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Influence of harvesting time on the phenolic composition, antioxidant activities and anti-cholinesterase potential of processed yellow yam (*Dioscorea cayenensis*)

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ABSTRACT

The present study seeks to evaluate the influence of the time of harvest on the phenolic composition, antioxidant activities and anti-cholinesterase action of processed yellow yam (Dioscorea cayenensis). HPLC-DAD analysis of the processed yellow yam revealed the presence of some phenolic acids and flavonoids. The result showed a higher amount of most of the identified phenolic compounds in the early harvested yellow yam. The result also showed a high level of most phenolic compounds in the raw yellow yam for both early and late harvested yam sample compared to their cooked counterpart. Similarly, the result of the antioxidant indices (total phenolic content, total flavonoid content, reducing power, ABTS and DPPH radical scavenging power) also ranked high in the early harvested and raw yellow yam sample compared to the late harvested and cooked yellow yam sample respectively. However, the anticholinesterase inhibitory action of the studied yellow yam revealed some level of inhibition only in the late harvested yellow yam with a slightly higher inhibition in the late harvested raw yellow yam compared to the cooked counterpart. Therefore the studied yellow yam when subjected to mild cooking process and harvested not very late could serve well as a functional food that could be harnessed in the management of free radical mediate diseases.

Key words: Harvesting Time, phenolic composition, antioxidant activities, anti-cholinesterase action.

INTRODUCTION

In recent years, there is an upsurge in the areas related to new developments in prevention of disease, particularly in the role of free radicals and antioxidants. Oxygen free radicals or, more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are formed in all cells as unwanted by-products of metabolism and as such can be regarded as "toxic agents" with regard to their potential for initiating intracellular damage [1]. It is increasingly being realized that many of the modern human diseases are due to oxidative stress initiated by over production of ROS [2]. In a normal healthy human body, the generation of pro-oxidants in the form of Reactive Oxygen Species (ROS) and Reactive Nitrogenous Species (RNS) are effectively kept in check by various antioxidants. Lack of antioxidants, to remove excess reactive free radicals, leads to different diseases, like cancer, neurodegenerative and inflammatory disorders [3]. Natural antioxidants from plant origin are more beneficial in reducing ROS levels, due to synergistic actions of wide range of bio-molecules such as phenolic compounds, vitamin C, vitamin E and phyto-micronutrients [4].

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants [5]. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects [6-8].The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity [9]. Phenolic compounds have been identified as the major determinant of antioxidant potentials of foods [10], and could therefore be a natural source of antioxidants.

Roots and tubers are the most important food crops of very ancient origin in the tropics and subtropics, associated with the human existence, survival, and their socio-economic history. Yam is one of the most important cultivated and commonly consumed tubers in Nigeria and regarded as medicinal food in traditional herbal medicine [11]. *Dioscorea cayenensis*, "yellow yam", is native to Africa, just as white yam "*Dioscorea rotundata*, though not as commonly consumed as white yam. Yam contains phytochemicals that can affect human health such as phyto-estrogen (isoflavones), saponins, terpenes, carotenoids [12-13]. Recent studies have shown that yam has antioxidant activities, anti-carcinogenic effect, antihypertensive effect, [14], and cholesterol lowering effects. Yam has high fiber content, this helps in weight control; it also helps to reduce cholesterol levels, thereby lowering risk of heart diseases. Yam is rich in carotenoid and it can reduce a woman's risk of developing ovarian cancer [12]. It is a great source of vitamin C which lowers blood pressure. Yams have also been used to treat menopausal symptoms folklorically. It has been discovered that yam can improve the status of sex hormones (estrogen), lipids and antioxidants [15].Yam tubers can be stored up to six months without refrigeration, which makes them a valuable resource during the period of food scarcity at the beginning of wet season [16].

