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NUTRITIONAL COMPOSITION AND ENZYME ACTIVITIES CHANGES OCCURING IN WATER YAM (*Dioscorea alata*) CULTIVAR "brazo" DURING THE POST-HARVEST STORAGE

Souleymane Sorh, Fankroma Martial Thierry Koné*, Siaka Binaté, Soumaïla Dabonné and Lucien Patrice Kouamé

Department of Food Science and Technology, University Nangui Abrogoua, Laboratory of Biocatalysis and Bioprocessing, 02 BP 801 Abidjan 02, Côte d'Ivoire.

*Corresponding author: fankrom@yahoo.fr

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ABSTRACT

The changes in nutritional composition and enzyme activities occurring during the post-harvest storage (0 to 7 months) of water yam (*Dioscorea alata*, cultivar "brazo") tubers grown in Côte d'Ivoire were investigated. Excepted ash, lipid, iron, phosphorus, potassium and sodium contents, some nutritional properties (moisture, protein, starch, magnesium and calcium) decreased significantly (P≤0.05), whereas reducing sugars increased during storage under prevailing tropical ambient conditions (31.25±3.00°C, 81.44±12.25% RH). The water yam contained no significant (P≤0.05) α-glucosidase, α-fucosidase, α-galactosidase, β-fucosidase, β-galactosidase, β-glucosidase, inulinase, invertase and xylanase activities during the storage period. The detected activities in *D. alata* were those of phosphatase, amylase and cellulase activities. However, the high phosphatase activity was found to decrease after 3 months storage (dormancy) and subsequently remained stable between 4 to 7 months storage. Contrasting this, low amylase and cellulase activities increased significantly. These enzymes are involved in the metabolic and physiological activities of yam tubers during the storage.

Keywords: *Dioscorea alata*, Yam tubers, Nutritional composition, Enzyme activities, Storage

INTRODUCTION

Yam (*Dioscorea* spp.) is a vegetatively propagated tuber food crop, belonging to the family *Dioscoreaceae* within the genus *Dioscorea*(Ezeocha and Oti, 2013).It is an energy-rich tuber and provides protein three times more superior than the one of cassava and sweet potato(Ezeocha and Ojimelukwe, 2012). Among the 600 species of the genus *Dioscorea*, six are cultivated for their edible corms, which constitute a staple food for many people in subtropical and tropical regions of the world(Coursey, 1983).The most economically important yam species are *Dioscorea rotundata*, *D. alata*, *D. cayenensis*, *D. bulbifera*, *D. trifida*, *D. opposita*, *D. dumentorum*, *D. japonica* and *D. hispida*(Riley *et al.*, 2006).In Côte d'Ivoire, yam plays an important role in food crop production with a production estimated at 5 million tons per year in 2001(Koné *et al.*, 2014) and the most cultivated species are *D. cayenensis-rotundata* complex and *D. alata*(Dje *et al.*, 2010).

Dioscorea alata commonly referred to as "winged yam", "water yam" or "greater yam" usually possesses tubers that are white, brown or brownish red in colour(Riley *et al.*, 2006).The water content of this tuber is usually high hence the name "water yam".Yam tubers of *D. alata* are also known for their high nutritional content, with crude protein content of 7.4%, starch content of 75-84%, and vitamin C content of13.0-24.7 mg/100g(Osagie, 1992). Moreover,*D. alata* is a crop with potential for increased consumer demand due to its low sugar content necessary for diabetic patients(Ezeocha and Ojimelukwe, 2012).

After harvesting, yam tubers are stored to preserve parts for consumption, for vegetative propagation, or preserved for the market when prices are higher. The storage ability of this yam is however restricted by the severe changes, which occur in tubers after harvest, and renders them unpalatable for human consumption. Indeed, fresh yams are difficult to store and are subject to deterioration during storage(Afoakwa and Sefa-Dedeh, 2001).Soon as yams are harvested at physiological maturity, they enter into dormancy.During such period, the tuber cells are metabolically quiescent(Osagie, 1992). This period assists storability since the longer the dormancy period in species of yam, the longer its storability. Oncethe dormancy is broken, sprouting inevitably resumes constituting an inroad to rapid carbohydrate metabolism and utilization, senescence and pathogenic invasion(Adeyemi, 2009). The termination of dormancy in yam leads to rapid biotransformation involving the release of hydrolases pre-existing in the tuber, as well as *de novo*

synthesis of enzymes involved in oxidative metabolism(Osagie, 1992).Moreover, the storage difficulties brought losses of 25% of the tuber production caused by physical and physiological factors(Girardin, 1996).The physical factors were temperature, relative humidity and the injuries.Sprouting, transpiration and respiration are physiological activities which depend on the storage environment mainly temperature and relative humidity(Osunde and Orhevba, 2009).Indeed, the metabolic activity is closely linked to those of the enzymes during the sprouting(Adesuyi, 1982). The role and influence of these enzymes could constitute an attractive biochemistry in the understanding of yams physiology from harvest to maturity as well as in storage since they were little studied in the whole yam tubers.

