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Changes in Some Enzymatic Activities in Different Tuber Parts of *Dioscorea alata* During Post-Harvest Storage

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Original Research Article

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Abstract: In this study, changes in some enzymatic activities in different tuber parts of "Florido" and "Bètè-bètè" cultivars belonging to *Dioscorea alata* species were investigated during post-harvest storage. Indeed, the studied enzymatic activities were those of acid phosphatase and *p*NP-glycosidases (α-glucosidase, α-fucosidase, α-galactosidase, β-glucosidase and β-galactosidase, α-mannosidase) and polysaccharidases (amylase, cellulase and inulinase). The results showed that the different tuber parts of both cultivars contained acid phosphatase and α-mannosidase activities. Besides, "Bètè-bètè" cultivar also possessed α-galactosidase. As for the polysaccharidases activities, the different parts of both cultivars had amylase and cellulase activities. Otherwise, the detected acid phosphatase, α-mannosidase and α-galactosidase activities in yam tuber part decreased with the storage time while the amylase and cellulase activities increased with the storage time. The enzymatic activities were found to be high in the distal tuber part for both cultivars. This work revealed that both cultivars didn't contain α-glucosidase, α-fucosidase, α-galactosidase, β-glucosidase, β-galactosidase and inulinase activities. Our results could constitute an attractive biochemistry in the understanding of yams physiology during post-harvest storage.

Keywords: *Dioscorea alata*, cultivar, enzymatic activity, tuber part, post-harvest storage

INTRODUCTION

Yam belongs to the genus Dioscorea. It is a major staple food for an estimated 60 million people in the region stretching from Ivory Coast to Cameroon, an area commonly referred to as "Yam Zone" of West Africa [1-2]. Yam tubers are an excellent source of carbohydrate, energy, vitamins (especially vitamin C), minerals and protein. Indeed, yams contain 80-90 % carbohydrates, 5-8 % protein and about 3.5 % minerals [3]. They are also high in starch content. Yam also plays a major role in the socio-cultural life of a wide range of smallholder households especially in the dominant production zone of West Africa and it is also recognized as a prestige food crop in many African countries [4]. Yams (*Dioscorea spp*) are the third most important tropical "root" crop after cassava and sweet potato [5]. Many different forms and cultivars of the edible yam species are available in different areas and it is likely that these differ in composition and nutritional values [6]. The most common problems faced by farmers are the losses of the produce during post-harvest and storage [7]. These losses are due to physical and physiological causes. The physical factors are temperature, relative humidity and the injuries. Sprouting, transpiration, respiration are physiological factors which depend on the storage environment mainly temperature and relative humidity [8]. Besides, the rot due to mould and bacteriosis, insects, nematodes and mammals was also physiological factor [9]. Indeed, these physiological factors affect the internal composition of tuber and result in destruction of edible material, which under normal storage conditions can often reach 10% after 3 months, and up to 25% after 5 months of storage [9]. The storage time of yam tuber under fresh shape is five (5) months and even on a longer time according to [10]. Morphologically yam tubers can be divided into three distinct regions, the 'head', 'middle' and the 'tail'. Yam tuber is generally cylindrical and often broader at the proximal end (head) and gradually tapers towards the distal end (tail) [11]. This has been showed by Dégras [12], who noted that yams tuber consists of three portions (the distal portion, the median portion and the proximal portion). Otherwise, the metabolic activity is closely linked to

those of the enzymes during the sprouting [13]. Some works on enzymatic activities were studied in the whole yam tuber. Indeed, Diopoh and Kamenan [14] and Houvet *et al*., [15] carried out the works on the amylases activities in the yams whole tubers. Sorh *et al.,* [16] were studied enzyme activities changes occurring in whole water yam tuber, cultivar "brazo" during the post-harvest storage. Research conducted on the tubers showed increasing levels of sugars and cell wall polysaccharides constituents and increases in texture during storage of tubers, with substantial decreases in moisture and starch contents [17]. This decrease in starch content could be due to result from the activities of constituent amylases, converting starches into sugars and causing textural changes in the tubers. Enzymatic profile of two cultivar of *Dioscorea cayenensis-rotundata* tuber parts during storage have been reported by Dabonné *et al.,* [18]. On the other hand, there is not information on enzymatic activities in *Dioscorea alata* cultivars tuber part during post-harvest storage. Therefore, it is necessary to evaluate enzymatic activities and then follow them during storage up to six (06) months.