The phytochemical content of plant foods is influenced by post-harvest factors including storage conditions and processing procedures and also by numerous pre-harvest factors, including genotype, root-stock, climatic conditions, agronomic practices and harvesting time [17-19]. Studies have also shown that phenolic compounds in plant foods may vary according to the growth stage, the part of the plant and the characteristics of the environment [20-21].

Therefore, the aim of this study is to evaluate the effect of time of harvesting on the phenolic composition, antioxidant activities and anti-cholinesterase action of yellow yam.

MATERIALS AND METHODS

2.1 Chemicals

2-Deoxy-D-ribose (Cat No - #D5899), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Cat No -#11557), 2,2-Diphenyl-1-picrylhydrazyl (Cat No - #D9132), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), hydrogen peroxide (H_2O_2), ferrous sulphate, potassium dichromate ($K_2Cr_2O_7$), Ferric chloride ($FeCl_3$), Methanol, Folin-Ciocalteu's phenol reagent, sodium bicarbonate, aluminum chloride, potassium acetate, sodium phosphate dibasic, sodium phosphate monobasic, di-sodium phosphate, potassium ferricyanide, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), orthophosphoric acid, quercitin, hydrochloric acid, sulphuric acid, chloroform, calcium chloride, vitamin C, tannic acid, sodium carbonate, aluminium chloride, gallic acid, quercetin, ascorbic acid, glacial acetic acid were obtained from Sigma chemical company, USA. The chemicals used were of analytical grades while the water was glass distilled.

2.2 Sample treatment and preparation

The tubers were peeled, chipped and washed to remove dirts and divided into two groups. The first group was boiled in a sterilised container at 100 ^{o}C until soft (as eaten) while the second group was left raw. Both groups were then sun dried for 3 days and milled into fine powder taking caution to avoid contamination. Both the cooked and raw samples were stored in a plastic container at room temperature in the Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria.

2.3 Preparation of methanolic extracts

Ten grams of yam flour was mixed with 80ml of methanol and left overnight. The suspension was filtered through whatman filter paper no 42. The filtrate was put in a measuring cylinder and then made up to 100ml mark with methanol. The filtrate was put inside amber bottle and stored at -4°C until it was used.

2.4 Quantification of phenolic compounds by HPLC-DAD

Reverse phase chromatographic analyses were carried out under gradient conditions using C18 column (4.6 mm x 150 mm) packed with 5µm diameter particles; the mobile phase was water containing 2% acetic acid (A) and methanol (B), and the composition gradient was: 5% of B until 2 min and changed to obtain 25%, 40%, 50%, 60%, 70% and 100% B at 10, 20, 30, 40, 50 and 80 min, respectively, following the method described by Amaral *et al*. [22] with slight modifications. Cooked and raw yellow yam was analyzed at a concentration of 15 mg/ml. The flow rate was 0.7 ml/min, injection volume 50 µl and the wavelength were 254 nm for gallic acid, 280 nm for catechin and epicatechin, 325 nm for chlorogenic, caffeic and ellagic acids, and 365 nm for isoquercitrin, quercitrin, quercetin, rutin, kaempferol and luteolin. The samples and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.025 – 0.300 mg/ml for isoquercitrin, quercitrin, quercetin, rutin, luteolin and kaempferol; and 0.040 – 0.250 mg/ml for gallic acid, chlorogenic acid, caffeic acid, ellagic acid, catechin and epicatechin. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 600 nm). All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by Sabir *et al*. [23] LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

2.5 Antioxidant indices

2.5.1 Total phenolic content (TPC)

The total phenolic content of the extracts was determined by the Folin-Ciocalteu assay as described by Waterman and Mole [24]. 500µL of Folin reagent was added and mixed with a solution containing 100µL of the extract and 2ml of distilled water. 1.5mL of 7.5% sodium carbonate was then added to the solution and the volume was made up to 10mL with distilled water. The mixture was left to stand for 2 h after addition of the sodium carbonate and the absorbance of the mixture was measured at 760 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer, USA). The standard used was tannic acid and the result was expressed as mg tannic acid equivalents per gram of the sample.

