*, 1982 [15], measured the concentration of specific phenolic compounds in dormant tubers (at harvest after vine senescence through to commencement of sprouting) and found higher concentrations in dormant tubers than in sprouting tubers. With *D. alata*, Ireland and Passam, 1984 [3] have shown that the concentration of batatasins is low during tuber development and high at vine senescence. To understand the role of phenolics in the initiation of dormancy in *D. rotundata*, investigations must cover the complete circle of dormancy, i.e., from some point after tuber initiation to sprouting).

Therefore, the objectives of this study were: 1) to examine

\* Corresponding author:

elsieile@yahoo.com (Elsie I. Hamadina)

Published online at <http://journal.sapub.org/plant>

Copyright © 2015 Scientific & Academic Publishing. All Rights Reserved

the changes in free phenolic compounds in white yam tubers between harvest (before and after vine senescence) and sprouting (*i.e.* Appearance of shoot bud on the surface of tubers; ASB), and 2) to determine the relationship between the concentration of phenolics, and the start and end of dormancy.

## 2. Materials and Methods

### 2.1. Experiment to assess the Presence of Phenolics in Young *D. rotundata* Tuber Tissues and *D. alata*

In this experiment, *D. rotundata* tubers weighing between 2 and 10 g were harvested at 76 DAP from plants that were just tuberising. The tubers were collected from selected plants grown under the conditions described in Section 2.2. Due to the small size of the tubers and thin peel, tubers weighing a total of 45 g fresh weight were shredded together. Extraction was done using 100% hot methanol + ethyl acetate, separating compounds in the crude extract on TLC plates developed in  $\text{CHCl}_3$ -MeOH (96:4) as well as in  $\text{CHCl}_3$ -HOAc (95:5), based on protocol described by Harborne, 1989 [20], as modified by Ile, 2004 [19].

Because phenolic dormancy promoting compounds are known to occur naturally in *D. alata*, the UV spectra and retention times (Rt), and retention factor (Rf) of compounds eluted from *D. alata* tubers were compared with those from *D. rotundata* tubers. Tubers of *D. alata* were peeled and separated into peel and pulp. Extraction was done in 100% hot methanol + ethyl acetate, and TLC plates developed in  $\text{CHCl}_3$ -MeOH (96: 4) and  $\text{CHCl}_3$ -HOAc (95:5), and the extract was also analysed by HPLC as described in Section 2.2.5.

### 2.2. Experiment to Determine Changes in Free Phenolics in *D. rotundata* Tubers during Tuber Development, Dormancy and Sprouting

Healthy, sprouting tubers of *D. rotundata* TDr 131 were planted in 25 cm pots containing a soil mixture of sterile sand: gravel: vermiculite and loam-less compost in a ratio of 4:2:4:1 (v/v) as well as 3 g/l Osmocote-plus fertilizer. Osmocote-plus is a slow release (90-120 d) fertilizer containing  $\text{N}+\text{P}_2\text{O}_5+\text{K}_2\text{O}+\text{MgO}$  at 15 + 11 + 13 + 2 + all essential trace elements (B, Cu, Fe, Mn, Mo, Zn) (Monro Horticulture, Goodwood, West Sussex UK). These tubers were themselves produced from tissue culture plantlets grown in pots. Planting was done in the month of May. Plants were grown under photoperiod and temperature conditions that simulated a June sowing date at Ibadan, Oyo State, Nigeria. Air temperature in the glasshouse was maintained at 32 °C during the day and 22 °C at night. Pots were watered regularly as required.

#### 2.2.1. Tuber Harvest, Storage Conditions and Sampling

Harvesting was done twice: at 127 DAP, when leaves and vine were still green, and at 176 DAP (main harvest), when

leaf senescence had began. The weight of the tubers ranged from 4 to 245 g in the first harvest and 13 to 173 g in the second harvest.

After harvest, tubers were kept in Saxcel growth cabinet until sprouting (*i.e.*, appearance of shoot bud on the surface of tubers). The growth cabinet was maintained at 12-hr photoperiod, photon flux density of  $0.017^\circ \text{ mmol m}^{-2} \text{ s}^{-1}$ , constant air temperature and relative humidity of 28 °C and 80%, respectively. Tubers were sampled at four dates for chemical analysis of phenolics:

- 127 DAP (during early tuber filling);
- 176 DAP (at vine senescence, tuber maturity);
- 246 DAP (during tuber storage); and
- 303 DAP (at first external sign of sprouting).

#### 2.2.2. Dehydration of Tissue Samples

At each sampling time, seven tubers were sampled. Each tuber was divided transversely into two parts forming proximal (head) and distal (tail) regions. Tuber portions weighing 50 g were sampled from each tuber region. The tuber portions were cut into small pieces and frozen in liquid nitrogen. Frozen samples were then freeze-dried in 50 ml Falcon tubes, for 48 h using a Birchover F.D (7.5 L capacity) freeze drier. After freeze-drying, samples were stored at -80 °C until tissue chemical analysis.