Thus, all these factors affect the nutritional quality of yam tubers. However, few studies have been carried out on the nutritional compositions and enzymes activities in yam tubers during the post-harvest storage period. The aim of this study was therefore to evaluate the quality of water yam (*D. alata*) cultivar "brazo" during the post-harvest storage by quantification of the proximate composition, mineral content and enzyme activities.

MATERIALS AND METHODS

MATERIALS

The cultivar of wateryam (*D. alata*) tubers named "brazo" was cultivated in experiment farm of University Nangui Abrogoua (Abidjan, Côte d'Ivoire)in West Africa. The field experiment was conducted in2006, 2007 and 2008. After harvesting, wateryam tubers were immediately transported to the laboratory and stored under prevailing tropical ambient conditions (31.25±3.00°C, 81.44±12.25% RH) for a period of 7 months of subsequent experiments. All other chemicals and reagents used were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO).

SAMPLING

Samples of stored tubers were collected at fixed time intervals (months 0, 1, 2, 3, 4, 5, 6 and 7). They were thoroughly washed with water, peeled, chopped into chips of 0.5 cm thickness, dried at 45°C in a ventilated oven (MMM MED CENTER) for 72 h. The dried chips were ground into flour in a hammer mill (BLENDOR) to pass through a 100µm sieve.

PROXIMATE COMPOSITION

Moisture contents were determined by drying in an oven at 105°C during 24 h to constant weight(AOAC, 1990).Ash contents were determined by incinerating flour (2 g) in a furnace at 550°C for 6 h, then weighing the residue after cooling to room temperature in a desiccator (AOAC, 1990). The ethanol-soluble sugars extraction was determined as described by Martinez-Herrera *et al.* (2006). The method described by Dubois *et al.* (1956)was used for the total sugar contents analysis. Reducing sugar contents were determined according to the method of Bernfeld (1955)using 3.5-dinitrosalycilic acid. Crude protein contents were calculated from nitrogen contents (N x 6.25) obtained using the Kjeldahl method (AOAC, 1990).Crude lipid contents were determined by continuous extraction in a Soxhlet apparatus for 8 h using hexane as solvent (AOAC, 1990).The starch content was determined by polarimetric method(BIPEA, 1976).

MINERAL COMPOSITION

Minerals of wateryam flour, such as calcium (Ca), iron (Fe), magnesium (Mg), potassium (K) and sodium (Na), were determined using AOAC(1990) method. Flour was acid-digested with a mixture of concentrated nitric acid (14.44 mol/l), sulfuric acid (18.01 mol/l) and perchloric acid (11.80 mol/l) and analyzed using an atomic absorption spectrophotometer (VARIAN, model AA-20). The total phosphorus of sample was estimated by the micro-calorimetric method described by Taussky and Shorr (1953). The results were expressed as mg/100g dry matter (mean ± SD) based on at least three replicate analyses.

ENZYMATIC ACTIVITY ASSAY

ENZYME EXTRACTION

Wateryam tubers (50 g) were ground in a grinder MOULINEX mark in 20 ml of NaCl 0.9% (w/v). The homogenate was subjected to sonication using a TRANSSONIC T420 for 10 min and then centrifuged at 6000 rpm for 30 min. The gotten supernatant was used as the crude extract and conserved at 4°C.

ENZYME ESSAY

Under the standard test conditions, *p*NP-glycosidases and *p*NP-phosphatase activities were measured by the release of *para*-nitrophenol (*p*NP). An essay mixture (250 µl) containing 75 µl of *p*NP-glycopyranoside or *p*NP-phosphate (5 mM) in 100 mM sodium acetate buffer (pH 5.0) with 50 µl enzyme solution, was incubated at 37°C for 10 min. The reference cell contained all reactants except the enzyme. The reaction was stopped by adding 2 ml of sodium carbonate (2%, w/v) and the absorbance of the essay solution was measured at 410 nm using a spectrophotometer GENESYS 5.