2.5.2 Total flavonoid content (TFC)

The total flavonoid content of the extracts was determined using a slightly modified method reported by Meda *et al*. [25]. Briefly, 0.5mL of appropriately diluted sample was mixed with 0.5mL methanol, 50µL of 10% $AlCl_3$, 50µL of 1mol L^{-1} potassium acetate and 1.4mL water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of each reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using quercetin as standard by making use of a seven point standard curve (0-40 µg/ml or 0-100 µg/ml), the total flavonoids content of samples was determined in triplicates and the results were expressed as mg quercetin equivalent per gram of the sample.

2.5.3 Ferric reducing antioxidant power (FRAP)

The reducing power of the extracts was determined by assessing the ability of each extracts to reduce $FeCl_3$ solution as described by Oyaizu [26]. Briefly, appropriate dilution of each extract (1ml) was mixed with 1ml of 200 mM sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. Each mixture was incubated at 50^{o}C for 20 min and then 1 ml of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 2ml of the supernatant was mixed with 2ml of distilled water and 0.4 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant power was determined in triplicate and expressed as mg ascorbic acid equivalent/g of the sample.

2.5.4 ABTS antiradical assay

An antioxidant activity of the extracts was determined using the 2, 2'-azinobis-(3- ethylbenzothiazoline-6-sulfonic acid) ABTS antiradical assay [27]. The $ABTS^{\bullet+}$ (mother solution) was prepared by mixing equal volumes of 8 mM ABTS and 3 mM potassium persulphate ($K_2S_2O_8$) (both prepared using distilled water) in a volumetric flask, which was wrapped with foil and allowed to react for a minimum of 12 h in a dark place. The working solution was prepared by adding 2.5 ml of the mother solution with 7.5 ml phosphate buffer (pH 7.4). A range of trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-carboxylic acid) standard solutions (100–1000 µM) were prepared in acidified methanol. The working solution (2.9 ml) was added to the methanol extracts (0.1 ml) or Trolox standard (0.1 ml) in a test tube and mixed with a vortex. The test tubes were allowed to stand for exactly 30 min. The

absorbance of the standards and samples were measured at 734 nm with a Lambda EZ150 spectrophotometer. The results which were determined in triplicates were expressed as µM Trolox equivalents/g sample, on dry weight basis

2.5.5 DPPH antiradical assay

The DPPH assay was done according to the method of Brand-Williams *et al*. [28], with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100mL methanol and then stored at -20^{o}C until needed. The working solution was obtained by mixing 10mL stock solution with 45mL methanol to obtain an absorbance of 1.1 units at 515 nm using the spectrophotometer. Phenol extracts (300µl) were allowed to react with 2700 µl of the DPPH solution for 6 h in the dark. Then the absorbance was taken at 515 nm. Results which were determined in triplicates were expressed in µM Trolox Equivalent/g sample. Additional dilution would be needed if the DPPH value measured was over the linear range of the standard curve.

2.6 Acetylcholinesterase and butrylcholinesterase inhibitory activity assay

Acetylcholine esterase (AChE) and butrylcholine esterase (BuChE) inhibitory activity was measured by the spectrophotometric method developed by Ellman *et al*. [29] with slight modifications. Briefly, 1mL of 10mM 5, 5'-Dithiobis-(2-Nitrobenzoic) acid (DTNB) dissolved in 10mM sodium phosphate buffer (pH 7.0) was added to 0.6ml of distilled water. 0.1ml of brain homogenate (enzyme source) and 0.1ml of the sample was then added to the mixture and incubated for 2 minutes at 25˚C before 0.2ml 8mM acetylcholine iodide (substrate) was added. The absorbance of the mixture was read at 412nm at intervals of 30 seconds for 5 minutes immediately after the substrate was added. For the control, 0.1ml of brain homogenate (enzyme source) was added to 1ml of 10mM DTNB dissolved in 10mM sodium phosphate buffer (pH 7.0) and 0.7ml of distilled water. The mixture was incubated at 25˚C for 2 minutes before 0.2ml 8mM of acetylcholine iodide was added and the absorbance was taken immediately. 1ml of distilled water and 1ml of 10mM DTNB was used as blank. The procedure was repeated using 8mM buytrlcholine iodide as substrate. The results were expressed in µmol min^{-1}mg $protein^{-1}$ using a molar extinction coefficient $13.6\times103M^{-1}cm^{-1}$.