MATERIAL AND METHODS

Plant material

Tubers of two yam cultivars were used in this study. Those are "Florido" and "Bètè-bètè" cultivars belonging *D. alata*. These yam tubers were harvested at physiological maturity (after drying of leaves and stems) in village fields (Douibo, Bomizambo and Koubi) in the department of Tiébissou (located in the center of Côte d'Ivoire, 284 kilometers from Abidjan, Latitude: 7.16306; Longitude: -5.22056). The tubers were without injury and 44.07 ± 4.46 cm long. After the harvest, they were immediately transported in a heap aired store and stored under prevailing tropical ambient conditions (26.56°C ± 3°C and 82% ± 5% RH) for a period of 6 months of subsequent experiments.

Sampling

Six (6) tubers of each yam cultivar were randomly selected every two months during for up to six (6) months. Each of them has been cut into three equal parts each representing the proximal part (tuber head), the median part (tuber middle) and the distal part (tuber tail).

Enzyme extraction

Each lot of tuber part (50 g) was 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 obtained supernatant was used as the crude extract and conserved at 4 °C.

Enzyme assay

Acid phosphatase activity was performed in a total volume of 210 µl, containing 125 µl of sodium acetate buffer (100 mM; pH 5.0), 50 µl of enzyme crude extract and 75 µl of substrate *p*NPP (5 mM). The reaction mixture was incubated at 37 °C for 10 min, then 2 ml of Na2CO3 2% (w/v) were added to stop the reaction and absorbance was measured at 410 nm using a spectrophotometer SPECTRONIC. Para-nitrophenol (*p*NP) was used as standard. Under the above experimental conditions, one unit of enzyme activity was defined as 1 µmol of *p*NP released per min. Specific activity was defined as the units of enzyme activity per mg of protein.

Under the standard test conditions, *p*NP-glycosidases activities were measured by the release of *p*-nitrophenol from the substrate *p*-nitrophenyl-glycopyranoside. An assay mixture (210 µl) containing 125 µl of sodium acetate buffer (100 mM; pH 5.0), 50 µl of enzyme crude extract and 75 µl of substrate *p*-nitrophenyl-glycopyranoside, was incubated at 37°C for 10 min. The control contained all reactants except the enzyme. The reaction was stopped by the addition of sodium carbonate (2 ml) at a concentration of 1 M, and absorbance of the reaction mixture was measured at 410 nm. Finally, specific activity has been determined such as in the previous case.

Polysaccharidases activities were assayed by the dinitrosalicylic acid procedure [30], using 1 % (w/v) polysaccharide (carboxymethylcellulose, inulin and starch) as substrate. The enzyme (50 µl) was incubated for 30 min at 37°C with 170 µl buffer (100 mM acetate, pH 5.0) and 80 µl polysaccharide. The reaction was stopped by addition of 300 µl dinitrosalicylic acid and heating in boiling water for 5 min. 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*-nitrophenol or glucose per min under the defined reaction conditions. Specific activity was expressed as units per mg of protein (U/mg of protein).

Protein assay

Protein concentrations were determined by method of Lowry *et al*., [19] using bovine serum albumin as a standard.

Statistical analysis

All analyses were performed in triplicates. Statistical significance was established using Analysis of Variance (ANOVA) models to estimate the effect of storage time on enzymatic activities of different yam tuber parts. Means were separated according to Duncan's multiple range analysis ($P \leq 0.05$), with the help of the software Statistica 7.1 (StatSoft Inc, Tulsa USA Headquarters).