Polysaccharidases activities were assayed by the 3.5-dinitrosalicylic acid (DNS) procedure(Bernfeld, 1955), using 1% (w/v) polysaccharide (carboxymethyl cellulose, sucrose, inulin, xylan and starch) as substrate. The enzyme (50 µl) was incubated for 30 min at 37°C with 170 µl sodium acetate buffer (100 mM, pH 5.0) and 80 µl polysaccharide. The reaction was stopped by adding 300 µlDNS solution and heating for 5 min in boiling water bath. The absorbance was read at 540 nm after cooling on ice for 5 min.

One unit of enzyme activity was defined as the amount of enzyme capable of releasing one µmol of *p*NP or glucose per min under the defined reaction conditions. Specific activity was expressed as units per mg of protein (Units/mg of protein).

STATISTICAL ANALYSIS

All analyses reported in this study were carried out in triplicates. Mean value and standard deviation were

calculated. Statistical significance was established using Analysis of Variance (ANOVA) models to estimate the effect of post-harvest storage on each nutrient and enzymatic activities in water yam tubers. Means were separated according to Duncan's multiple range analysis (P≤0.05), with the help of the software STATISCA 7.1 (Stat Soft Inc, Tulsa USA Headquarters).

RESULTS AND DISCUSSION

BIOCHEMICAL COMPOSITION

The proximate composition of wateryam (*D. alata*) cultivar "brazo" during 7 months of storage are showed in Table 1. The post-harvest storage led to a significant (P≤0.05) variation in the biochemical composition.

The high moisture content of wateryam tubers *D. alata* "brazo" at harvest decreased from 68.38 to 60.00%, representing 8.38% moisture loss after 7 months of storage. This result is comparable to those obtained by Sefa-Dedeh and Afoakwa (2002) who reported moisture loss of about 8% in trifoliate yam *D. dumetorum* tubers even 72 h after harvest. In the study of Trèche and Agbor-Egbe (1996), a moisture loss of 31% and 35% was observed after 110 days of storage for *D. rotunda* and *D. dumetorum*, respectively. It has been reported that all tubers are generally prone to rapid moisture loss on storage(Sreerag *et al.*, 2014). The rapid dehydration could be due to the little difference in the storage environment mainly temperature and relative humidity(Passam *et al.*, 1978).High respiratory activity of tubers on storage contributes to the severe water loss from the tissues(Sreerag *et al.*, 2014).

Table 1 - Effect of post-harvest storage on proximate composition (g/100g dry matter) of yam tubers *D. alata* cultivar "brazo"

Storage time (month)	Moisture	Ash	Proteins	Lipids	Reducing sugars	Total sugars	Starch
0	68.38±1.43^{ab}	1.78±0.03^{a}	5.78±0.36^{a}	0.76±0.10^{a}	1.30±0.31^{a}	3.50±0.10^{d}	87.70±4.70^{a}
1	69.80±0.44^{b}	1.75±0.06^{a}	5.81±0.33^{a}	0.70±0.07^{a}	1.60±0.08^{b}	2.67±0.05^{bc}	84.40±4.01^{b}
2	65.80±1.29^{ab}	1.74±0.04^{a}	5.83±0.14^{a}	0.72±0.11^{a}	1.79±0.06^{c}	2.60±0.14^{b}	81.20±2.68^{c}
3	65.30±2.00^{a}	1.73±0.04^{a}	5.44±0.14^{b}	0.68±0.08^{a}	1.97±0.07^{d}	2.40±0.11^{a}	78.02±3.08^{d}
4	63.70±1.43^{ab}	1.64±0.03^{a}	5.40±0.45^{ab}	0.68±0.07^{a}	2.06±0.31^{d}	2.40±0.10^{a}	74.24±1.85^{e}
5	63.20±0.44^{ab}	1.64±0.06^{a}	5.40±0.33^{ab}	0.65±0.10^{a}	2.15±0.08^{d}	2.40±0.05^{a}	71.06±2.98^{f}
6	61.40±1.30^{a}	1.60±0.04^{a}	5.57±0.14^{ab}	0.68±0.07^{a}	2.33±0.06^{e}	2.60±0.34^{b}	64.14±4.01^{g}
7	60.00±2.00^{a}	1.69±0.04^{a}	5.44±0.14^{ab}	0.69±0.10^{a}	2.50±0.07^{f}	2.72±0.41^{c}	58.51±5.05^{h}

Note: Means with different letter for the same parameter are significantly different at the 5% level according to Duncan's multiple range test.