2.7 Statistical analysis

All the analyses were run in triplicates. Results were then computed using Microsoft Excel software (Microsoft Corporation, Redmond, WA) and followed by one –way Anova Duncan's Multiple Range Test (DMRT) to compare the means that showed significant variation by using SPSS 11.09 for windows. The significance level was set at $p < 0.05$.

RESULTS

3.1 HPLC-DAD PHENOLIC ESTIMATION

The HPLC-DAD quali-quantitative estimates of phenolic compounds of early harvested (August, 2014) and late harvested (December, 2014) raw and cooked yellow yam (*Dioscorea cayenensis*) is as shown Figure 1(a-d) and Table 1. The qualitative estimates (Figure 1a-d) revealed the presence of gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, epicatechin, rutin, quercetrin, quercetin and kaempferol in the raw and processed yellow yam samples harvested during two distinct period of the year. The identified phenolic compounds were essentially phenolic acids and flavonoids. The result revealed a higher level of most of the identified phenolic compounds in the early harvested raw yellow yam compared with the early harvested cooked yellow yam with the exception of catechin, chlorogenic acid, ellagic acid and quercetin. Similarly, the same trend was observed in the late harvested raw yam with higher amount of the quantified phenolic compounds in the raw yellow yam compared with the cooked yellow yam, with exception of rutin, quercetin and kaempferol.

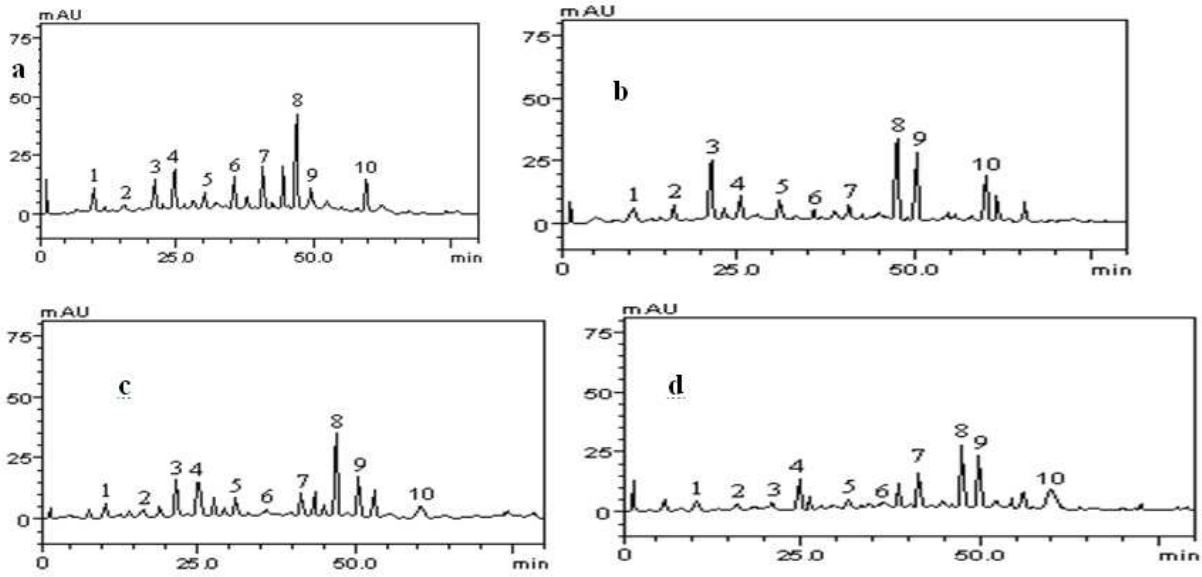

Figure 1 – HPLC-DAD chromatographic profiles of (a). Raw (b). Cooked early harvested yellow yam and (c). Raw (d). Cooked late harvested yellow yams: Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), epicatechin (peak 6), rutin (peak 7), quercitrin (peak 8), quercetin (peak 9), kaempferol (peak 10)