#### 2.2.3. Extraction of Phenolics in Freeze-dried Samples

The freeze-dried samples were first weighed for the determination of dry matter content and then samples were ground (in a laboratory mill A-10) for one to two minutes. Powder samples weighing 9g was used for the extraction of phenolics. Extraction was done with 100 ml 70% methanol that was brought to boil and allowed to stand for 3h. The extract was filtered through glass wool to remove debris. Samples were rinsed two to three times with 50ml methanol or until the extract was colourless. The methanol was evaporated under vacuum at 40 °C. The aqueous extract was transferred to a separating funnel to separate the free phenolic fraction from those of other dissolved compounds. The extract was partitioned using ethyl acetate. Free phenolics were extracted into the upper ethyl acetate layer [20], which was collected in a conical flask. This procedure was repeated until no colour was observed in the ethyl acetate layer. The combined ethyl acetate fractions were reduced to dryness by rotary evaporation at 40 °C. The residue was dissolved in 2 ml absolute methanol with the help of sonication. The extract was then collected into a 5ml glass tube and stopped tightly to prevent evaporation. The process was repeated, to rinse the flask, using another 2ml absolute methanol and combined with the first 2 ml.

#### 2.2.4. Qualitative Analysis of Phenolics

Fifteen spot applications of crude extract were applied on Merck silica gel TLC sheets. A preliminary experiment showed that 15 spot applications was better than 5 or 10. The silica gel sheets were developed in  $\text{CHCl}_3$ -MeOH (96: 4),

based on results from the experiments reported in 2.3.2.

Colour reagents (vanillin in 2-tetraoxosulphate-6 acid, folin and ciocaleau (F+C), and F+C + ammonia) were used to confirm that the compounds separated from the extract were phenolic [20]. Two standards were run alongside the extracts; a stilbene (*i.e.* resveratrol) and a phenolic acid (*i.e.* ferulic acid). The standards were denoted as Std 1 and Std 2 respectively.

### 2.2.5. Quantification of Phenolic Compounds

The HPLC procedure used in this experiment is as described in section 2.1. Seventy (70)  $\mu$ l of crude extract (from the 4 ml crude extract) was filtered through a Gelman Acrodisc 13 nylon membrane filter of pore size 0.45  $\mu$ m (HPLC certified). An injection volume of 40  $\mu$ l of the filtered extract was used for the HPLC analysis.

The HPLC instrument used was a Watters 600 multi-solvent delivery system fitted with a Watter 994 photodiode array detector. The column was a Watter Bondapak Phenyl C18 of dimension 4 mm internal diameter (ID) x 30 cm. Solvent A was 2% acetic acid and solvent B was methanol: acetic acid: water in the ratio of 18:1:1. The flow rate was 1 ml/min and the temperature 25°C. The slow eluting, more polar programme started with a mixture of 75% A: 25% B changing to 100% B over 35 mins in a linear mode. The faster eluting slightly less polar programme started with 65% A: 35% B changing to 100% B over 35 mins in a linear mode. In both cases, the UV detection was at 264 and 272 nm, which are the peak absorbance reported for the phenanthrenes [15] of which batatasins I is one, and the diode array scan was over the range of 200 to 400 nm.

### 2.2.6. Experimental Design and Data Analysis

There were four sampling times with seven replications (number of tubers sampled) per sampling time. Total free phenolics per sample was estimated as the summation of the

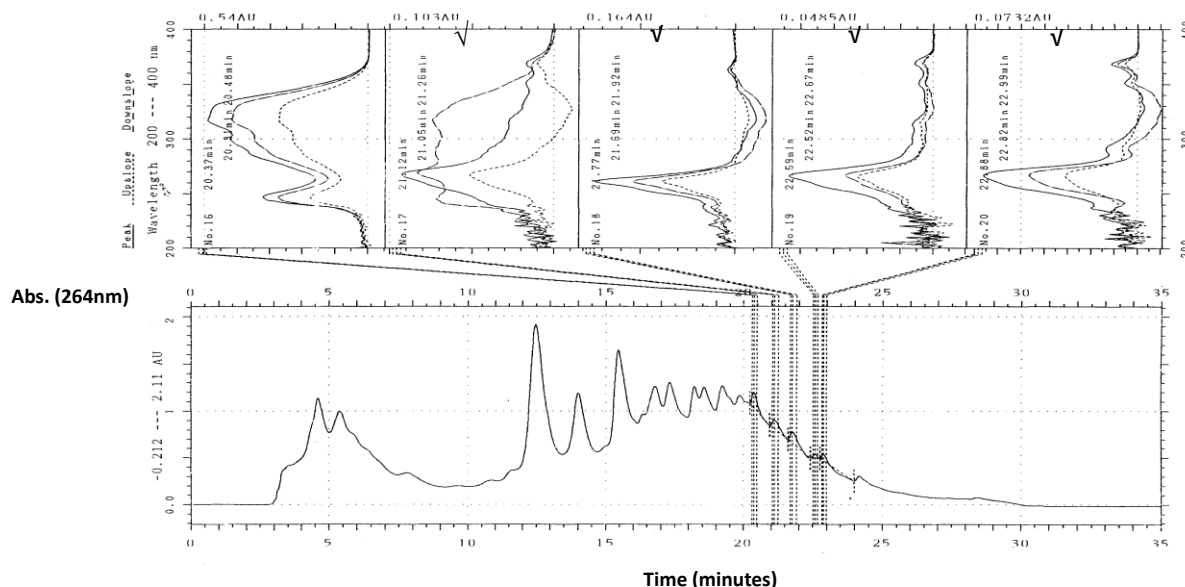
peak heights of all the compounds [20] detected per 40  $\mu$ l crude extract. The result was expressed as absorbance units/g of dry weight. Mean total free phenolics per sampling date (in Abs. units/g dry wt.) was the mean of seven replicates. The significance of the differences over sampling dates was determined using the SAS GLM procedure.