RESULTS

Acid phosphatase and *p*NP-glycosidases activities

The different tuber parts of *Dioscorea alata*, "Florido" cultivar had acid phosphatase and α-mannosidase activities (Fig-1). These enzymatic activities in the distal part were higher than those obtained in other yam tuber parts. Indeed, the obtained values of acid phosphatase and α-mannosidase activities in distal tuber part ranged from 0.04 to 0.01 UI/mg of protein and from 0.04 to 0.00 UI/mg of protein respectively. The both enzymatic activities decreased meaningfully (P≤0.05) with the storage time. This decrease was more marked in the second month of storage time. From fourth month of storage time, detected enzymatic activities in proximal and median parts decreased up to 0.00 UI/mg of protein in the sixth month while those of distal tuber part stabilized from fourth month to sixth month of storage time. Acid phosphatase activity disappeared in the proximal portion after four (4) months of storage. Moreover, the results indicated that the different tuber parts of *Dioscorea alata*, "Florido" cultivar didn't contain α-glucosidase, α-fucosidase, α-galactosidase, β-glucosidase and β-galactosidase activities. Statistical analyzes showed that the detected enzymatic activities differed significantly (P≤0.05) between them.

As for the tuber parts of *Dioscorea alata*, "Bètè-bètè" cultivar, the results indicated that acid phosphatase, α-mannosidase and α-galactosidase activities were detected in the three tuber parts during storage (Fig-2). The highest enzymes activities were observed in distal tuber part. Indeed, acid phosphatase, α-mannosidase and α-galactosidase activities varied from 0.04 to 0.02 UI/mg of protein, from 0.04 to 0.02 UI/mg of protein and from 0.03 to 0.00 UI/mg of protein respectively. These enzymatic activities decreased significantly (P≤0.05) during post-harvest storage. From fourth month of storage, α-galactosidase activity disappeared in the three tuber parts. Furthermore, the study showed that there also weren't α-glucosidase, α-fucosidase, β-glucosidase and β-galactosidase activities in different tuber parts of "Bètè-bètè" cultivar as in tuber parts of "Florido" cultivar. Statistical tests showed that the revealed enzymatic activities differed meaningfully (P≤0.05) between them.

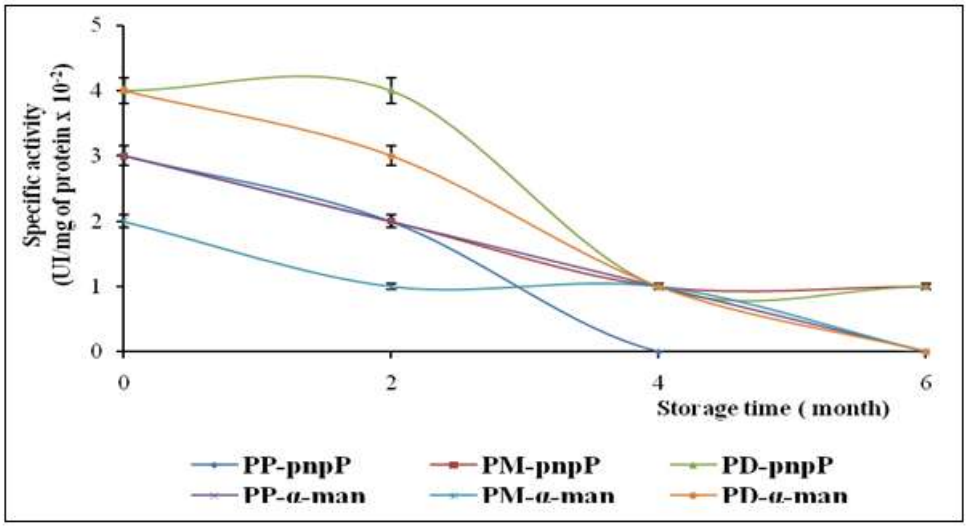

Fig-1: Acid phosphatase and α-mannosidase activities in the different parts of *Dioscorea alata* "Florido" cultivar tuber during the post-harvest storage.