Table1– Quantitative Estimates of Phenolic Compounds of Early and Late Harvested Yellow Yam

Compounds	*Yam Yellow*				LOD	LOQ
	EHRYY (mg/g)	EHCYY (mg/g)	LHRYY (mg/g)	LHCYY(mg/g)	µg/mL	µg/mL
Gallic acid	3.56±0.02	1.65±0.01	1.62±0.01	1.49±0.03	0.013	0.045
Catechin	0.91±0.01	1.59±0.03	0.94±0.01	0.85±0.01	0.021	0.078
Chlorogenic acid	3.87±0.01	4.83±0.01	4.07±0.03	0.81±0.01	0.030	0.102
Caffeic acid	4.15±0.03	2.71±0.01	3.91±0.02	3.59±0.02	0.019	0.062
Ellagic acid	1.73±0.02	2.64±0.01	1.65±0.03	1.48±0.01	0.027	0.089
Epicatechin	3.89±0.01	0.87±0.03	0.89±0.01	0.79±0.03	0.008	0.025
Rutin	4.18±0.01	1.45 ± 0.03	1.74±0.02	4.36±0.01	0.020	0.067
Quercitrin	9.13±0.02	7.29±0.01	7.18±0.01	6.15±0.02	0.033	0.115
Quercetin	3.52±0.01	6.01±0.02	4.69±0.03	5.97±0.01	0.015	0.059
Kaempferol	4.08±0.03	3.95±0.01	1.71±0.01	3.25±0.01	0.028	0.096

Results are expressed as mean ±standard deviations (SD) of three determinations. LOD= Limit of Detection, LOQ= Limit of Quantification. EHRYY= Early Harvested Raw Yellow Yam, EHCYY = Early Harvested Cooked Yellow Yam, LHRYY= Late Harvested Raw Yellow Yam, LHCYY= Late Harvested Cooked Yellow Yam.

3.2 ANTIOXIDANT INDICES

Studies have attributed that antioxidant properties are due to the presence of phenolic acids and flavonoids [30-31]. The antioxidant indices of processed early and late harvested yellow yam are as shown in Table 2. The result showed a higher phenolic content in the early harvested yellow yam (Raw: 79.32±7.53; Cooked: 47.34±6.63) compared with the late harvested counterpart (Raw: 44.69±13.02; Cooked: 37.81±6.15. The result also showed a reduced phenolic content after cooking. Similarly, higher flavonoid content and ferric reducing antioxidant power was recorded for both raw and early harvested yellow yam.

DPPH radicals react with suitable reducing agents, during which the electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up. Similarly, the decolorization of $ABTS^{+}$ cation radical is an unambiguous way to measure the antioxidant activity of phenolic compounds. ABTS chemistry involves direct generation of ABTS radical mono cation with no involvement of any intermediary radical, it is a decolorization reaction and thus the radical cation assay is performed prior to addition of antioxidant test system, rather than the generation of the radical to occur continuously in the presence of antioxidants. The two assays have been used widely to evaluate the antioxidant activity of various natural products.

The result of the antiradical action also revealed high ABTS and DPPH radical scavenging activities for both raw and early harvested yellow yam compared to the cooked and early harvested yellow yam.