To determination the duration from planting to sprouting, tubers were arranged in a growth cabinet following a completely randomised design (CRD) with three replications and 10 tubers per replicate. The SAS, product limit procedure was used to determine the 50<sup>th</sup> percentile duration from planting to sprouting. However, because there were no censored data, *i.e.* all tubers sprouted within the experimental period, the mean and standard error values are presented.

## 3. Results and Discussion

### 3.1. Assessment of Phenolics in Crude Extracts of Young, Developing *D. rotundata* and *D. alata* Tuber Tissues

Using TLC, three major fluorescent bands were observed at  $R_f$  0.15, 0.44 and 0.91 respectively in crude extract of young *D. rotundata* tuber tissue. These  $R_f$  were similar to the  $R_f$  values obtained by Hashimoto *et al.*, 1974 [10]; Hashimoto and Tajima, 1978 [21]; Ireland *et al.*, 1981, 1984 ([13, 22]) for batatasins. Using HPLC, the concentration of phenolic compounds per 40  $\mu$ l crude extract was generally low, ranging from 0.005 to 0.12 AU, and lower than that obtained in sprouting *D. rotundata* tuber tissue. The UV spectrum of some compounds with minor peaks were detected this study. They were eluted at about 19.64, 20.95, 21.77, and 22.63 mins and at wavelengths between 300 and up to 350 nm. The results showed that young (bulking) tubers contain phenolic compounds similar to those found in mature sprouting *D. rotundata* tubers.



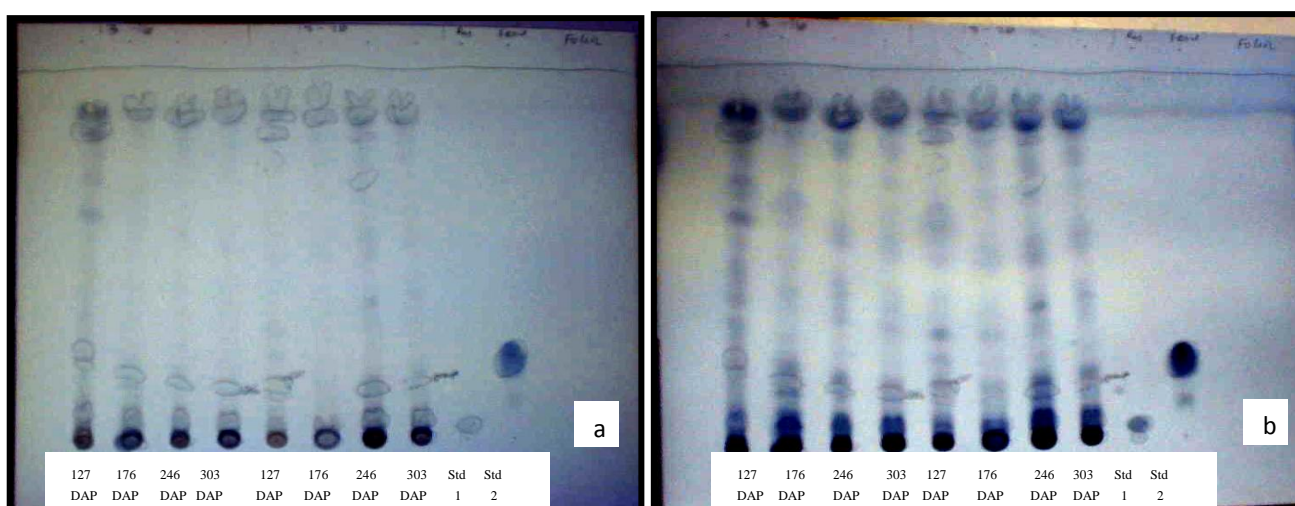
**Figure 1.** HPLC scan of some compounds detected in *D. alata* crude extract with 'in flight' UV spectrum. (Ticked (✓) spectrum refer to compounds that appear to be in the phenanthrenes, such as batatasin 1)

In *D. alata*, peak heights were mostly high, ranging from 0.2 to 2.11 AU per 40  $\mu$ l crude extract. The peaks that were marked as potential batatasins in the sprouting and young *D. rotundata* tuber tissues were also present in *D. alata*, and were eluted at similar retention times ( $R_t$ ) (Figure 1). The relative consistency in their retention times further suggests that the protocol was satisfactory for determining the presence and changes in specific phenolic compound in *D. rotundata*. The spectrum absorbance of some other peaks and their  $R_t$ , i.e. 10.85, 12.59, 14.01, and 16.78 mins, suggest the presence of stilbenoids. Although the retention times with which these comparisons were made were mostly derived from analysis using gas-liquid chromatography, GLC ([12], [23]), the presence of similarities between them suggested a need for further investigation.