PP: proximal part; PM: median part; PD: distal part

α-man : α-mannosidases activity ; *p*NPP : phosphatases activity

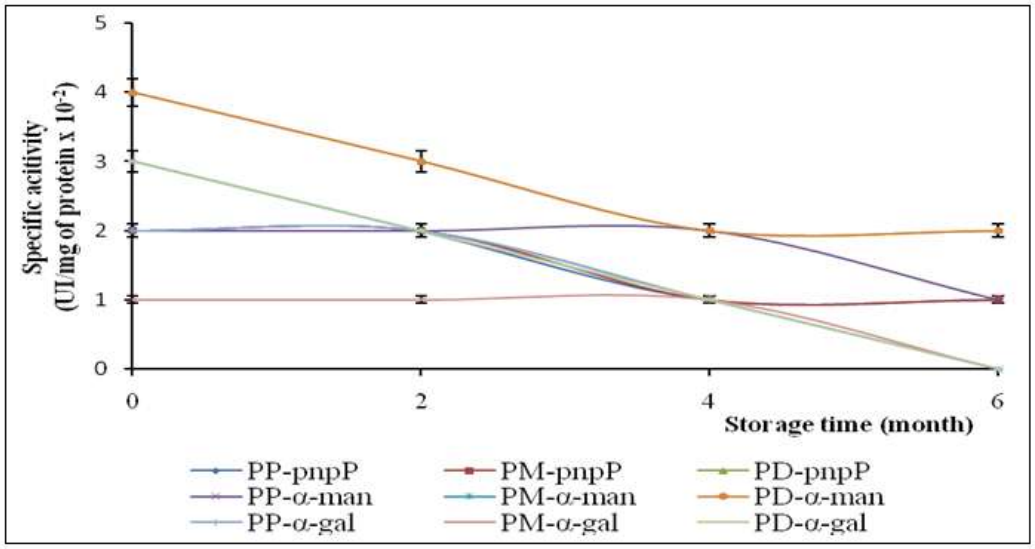

Fig-2: Acid phosphatase and α-mannosidase activities in the different tuber parts of *Dioscorea alata* "Bètè-bètè" cultivar during the post-harvest storage.

PP: proximal part; PM: median part; PD: distal part

α-man: α-mannosidases activity ; *p*NPP: phosphatases activity

Polysaccharidases activities

The polysaccharidases activities such as Amylase, inulinase and cellulase activities were studied in the different yam tuber part during post-harvest storage (Fig 3-6). The results revealed the absence of inulinase activities in the three tuber part during post-harvest storage whatever the variety is. One the other hand, amylase and cellulase activities were observed in the different yam tuber part of both cultivar of *Dioscorea alata* species. These enzymes activities in distal tuber part were found to be higher than those observed in other yam tuber part for "Bètè-bètè" and "Florido"cultivars. Indeed, amylase and cellulase activities in distal tuber part of "Bètè-bètè" cultivar ranged from 0.04 to 0.08 UI/mg of protein and from 0.02 to 0.06 UI/mg of protein respectively (Fig 3 and 4). As for the distal tuber part of "Florido" cultivar, amylase and cellulase activities varied from 0.01 to 0.05 UI/mg of protein and from 0.02 to 0.07 UI/mg of protein respectively (Fig 5 and 6). Otherwise, Statistical analyzes indicated that these enzymatic activities in different yam tuber part increased significantly ($P \leq 0.05$) during post-harvest storage for the both cultivars.