Storage did not cause any significant (P≤0.05) changes in levels of ash, crude protein and lipid (Table 1). The ash and crude protein level of water yam *D. alata* "brazo" varied from1.78% (month 0) to 1.69% (month 7) and 5.78% (month 0) to 5.44% (month 7), respectively. Changes in ash and/or crude protein contents have been reported during the post-harvest storage of yam tubers after 56 days (Medoua *et al.*, 2005), 110 days (Trèche and Agbor-Egbe, 1996), 2 months (Ezeocha and Oti, 2013), 4 months (Sreerag *et al.*, 2014) and 6 months (Osunde and Orhevba, 2009). The slight decrease in protein content may have been affected by tannins reported to form complexes with protein, limiting their availability (Osunde and Orhevba, 2009).

Lipid contents (0.68 to 0.76%) of *D. alata* "brazo" were in agreement with those reported by Amani and Kamenan(2003)for Ivorian yam varieties "*Florido*" and "*Kponan*" (0.70-1.10%). They were found to be relatively higher than those of two traditional Nigerian yam barns (0.18-0.27%)(Osunde and Orhevba, 2009) and two edible Ivorian yams *D. alata* and *D. cayenensis-rotundata* (0.20%)(Dje *et al.*, 2010) stored 6 months after harvest.

The reducing sugars contents of wateryam *D. alata* "brazo" ranged from 1.30 to 2.50% and appeared to increase significantly (P≤0.05) during the post-harvest storage times. This increase may be due to the yam starch hydrolysis in to simple sugars (Panneerselvam and Jaleel, 2008). The reducing sugars level in wateryam *D. alata* "brazo" was found to be higher than those of several tropical yams from the south pacific region (0.12 to 1.03%) (Bradbury, 1988). It has been suggested that reducing sugars in flour may cause caking and damping during their storage because of sugar's hygroscopic property. Indeed, sugars may be desirable in bakery products like bread and cake where the tenderizing effects positively affect texture and where sugars serve as substrate for fermentation of the dough (Koné *et al.*, 2014).

The initial total sugars content (3.50%) of wateryam *D. alata*"brazo" gradually reduced to 2.40% after 3-5 months of post-harvest storage, and slightly increases to 2.72% at the final storage period (7

months).The total sugars average contents (2.40-3.50%) of *D. alata*"brazo" during storage were similar to those reported for yam *D. dumetorum* ("*Jakiri*" cultivar) (2.8-4.5%) after 19 weeks storage(Treche, 1989), for yams *D. alata* ("*Florido*" variety) (2.48-9.10%) and *D. cayenensis-rotundata* ("*Krenglè*" variety) (2.40-8.17%) after 6 monthsstorage (Dje *et al.*, 2010).

Table 1 shows that starch represented the largest single constituent of wateryam *D. alata*"brazo". Its level decreased significantly (P≤0.05) during the post-harvest storage from 87.70% (month 0)to 58.51% (month 7).During storage, stored starch is hydrolyzed to sugars for physiological activities such as respiration hence the increases in sugar content with corresponding decreases in starch content. It is known that sugar and starch exist in a state of dynamic equilibrium during storage. There are reports in the literature of starch breakdown, mainly glucose and sucrose to maltose and fructose during storage of tubers of *D. alata* and *D. rotundata*(Wireko-Manu *et al.*, 2013). Passam *et al.*(1978) obtained similar result and attributed it to the metabolic processes in yam, which convert starch to sugar. Such degradation of starch from yam tubers during storage was also reported in the literature (Sefa-Dedeh and Afoakwa, 2002; Sreerag *et al.*, 2014).