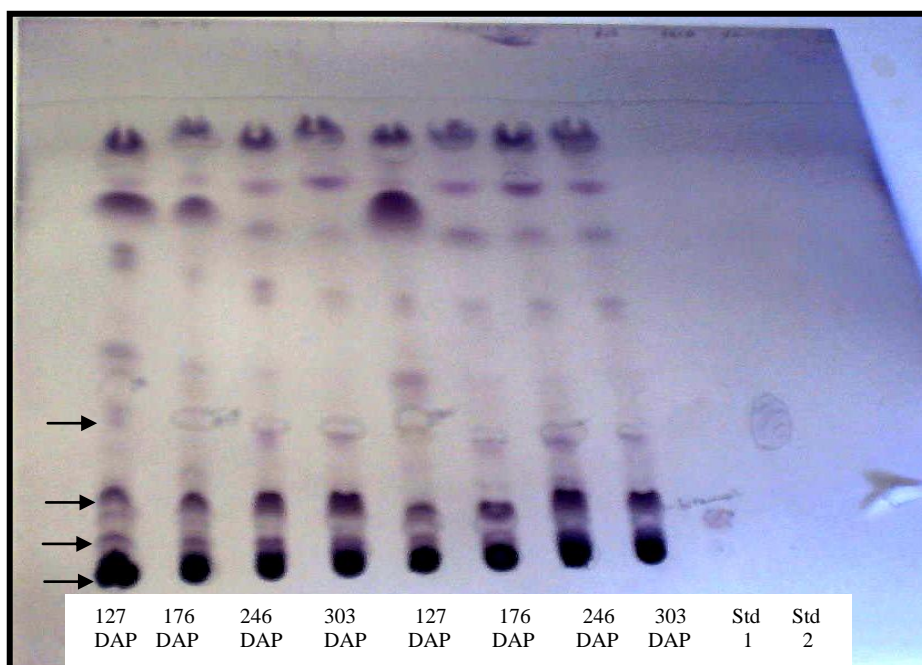
### 3.2. Determination of Changes in Free Phenolics in *D. rotundata* Tubers through Dormancy and Sprouting

#### 3.2.1. Weight of Tubers at Harvest and Duration from Planting to Sprouting in the Control

The mean weight ( $\pm$ SE) of tubers harvested at 127 DAP was lower ( $53.6 \text{ g} \pm 8.39$ ) than those at 176 DAP ( $165.5 \text{ g} \pm 14.3$ ). The first external sign of sprouting was observed by 295 DAP, corresponding to the month of February. The mean duration from planting to sprouting was  $313 \pm 3.3$  DAP and  $310 \pm 1.58$  DAP for tubers harvested at 127 and 176 DAP respectively. Therefore, irrespective of date of harvest or tuber weight at harvest, tubers were observed to sprout at about the same time.



**Figure 2.** Colour reaction test for the presence of phenolic compounds in bands separated on TLC. (a), blue upon spraying with F+C and (b), intensified colour upon fuming with  $\text{NH}_3$



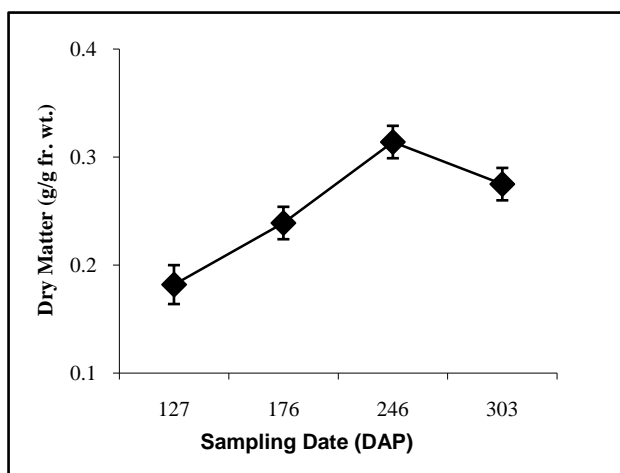
**Figure 3.** Colour reaction test with vanillin/  $\text{H}_2\text{SO}_4$  reagent for the presence of various classes of phenolics in crude extract. Arrows point to some blue, yellow or greyish colour bands

### 3.2.2. Colour Reaction Test for the Presence of Phenolic Compounds in Crude Extract

All bands on TLC sheet, including those for Std. 1 and Std. 2, turned blue upon spraying with F+C reagent (Figure 2a) and the colour intensified when fumed with  $\text{NH}_3$  (Figure 2b). This suggests that the extracts in the bands were phenolic compounds [20]. The colour reaction with vanillin in  $\text{H}_2\text{SO}_4$ , was mostly pink. However, a number of other coloured bands ranging from blue, yellow to greyish blue were observed, and the quantity of some of these compounds varied with sampling date (Figure 3). The presence of the various colour bands indicate that the compounds in crude extract belong to different classes of phenolics [20].

### 3.2.3. Changes in Tuber Dry Matter and Total Free Phenolics during Dormancy and Sprouting

Dry matter content of freeze-dried samples varied significantly ( $p=0.003$ ) with sampling date; increasing linearly from 127 to 246 DAP, and then declined by 303 DAP (Figure 4), when sprouting loci were seen on several tubers. The reverse was the case for mean free phenolics content (AU/g dry wt.). Tubers that were harvested prior to leaf senescence (127 DAP) contained about twice as much free phenolics as compared to those at 246 or 303 DAP (Table 1).