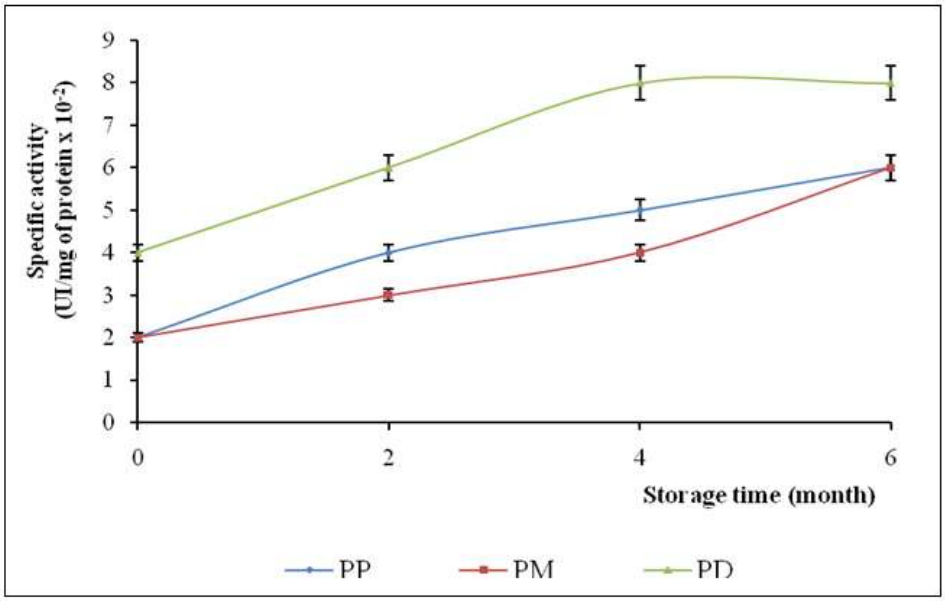

Fig-3: Amylase activity in the different tuber parts of *Dioscorea alata* "Bètè-bètè" cultivar during the post-harvest storage

PP: proximal part; PM: median part; PD: distal part

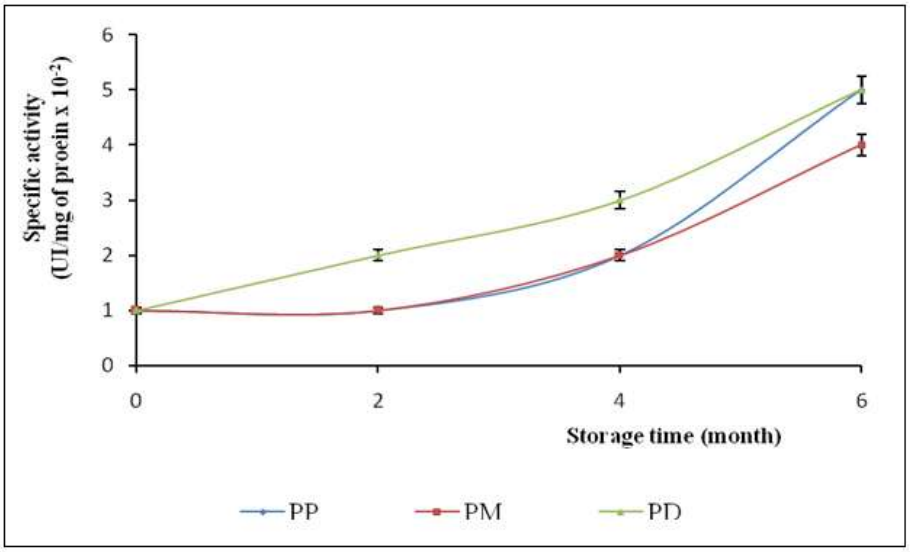

Fig-4: Amylase activity in the different tuber parts of *Dioscorea alata* cultivar "Florido" during the post- harvest storage