Table 2: Antioxidant Indices of Early Harvested and Late Harvested Yellow yam

Sample	Total phenolic content (mg TAE/g)	Total flavonoid content (mg QE/g)	Reducing power (mg AAE/g)	$ABTS^{\cdot+}$ (µM TE/g)	$DPPH^{\cdot}$ (µM TE/g)
EHRYY	79.32± 7.53	2.12± 0.03	33.028± 2.97	421.71± 46.65	70.78± 1.36
EHCYY	47.34±6.63	0.91± 0.08	23.59± 3.70	289.19± 56.14	61.36 ± 1.14
LHRYY	44.69±13.02	1.041± 0.07	12.797±1.82	307.75± 33.60	41.09± 0.43
LHCYY	37.805±6.15	0.65± 0.07	9.656± 0.97	202.97±95.55	37.29± 0.48

Values represent mean ± standard deviation of triplicate experiments. TAE= Tannic Acid Equivalent; QE= Quercetin Equivalent; AAE= Ascorbic Acid Equivalent; TE=Trolox Equivalent. EHRYY= Early Harvested Raw Yellow Yam; EHCYY = Early Harvested Cooked Yellow Yam; LHRYY= Late Harvested Raw Yellow Yam; LHCYY= Late Harvested Cooked Yellow Yam.

3.3 ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITY

The anticholinesterase action of processed early and late harvested yellow yam is as shown in Figures 2-3. The result showed no acetylcholinesterase and butryrylcholinesterase inhibitory action for the early harvested yellow yam but a substantial inhibitory action was recorded for the late harvested yellow yam. The result also showed a slightly higher inhibitory action in the late harvested raw yellow yam compared with the cooked counterpart.

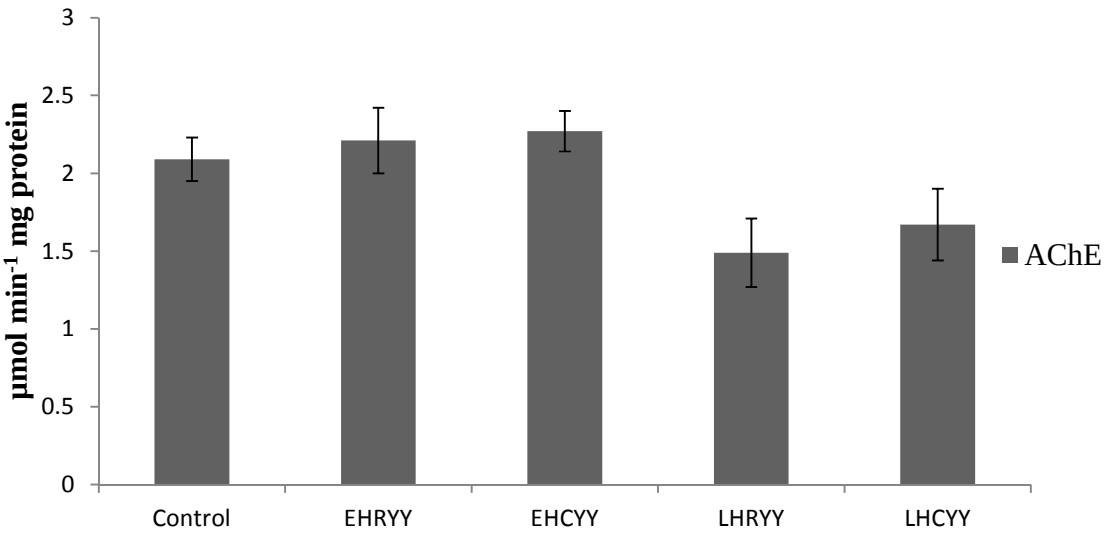

Figure 2: Effect of early and late harvested yellow yam extracts on brain acetyl- cholinesterase (AChE). EHRYY = Early Harvested Raw Yellow Yam; EHCYY = Early Harvested Cooked Yellow Yam; LHRYY = Late Harvested Raw Yellow Yam; LHCYY = Late Harvested Cooked Yellow Yam