**Figure 4.** Changes in mean dry matter content of freeze dried tuber tissue during dormancy (before sprouting)

**Table 1.** Changes in mean total free phenolics (AU units/g dry wt.) over sampling dates

Sampling Date	Mean (AU units/g dry wt.)	SE
127 DAP	2.457	0.69
176 DAP	1.797	0.30
246 DAP	1.554	0.29
303 DAP	1.635	0.30

n = 7 tubers per date

### 3.2.4. Changes in Individual Phenolic Compounds in Crude Extract

The UV absorbance presented are those monitored at

264nm. The number of compounds eluted per sample varied from 15 to 23, and the peaks exhibited could be classified as either major or minor (Figure 5). Compounds were differentiated based on retention time ( $R_t$ ) and UV absorbance spectrum. The change in concentration of individual compounds over sampling date was traced to determine any systematic changes.

Five compounds were found to exhibit major peak heights (AU) in most crude extracts and these were detected at  $R_t$  2.95 to 3.04, 3.12 to 3.32, 3.57 to 3.71, 5.11 to 5.68 and 12.95 to 13.77 mins (Figure 7). However, only two of them (Compounds I detected at 270 nm after 2.95 to 3.04 mins and II detected at 262 nm after 3.41 to 3.71 mins) were found to decline in concentration over sampling date (Table 2). The UV absorbance spectra of these two compounds (Figure 6) compared with those of known compounds suggest that may be simple phenols or phenolic acids. A comparison of the UV spectrum absorbance for compounds III and IV with those of known compounds did not give a clear indication of their identity.

**Table 2.** Changes in mean concentration (AU units/g dry wt.) of Compound I; detected at  $R_t$  2.95 to 3.04, and compound II; detected at  $R_t$  3.57 to 3.71

Sampling date	Compound I		Compound II	
	Mean	SE	Mean	SE
127 DAP	0.299	0.09	0.778	0.06
176 DAP	0.188	0.04	NA	NA
246 DAP	0.121	0.01	0.243	0.09
303 DAP	0.138	0.06	0.121	0.05

n = 7 per sampling date; NA, Not available

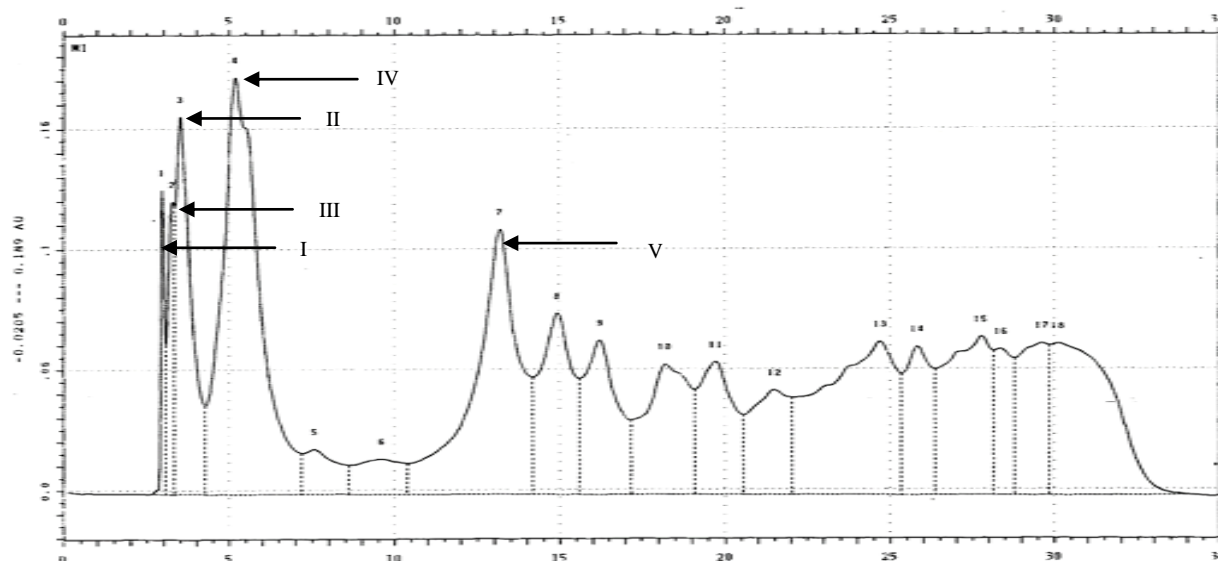
On the other hand, UV spectrum absorbance of compound V (Figure 7) may correspond to stilbenes or the phenolic acid, ferulic acid. Comparing the UV absorbance spectrum of an unknown compound with that of known compounds is a good way of identifying unknown compounds [24]. Most phenols and phenolic acids show one major UV absorption at wavelengths ranging from 266 to 295 nm and 235 to 305 nm respectively ([20], [24]). Based on these findings, there is a need to conduct more studies to characterize some of the phenolic compounds in yam tissues. Knowledge of these phenolic compounds is important because phenols and phenolic acid are known to affect a wide range of plant activities. For example ferulic acid and coumarins are known to promote germination in barley seeds [25]. Ellagic acid and gallic acid act as feed deterrents while some even hinder photosynthesis via the inhibition of chloroplast function [24]. Nevertheless, the UV spectrum scan of compounds I and II did not prove that they are either ferulic acid, gallic acid, ellagic acid or any of the M-, O-, or P-coumarins.