PP: proximal part; PM: median part; PD: distal part

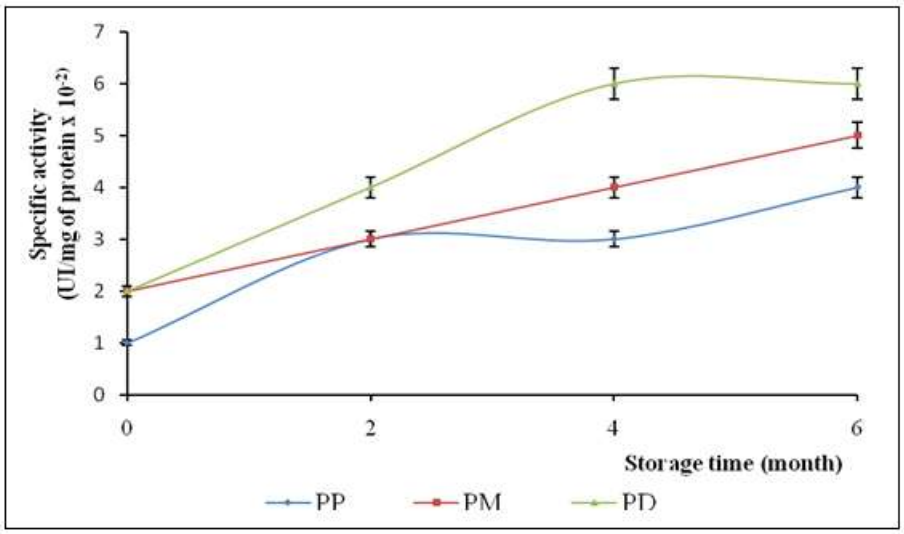

Fig-5: Cellulase activity in the different parts of *Dioscorea alata* "Bètè-bètè" cultivar tuber during the post-harvest storage

PP: proximal part; PM: median part; PD: distal part

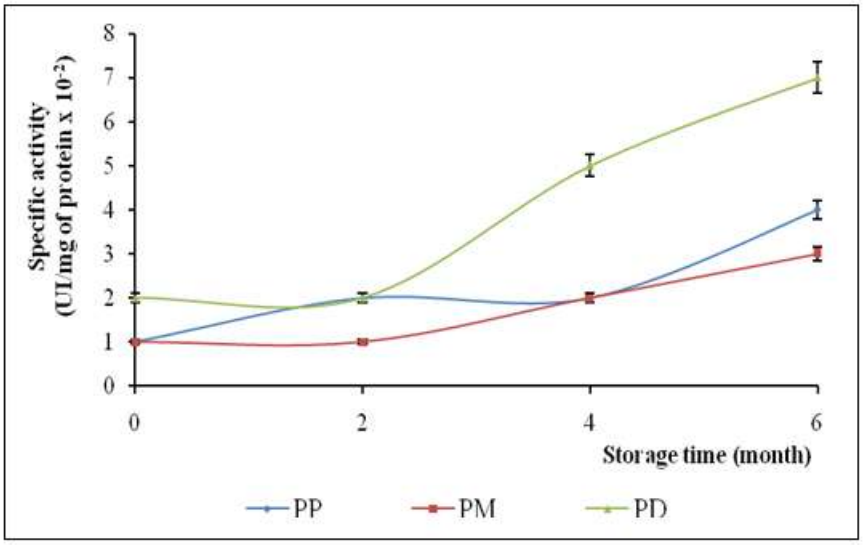

Fig-6: Cellulase activity in the different parts of *Dioscorea alata* cultivar "Florido"tuber during the post-harvest storage

PP: proximal part; PM: median part; PD: distal part

DISCUSSION

The Acid phosphatase activity and the *p*NP-glycosidases activities such as α-mannosidase and α-galactosidase activities were observed in all tuber parts of "Florido" and "Bètè-bètè" cultivars (*Dioscorea alata*). The results showed that these enzymatic activities decreased significantly ($P \leq 0.05$) during the post-harvest storage. Similar studies were carried out by Dabonné *et al.*, [18], who reported the decrease of acid phosphatase activity and the *pNP*-glycosidases activities in different tuber parts of "Kangba" and "Krenglè" cultivars (*Dioscorea cayenensis-rotundata*) during post-harvest storage. Our results were agreement with the findings of Sorh *et al.* [16], who indicated the decrease of Acid phosphatase and *p*NP-glycosidases activities in whole tuber of "brazo" cultivar (*D. alata*) during post-harvest storage. The strong presence of acid phosphatase activity in the different yam tuber parts during the pos-harvest storage could probably be the consequence of the use of acid phosphate as supplier of inorganic phosphate (Pi) during the phosphorolyse [20]. This author showed the enzyme decrease in the whole tubers of *Dioscorea cayenensis-rotundata* species. Moreover, the phosphatase activity decrease during storage may result from the strong depletion of phosphorylated macromolecules in the constitution of yam tuber. The low α-mannosidase activity found in different tuber part of both yam cultivars is linked to the presence of polymer of mannose ranging from 93.90 % to 95.40% of mucilage content in the yam tuber [21]. It could intervene in the hydrolysis of these molecules. Otherwise, the highest enzyme activities in distal yam tuber parts is due to the fact that this region is the youngest and most active region during dormancy, appear to be more favoured by the conditions under natural Light storage [9].