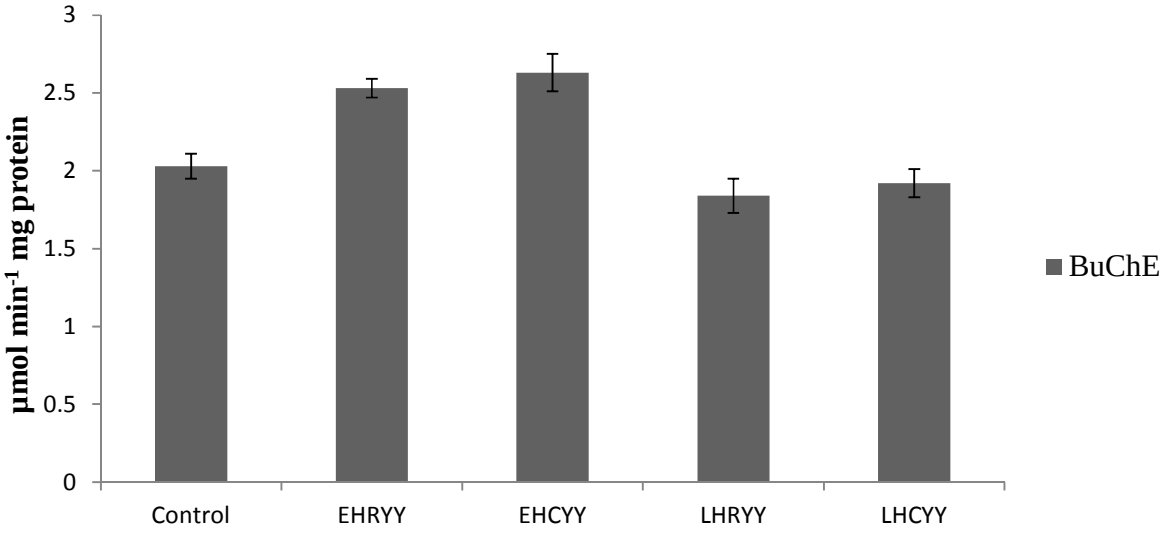

Figure 3: Effect of early and late harvested yellow yam extracts on brain butyrylcholinesterase (BuChE). Keys: EHRYY = Early Harvested Raw Yellow Yam; EHCYY = Early Harvested Cooked Yellow Yam; LHRYY = Late Harvested Raw Yellow Yam; LHCYY = Late Harvested Cooked Yellow Yam

DISCUSSION AND CONCLUSION

Over the years, exploration of natural products has been on the increase leading to the identifications of plant products beneficial to mankind. Interest in the health benefits produced by phenolic compounds has increased in recent years because of their proven potent antioxidant capacity [32]. Phenolic compounds exhibit different biochemical and pharmacological properties [33], and the total antioxidant activity of plant foods is related to their phenolic content [34]. Phenolic compounds have attracted much interest recently because *in vitro* studies suggest that they have a variety of beneficial biological properties like anti-inflammatory, anti-tumor and anti-microbial activities [35-38].

Previous report has identified cyanidin-3- glucoside, catechin, procyanidin dimers 'B-1' and 'B-3', coumarin, quercetin and gallic acid as phenolic constituents of *Dioscorea alata* tubers [39-40]. Phenolic compounds are commonly found in plants and have been reported to have several biological activities [41-43]. The result revealed a higher level of most of the identified phenolic compounds in the early harvested raw yellow yam compared with the early harvested cooked yellow yam with the exception of catechin, chlorogenic acid, ellagic acid and quercetin. Similarly, the same trend was observed in the late harvested raw and cooked yam with higher amount of the quantified phenolic compounds in in the raw compared with the cooked yellow yam, with exception of rutin, quercetin and kaempferol. This observation agrees with the report of Ukom *et al*. [44] which showed that cooking decrease the polyphenol content in *Xanthoma maffa* tuber. Similar observation was made by Didier *et al*. [40] who reported decreasing phenolic compounds in the tubers of Dioscorea *alata* after cooking. The result further showed higher phenolic compounds in the early harvested yellow yam compared to their late harvested raw and cooked counterparts. This observation is in agreement with the result of Remorini *et al*. [45]. They reported that the higher maturity of fruit cause a lower phenolic content and postulated that this may be attributed to the series of chemical and enzymatic changes like glycoside hydrolysis by glycosidase, phenolic compounds oxidation by phenol oxidase and polymerization of free phenolic compounds [45]. It has also been reported that phenolic compounds such as flavanol and cyaniding-3-glucoside of nectarine gold cultivar decrease during fruit maturity [46].