Compounds VI to X exhibited minor peak heights detectable at  $R_t$  7.48 to 7.79, 9.92, 19.49, 20.95 and 27.01 mins respectively. However, none of these compounds varied in a systematic way over the sampling period. Nevertheless, the UV spectrum and retention times for

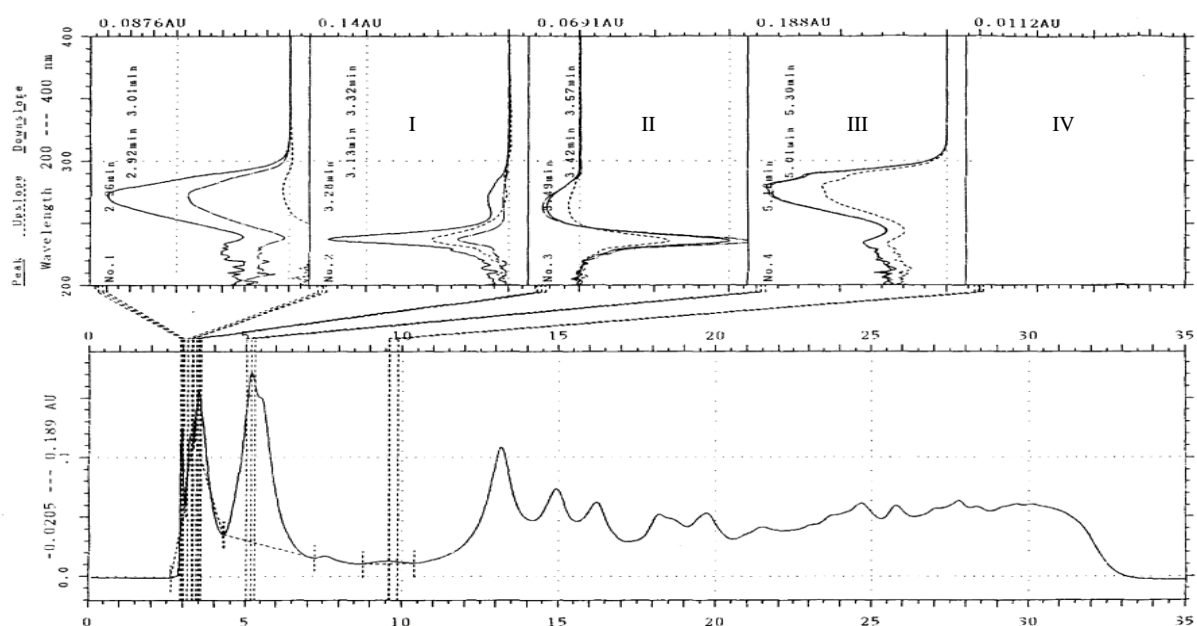
compounds VII, IX and X were similar to those of phenanthrenes and other stilbenoids such as the bibenzyls. These compounds are also similar (as they exhibited at least two or more major peak absorbance and a number of minor peaks between 220 and 350 nm) to those detected in sprouting and young *D. rotundata* tubers and *D. alata*.

The decline in total free phenolics between harvest at 176 DAP, i.e. after vine senescence and 303 DAP, when sprouting began suggested that free phenolics may have a role in the release of *D. rotundata* tuber dormancy. The decline in Compound I between 176 and 303 DAP and in

Compound II between 246 and 303 DAP, suggested that they may be associated with the release of dormancy since they may be breaking down to allow for sprouting. It is however, unclear whether they exhibit growth inhibit activity in *D. rotundata* tubers since the research protocol did not test for their effect on shoot growth nor did it include the characterization of their chemical structure. Ireland and Passam, 1984 [3] and Hashimoto *et al.*, 1972 [10] have also reported a decline in batatasins between harvest at vine senescence and sprouting