MINERAL COMPOSITION

The mineral analysis of wateryam *D. alata*"brazo" during the post-harvest storage is presented in Table 2. The results reveal that the magnesium and calcium contents differed significantly (P≤0.05) during the post-harvest period studied. Magnesium was found to be the most abundant mineral, ranging from 230 to 266 mg/100g dry matter (DM) over 7 months of post harvest storage. These levels were higher than those obtained by Dje *et al.*(2010) in edible yamtubers *D. alata* ("*Florido*" variety) (38.15-57.32mg/100g dry weight) and *D. cayenensis-rotundata* ("*Krenglè*" variety) (25.35-38.33mg/100g dry weight) stored during 6 months. From a nutritional point of view, wateryam *D. alata*"brazo" may be considered as a good source of calcium indicated from our study. The calcium contents, ranging from 148 to 185 mg/100g DM, were higher than reported values for several yam species (Bradbury, 1988; Bhandari *et al.*, 2003; Sahoré and Amani, 2005; Dje *et al.*, 2010). Most of the calcium in yam tubers occurs principally as raphides as calcium oxalate, and also within starch grains where it serves storage functions and acts as nuclei for starch deposition (Okoli and Green, 1987).This study shows also that iron was the least abundant mineral in wateryam *D. alata*"brazo" and the post-harvest storage did not cause any significant changes (P≤0.05) in the levels of the other minerals such as phosphorus, potassium, sodium and iron. The level of potassium (around 90.00 mg/100g DM) appeared to be lower than values reported by other researchers for some yam species (Wanasundera and Ravindran, 1994; Sahoré and Amani, 2005). The mean values of phosphorus (41.00 mg/100g DM), sodium (20.00mg/100g DM) and iron (10.52 mg/100g DM) contents obtained from wateryam *D. alata*"brazo" are relatively comparable to those reported in previous works (Bradbury, 1988; Trèche and Agbor-Egbe, 1996; Bhandari *et al.*, 2003). This discrepancy possibly could be due to the species origin, the type of soil it was harvested from, the maturity of the cropand environmental differences. It is quite clear from our results that wateryam (*D. alata*"brazo") tubers studied has a potential for long-term storage under tropical ambient conditions without causing highly significant changes in their mineral composition.

Table 2 - Effect of post-harvest storage on mineral composition (mg/100g dry matter) of yam tubers *D. alata* cultivar "brazo"

Storage time (month)	Mg	Ca	P	K	Na	Fe
0	266±24.93^{b}	185±18.14^{d}	41±0.48^{a}	90±0.01^{a}	20±0.01^{a}	10±1.41^{a}
1	230±20.12^{a}	172±14.35^{cd}	40±1.74^{a}	90±0.01^{a}	20±0.01^{a}	11±1.38^{ab}
2	241±17.25^{ab}	148±17.03^{a}	40±1.70^{a}	90±0.01^{a}	20±0.01^{a}	10±1.40^{a}
3	263±22.51^{ab}	182±17.11^{abcd}	44±1.93^{a}	90±0.01^{a}	20±0.01^{a}	13±1.48^{b}
4	255±20.30^{ab}	171±17.52^{bcd}	39±0.98^{a}	90±0.01^{a}	20±0.01^{a}	10±1.20^{a}
5	240±18.42^{ab}	166±12.60^{abcd}	40±0.68^{a}	89.5±0.01^{a}	20±0.11^{a}	10.5±0.10^{a}
6	236±15.60^{ab}	155±13.02^{abc}	42±1.01^{a}	89±0.01^{a}	18±0.11^{a}	10±1.10^{a}
7	232±14.80^{a}	150±14.50^{ab}	41±0.75^{a}	89±0.01^{a}	18±0.01^{a}	9.7±1.08^{a}

Note: Means with different letter for the same parameter are significantly different at the 5% level according to Duncan's multiple range test.

***p*NP-GLYCOSIDASES, *p*NP-PHOSPHATASE AND POLYSACCHARIDASES ACTIVITIES**

Enzymes activities inwateryam *D. alata*"brazo" are significantly (P≤0.05) different during the post-harvest storage period (Fig. 1 and 2). α-Glucosidase, α-fucosidase, α-galactosidase, β-fucosidase, β-galactosidase and β-glucosidase activities were very low and decrease during the storage, like wise some of them totally disappear after 2 months storage (Fig. 1). However, the most elevated activity was observed with phosphatase activity. Indeed, the initial phosphatase activity (20.70Units/mg) of wateryam *D. alata*"brazo" increases significantly

(P≤0.05) to reach its optimal activity (36.53 Units/mg) at 3 months storage (dormancy), and then gradually decreases to stabilize between 4 to 7 months storage (13.10 Units/mg) (Fig. 1).

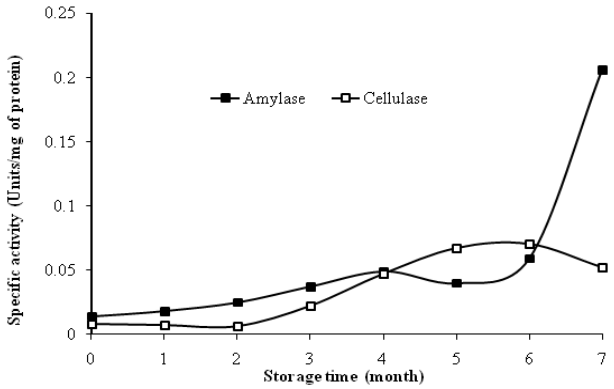

Figure 1. Amylase (■) and cellulase (□) activities in yam tuber *D. alata* cultivar "brazo" during the post-harvest storage.