The higher phenolic content observed in the early harvested yam compared to the late harvested counterpart is in tandem with the observed trend in the quantitative estimation of some phenolic compounds where higher level of the identified compounds were reported for the early harvested yam compared with the late harvested yam [45-46].

The reduction in total phenolic compounds during cooking might be due to cooking treatment which may destroy some heat sensitive phenolic compounds [13, 47, 44]. The reduced total flavonoid content is in agreement with the

report of Ezeocha and Ojimelukwe [48]. They reported that flavonoids are significantly reduced in boiled tubers of water yam. Flavonoids are potent water- soluble antioxidants which prevent cell damage and have strong anti-haemorhoidal activity [49]. Flavonoids have been found to possess antioxidant and anti-inflammatory activities and were also useful for sexual stimulation [50]. Flavonoids had the record of being a powerful water soluble free radical scavengers and powerful antioxidants which could prevent oxidative cell damage, have a potent anticancer activity and inhibited tumour growth [51]. It also contains hydroxyl functional group, which are responsible for antioxidant effect in some medicinal plants. The higher ABTS and DPPH radical scavenging activities observed for both raw and early harvested yellow yam may be ascribed to strong positive correlation existing between total phenolic content and the antioxidant activities of plant foods [52-53].

Inhibition of cholinesterase is a promising approach for the treatment of Alzheimer's disease (AD) and for possible therapeutic applications in the treatment of Parkinson's disease, ageing, and myasthenia gravis [54]. Plant alkaloids are best known for inhibiting cholinesterase enzymes, however recent reports has indicated new classes of cholinesterase-inhibiting phytochemicals such as coumarins, flavonols, terpenoids, especially monoterpenes [55-58], thus making a phenolic compounds containing plant foods a good candidate in the management and prevention Alzheimer's disease, Parkinson's disease, ageing, and myasthenia gravis. The reduced anticholinesterase after cooking might be due to the destruction of some heat sensitive bioactive compounds [13, 47, 44].

Previous report showed the cholinesterase inhibitory action of methanolic extract of *Dioscorea bulbifera* L [59]. The cholinesterase inhibitory action that was enhanced in late harvested yam may be attributed to chemical modification and enzymatic changes of phenolic compounds and other phytochemical that occurred during aging [45], which in turn could have a positive effect on the bioactivity of the phyto-constituents.

CONCLUSION

The result of this investigation revealed a higher phenolic content, and radical scavenging activities in the early harvested (August) yellow yam compared to the late harvested (December) yellow yam. The result also revealed that cooking brings about a marked reduction in the phenolic content and antioxidant activities in the studied yellow yam and also a slight reduction in the acetylcholinesterase and butyrylcholinesterase inhibitory action of late harvested yellow yam. Furthermore, the anti-cholinesterase action of the studied yam showed no observable activity for the early harvested (August) yellow yam but a substantial inhibitory action in the late harvested (December) yellow yam. Therefore it could by hypothesized from the result that the studied yellow yam when subjected to mild cooking process and harvested not too late (somewhere around October) so as not to prolong the chemical modification and enzymatic changes that occurs in plant during aging and maturity, would make the studied yam serve better as a functional food, and therefore, could be used in the management of free radical mediate diseases.

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