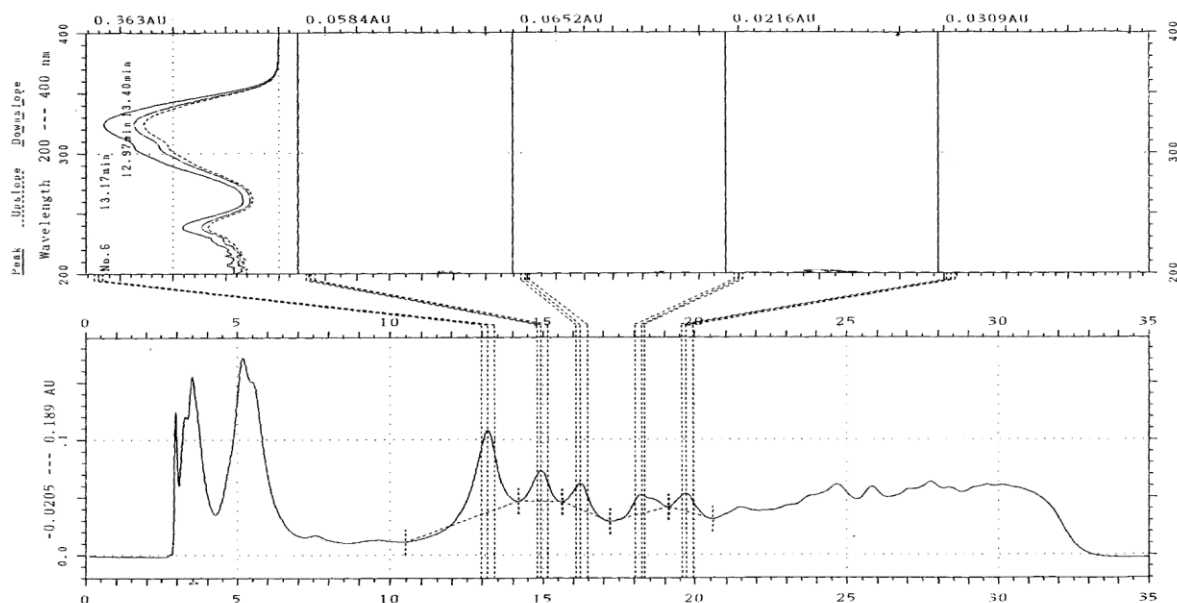


**Figure 5.** Peak absorbance and retention times for phenolic compounds in *D. rotundata* crude extract. (Arrows point to peaks for Compounds I to V)



**Figure 6.** UV spectrum scan for compounds I to IV with peak absorbance at 270 nm after 2.96 mins, 262 nm after 3.49 mins, 240 and 270 nm after 3.28 mins, and 272, 280, 288sh nm after 5.18 mins respectively





**Figure 7.** UV spectrum scan for compound V. Peak absorbance was at 304, 324 nm after 13.17 mins

No individual compound was found to be lower in concentration prior to that after vine senescence. Indeed, total free phenolic content was also higher at 127 DAP (prior to vine senescence) than at vine senescence (176 DAP) or later. If the pattern of change in these two compounds and total free phenolics relate with dormancy in yam, then, the presence of a higher level even before vine senescence suggests that dormancy begins at some point long before vine senescence rather than at vine senescence as suggested by Ireland and Passam, 1984 [3] and Hashimoto *et al.*, 1972 [10].

The *in vitro* study of Hamadina *et al.*, 2010 [27] and the anatomical study of Ile *et al.*, 2006 [18] also suggest that yam tuber dormancy begins prior to vine senescence. Further, the fact that the tubers that were harvested at 127 DAP did not sprout until 313 DAP, confirms that they were already dormant ([28], [29]). Since dry matter per fresh weight is known to increase rapidly during tuber development [30], and batatasins are higher in the skin than the pulp [3], the lower concentration of batatasins observed by Ireland and Passam, 1985 [3] during the early stages of tuber development was likely due to differences in dry matter content and/or to the thin un-suberized skin present at these stages compared to mature tubers. Thus, it may be more useful to express the concentration of phenolics on dry weight bases than on fresh weight bases.

## 4. Conclusions

In summary, the level of phenolic compounds in *D. rotundata* is higher in developing tubers than at tuber maturity/ vine senescence. This suggests that developing tubers are already dormant. Whether they are involved in the initiation of dormancy, which begins at tuber formation or during tuber development rather than at that at vine

senescence remains to be investigated. After harvest, the concentration of phenolics decline but no individual phenolic compound declined in any clear pattern in this study. It is therefore suggested that phenolics may be involved in the release of dormancy.

## ACKNOWLEDGEMENTS

The authors wish to acknowledge Prof. Nick H. Battey, Mrs. J. Greenham, and staff of Phytochemistry Laboratory, Department of Plant Science, University of Reading, UK.

## REFERENCES

- [1] Gumbs, F. A. and Ferguson, T. U. (1976). Effect of tuber yield of the exposure of yam tubers to light. *Agronomy Journal*, 68, 831-832.
- [2] Enyi, B. A. C. (1973). Growth, Development and Yield of Some Tropical Root Crops. In: *Proceedings of the Third symposium of International Society of Tropical Root Crops held at IITA, Ibadan, Nigeria* (ed C. L. A. Leakey), pp.87-97. IITA, Ibadan.
- [3] Ireland, C. R. and Passam, H. C. (1984). The level and distribution of phenolic plant growth inhibitors in yam tubers during dormancy. *New Phytologist*, 97, 233-242.
- [4] Coursey, D. G. (1965). The role of yams in West African food economics. *World Crops*, 17, 74-82.
- [5] Onwueme, I. C. (1984). Yam. In: *The Physiology of Tropical Field Crops* (eds P. R. Goldsworthy and N. M. Fisher), pp. 569-585. John Wiley and Sons Ltd, London.
- [6] Osagie, A. U. (1992). *The Yam Tuber in Storage*. Post Harvest Research Unit, Department of Biochemistry, University of Benin, Nigeria.