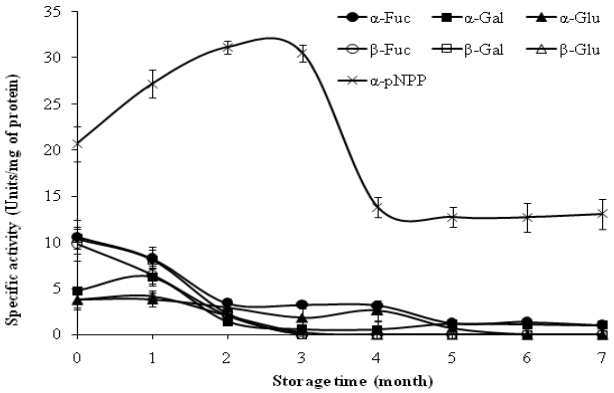

Figure2. Enzyme activities in yam tuber *D. alata* cultivar "brazo" during the post-harvest storage. Symbols: α-fucosidase (●), α-galactosidase (■), α-Glucosidase (▲), β-fucosidase (○), β-galactosidase (□), β-glucosidase (△) and phosphatase (×) activities.

As regards the polysaccharide activities, no significant (P≤0.05)inulinase, invertase and xylanase activity was observed during the storage period studied. However, wateryam *D. alata*"brazo" containlow amylase and cellulase activities (Fig. 2). These two activities were found to slightly increase significantly (P≤0.05) during storage to reach an optimal value of 0.21 Units/mg at the final storage period (7 months) for amylase, while the highest cellulase activity (0.07 Units/mg) was observed at 6 months storage.

Starch and cellulose are two polymers of glucose, which are major constituents of yam tuber. They are the target substrates of amylase and cellulase, respectively. These enzymes are involved in the metabolic and physiological activities of yam tubers during the storage like germination(Dabonné *et al.*, 2011). The increase of cellulase activity causes the progressive degradation of cellulose, which is the main component of fiber in the tuber(Kouadio, 2004).During the post-harvest storage, the starch breakdown can occur by either of two pathways: the hydrolytic (amylolytic) and the phosphorolytic pathways (Weise *et al.*, 2011). For wateryam *D. alata* "brazo", our results show that the phosphorolytic pathway predominates during the dormancy period, and the amylolytic pathway begins as soon as the dormancy is broken. Knoth(1993) reported that the duration of natural dormancy fluctuate according to the variety of yam between 4-18 weeks. The stability of phosphatase activity observed between 4 to 7 months storage could be explained by the fact that the starch degradation occurs by the two pathways. In yam, starch hydrolysis partly involves α-amylase whose initial dormancy induced low activity at the onset of storage gradually culminated in enhanced activities after breakage of dormancy. During the post-harvest storage of tubers from taro *Xanthosoma sp*.cultivar "atoumbou oronô", Kouadio(2004) stipulated that the increase in amylase activity demonstrates the dominance of the amylase pathway. Kamenan (1984) showed that the strong presence of phosphatase activity in yam tubers *D. cayenensis-rotundata* during the storage could probably be the consequence of the use of the acid phosphate as supplier of inorganic phosphate (Pi) during the phosphorolyse.

CONCLUSION

This study revealed the effects of post-harveststorage on some nutritional properties and enzymes activities inwateryam tubers *D. alata*"brazo". Proximate composition like moisture, protein and starch contents decreased significantly (P≤0.05) during post-harvest storage period, while reducing sugars increased. However, ash and lipid contents were not affected significantly (P≤0.05) by the storage period. Wateryam *D. alata* "brazo" was also found to be fairly good sources of dietary minerals, with high magnesium and calcium contents. A selective presence of enzymatic activity was observed during the yams' storage. This activity varied significantly (P≤0.05) during the storage period and phosphatase activity was found to be the best expressed activity. These enzymes are really involved in the metabolic and physiological activities of yam tubers during the storage. However, understanding the role of these enzymes in yam physiological processes could be an attractive biochemistry. The results suggest that, wateryam *D. alata* "brazo" has a good potential to be used in food industry and it is necessary to take into consideration the state of the tubers during the post-harvest storage.

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