As for the α-glucosidase, α-fucosidase, β-glucosidase and β-galactosidase, there wasn't in the different yam tubers parts during post-harvest storage. α-glucosidase logically hydrolyses maltose and the maltodextrins that are the last products of the starch hydrolysis by amylase. But it seems that during the six months of storage enough maltose and maltodextrin was not produce to allow expression of α-glucosidases. In general, this absence would imply the absence of substrates for these enzymes. Similar results have been reported by Dabonné *et al*., [18] on different tuber parts of "Kangba" and "Krenglè" cultivars (*Dioscorea cayenensis-rotundata*).

Starch and cellulose are two polymers of glucose, which are major constituents of yam tuber. They are the target substrates of amylase and cellulose respectively. These enzymes are involved in the metabolic and physiological activities of yam tubers during the storage like germination [18]. Amylase and cellulase activities increased in all tuber parts "Florido" and "Bètè-bètè" cultivar (*Dioscorea alata*) during post-harvest storage. This increase suggests that they would be actively involved in the metabolic and physiological activities of yam tubers during post-harvest storage. Indeed, the presence of amylases activities in tubers and their increase during post-harvest conservation suggests a possible increased hydrolysis of starch. Indeed, the changes in the starch and sugar contents are attributed to the hydrolysis of starch to sugar during storage. This assertion has been confirmed on *D. dumetorum* tubers after harvest by Afoakwa and Sefa-Dedeh [17], who noted that the changes in amylase enzyme activities result in the hydrolysis of starches into sugars. According to Osagie [10], sugar and starch exist in a state of dynamic equilibrium during storage. Moreover, Degradation of starch appears to involve the cooperative attack by phosphorolytic and hydrolytic

activities [22, 23]. Similar trends have been recorded by Sorh *et al*., [16], who showed the changes in amylases actitivies and their increase with the storage time. Amylase activities would therefore be negatively correlated with the starch levels in the different parts of the yam tubers and positively with the total and reducing sugar levels in the same tissues because their contents increase during post-harvest conservation [24].

Otherwise, the presence of cellulase activity and its increase during post-harvest storage would suggest the existence of natural fibers in the yams tubers studied [25], because cellulose is the essential constituent of these fibers. For example, cellulase could be used to hydrolyze fibers during post-harvest storage. This assertion was supported by Gohl [26] and Kouadio [27], who indicated that the increase of cellulase activity causes the progressive degradation of cellulose, which is the main component of fiber in the tuber. In general, the increase of amylase and cellulase activities would be stimulated by the phenomenon of germination according to Ikediobi and Oti [28].

Inulinases activity didn't detect in yam tubers studied during post-harvest storage. This lack of activity could be explained by the absence or low level of inulin in yam tubers. Indeed, inulin is a fructose polymer belonging to the class of dietary fiber called fructans. It is used by plants such as chicory root, Jerusalem artichokes or dahlias onions, burdock, big elk and Echinacea as a source of energy storage in the same way as starch. Thus, plants that don't store starch, synthesize and accumulate it [29].

CONCLUSION

Profiles of changes some enzymatic activities were investigated during post-harvest storage. The results of this work indicated that there were Acid phosphatase activity and the *pNP*-glycosidases activities such as α-mannosidase and α-galactosidase activities in proximal, median and distal parts of "Florido" and "Bètè-bètè" cultivars (*Dioscorea alata*). These parts also contained amylase and cellulase activities. During post-harvest storage, acid phosphatase and *p*NP-glycosidase activities in different yam tuber part decreased while amylase and cellulase activities increased. This study revealed that the different tuber part of both yam cultivar didn't contain α-glucosidase, α-fucosidase, β-glucosidase and β-galactosidase and Inulinase activities. Results from our research will provide data about shelf life of yams and could constitute an attractive biochemistry in the understanding of yams physiology during post-harvest storage.

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