- [7] Orkwor, G. C. and Ekanayake, I. J. (1998). Growth and Development. In: Food Yams: Advances in Research (eds G.C. Orkwor, R. Asiedu and I. J. Ekanayake), pp. 39-62. NRCRI and IITA, Ibadan, Nigeria.
- [8] Waitt, A. W. (1965). A key to some Nigerian varieties of yam (*Dioscorea rotundata*). Federal Department of Agric. Research, Memorandum, 60, Lagos, Nigeria.
- [9] Hamadina, E.I. and G. O. Eze, 2014. Pre Tuber Application of Fluridone: Effect on Vegetative Growth and Seed Tuber Dormancy in Yam (*D. alata*). Am. J. of Exp. Agric/ Vol.: 4 (4): 415-426.
- [10] Hashimoto, T., Hasegawa, K. and Kawarada, A. (1972). Batatasin: new dormancy-inducing substances of yam bulbils. Planta, 108, 368-374.
- [11] Hemberg, T. (1985). Potato Rest. In: Potato Physiology (ed P. H. Li), pp.353 -388 Academic Press, New York.
- [12] Gorham, J. (1995). The Biochemistry of the Stilbenoids. (eds M. Tori and Y. Asakawa). London, Chapman and Hall.
- [13] Ireland, C. R., Schwabe, W. W. and Coursey, D. G. (1981). The occurrence of batatasins in the Dioscoreaceae. Phytochemistry, 20, 1569-1571.
- [14] Farooqi, A. H. A., Shukla, Y. N., Sharma, S. and Bangerth, F. (1989). Endogenous inhibitors and seasonal changes in Absciscic acid in *Dioscorea floribunda* Mart. and Gal. Plant Growth Regulators, 8, 225-232.
- [15] Coxon, D. T., Ogundana, S. K. and Dennis, C. (1982). Antifungal phenanthrenes in yam tubers. Phytochemistry, 21, 1389-1392.
- [16] Glass, A. D. M. (1975). Inhibition of phosphate uptake in barley roots by hydroxy-benzoic acids. Phytochemistry, 14, 2127-2130.
- [17] Escher, B. I., Snozzi, M. and Schwarzenbach, R. P. (1996). Uptake, speciation, and uncoupling activity of substituted phenols in energy transducing membranes. Environmental Science and Technology, 30, 3071-3079.
- [18] Ile E. I., Craufurd, P. Q., Batty, N. H. Asiedu R., 2006, Phases of Tuber Dormancy in Yam (*Dioscorea rotundata* Poir), Annals of Botany 97: 497-504.
- [19] E. I., Ile, Control of Tuber Dormancy and Flowering in Yam (*Dioscorea rotundata* Poir.). PhD Thesis, The University of Reading, Reading, UK, 2004.
- [20] Ile E. I., Craufurd, P. Q., Batty, N. H. Asiedu R., 2006, Phases of Tuber Dormancy in Yam (*Dioscorea rotundata* Poir), Annals of Botany 97: 497-504.
- [21] Harborne, J. B. (1989). General Procedures and Measurement of Total Phenolics. In: Methods of Plant Biochemistry, Volume 1, Plant Phenolics. (eds J.B. Harborne), pp.1-28. Academic Press UK.
- [22] Hashimoto, T. and Tajima, M. (1978). Structures and synthesis of the growth inhibitors batatasins IV and V, and their physiological activities. Phytochemistry, 17, 1179-1184.
- [23] Ireland, C. R. and Passam, H. C. (1985). Effect of exogenous batatasin, batatasin analogues and gibberellins on the dormancy of stored yam tubers. Tropical Agriculture, 62, 41-46.
- [24] Gorham, J. (1989). Stilbenes and Phenanthrenes. In: Methods of Plant Biochemistry, Volume 1, Plant Phenolics (eds J. B. Harborne), pp 159-196. Academic Press, UK.
- [25] Van Sumere, C. F. (1989). Phenols and Phenolics Acids. In: Methods in Plant Biochemistry. Vol. 1. Plant Phenolics (eds P. M. Dey and J. B. Harborne), pp.29-73. Academic Press London.
- [26] Van Sumere, C. F., Cottenie, J. De Greef, J. and Kint, J. (1972). Biochemical Studies in Relation to the Possible Germination Regulatory Role of Naturally Occurring Coumarin and Phenolics. In: Recent Advances in Phytochemistry (eds V. C. Runeckles and J. E. Watin), vol. 4, pp.165-221. Appleton Century-Crofts, New York.
- [27] Passam, H.C. , S.J. Read and Rickard, J. E., 1978, The respiration of yam tubers and its contribution to storage loss Trop. Agric. 55, 207 -214.
- [28] Hamadina, E.I., Craufurd, P.Q., Battey, N. H. and Asiedu, R. 2010. In vitro microtuber initiation and dormancy in yam. Annals of Appl. Biol. 157: 203-212.
- [29] Okoli, O. O. (1980). Parameters for Selecting Parents for Yam Hybridization. In: Tropical Root Crops: Research Strategies for the 1980s, Proceedings of the First Triennial Symposium of the International Society for Tropical Roots Crops. Africa Branch September 1980 (eds E. R., Terry, K. O. Oduro and F. Caveness), pp.163-165. IDRC Publication.
- [30] Swanell, M. C., Wheeler, T. R., Asiedu, R. and Craufurd, P. Q. (2003). Effect of harvest date on the dormancy period of yam (*Dioscorea rotundata*). Tropical Science 2003, 43, 103-107.
- [31] Ketitu, A. O. and Oyenuga, U. A. (1973). Changes in the carbohydrate constituents of yam tuber during growth. Journal of Science, Food and Agriculture, 24, 367-373.