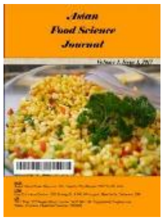

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Nutritional and *In vitro* Glycemic Properties of Selected Indigenous Tubers

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Authors' contributions

This work was carried out in collaboration between all authors. Author JHG designed the study, wrote the first draft of the manuscript and managed the analyses of the study. Author VNE wrote the protocol and managed the literature. Author ABA managed the analyses of the study and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Tacca involucrata, *Dioscorea angawa* and *Dioscorea bulbifera* are tubers which are usually boiled or roasted and consumed without sauce by the rural population in the Northern part of Nigeria. This study was conducted to evaluate the nutritional and *in vitro* glycemic properties of three indigenous tubers (*Tacca involucrata*, *Dioscorea angawa* and *Dioscorea bulbifera*). These tubers were subjected to processing. A batch was processed into raw flours, another batch was boiled for 30 min and another batch boiled for 1 h after which the samples were dried at 60°C for 48 h to obtain boiled treated flour samples. The flour samples were then analysed for proximate composition, minerals, amylose/amylopectin content, alpha amylase and alpha glucosidase using -standard methods. The results of the study indicated that boiling significantly ($P<0.05$) increased the carbohydrate content of the raw flour samples from 79 to 81%, 79 to 84% and 85 to 88% for *Dioscorea angawa*, *Tacca involucrata Dioscorea bulbifera* resspectively. Amylopectin content increased from (69.38±0.02%, 70.80±0.02%, 71.95±0.01%) in the raw samples to (76.56±0.05%,

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74.50±0.01%, 73.32±0.01%) after 1 h boiling for *Dioscorea angawa*, *Dioscorea bulibifera*, *Tacca involucrata*, respectively. *In vitro* Alpha amylase increased its activity from (38.27±0.01 mg/g, 49.16±0.06 mg/g, 53.51±0.01 mg/g) in the raw samples to (70.29±0.5 mg/g, 65.93±0.01 mg/g, 118.76±0.03 mg/g) after 1 h boiling for *Dioscorea angawa*, *Dioscorea bulbifera* and *Tacca involucrata*, respectively and *in vitro* alpha glucosidase activities of the tubers increased from (566.11±0.01 mg/g, 603.25±0.01 mg/g, 644.43±0.04 mg/g) in the raw samples to (822.03±0.07 mg/g, 992.14±0.01 mg/g 1014.12±0.01 mg/g) in samples boiled for 1 h. Boiling these tubers for 1 h increased the rate of activity of the in vitro enzymes involved in the breaking down of starch to simple sugars.

Keywords: Tacca involuncrata; Dioscorea bulbifera; Dioscorea angawa; nutritional; glycemic properties.

1. INTRODUCTION

Roots and tubers are the most important food crops since time immemorial in the tropics and subtropics [1]. Roots and tubers refer to any growing plant that stores edible material in subterranean root, corm and tuber [2]. *Tacca involuncrata*, *Dioscorea bulbifera* and *Dioscorea angawa* are indigenous tubers common to the rural farmers in Benue state. *Tacca involucrata* also known as Amura plant is a crop found in the family of Taccaceae of the genus Tacca. Tacca involucrata is a crop popular for its natural starch with almost zero fat [3]. This crop is usually processed into flour (starch) which is used for the preparation of sauce and also porridge compared to yam porridge.

Angawa is among the *Dioscorea* spp, which are climbing plants with globorous leaves and twining stems, which coil readily around a stake. The underground tubers maybe in single or in cluster weighing several kg with individual yields measuring 13-17 cm long and 6-8 cm wide. The angawa harvested single could have a tuber yield of 20-25 cm long and 20-25 cm wide and usually seen to be winged. It is usually grown in the form of mixed cropping; its maturity and harvesting period is done at the same time as water yam. The tubers are usually washed and boiled whole together with the peels, and peeled with knife or crushed with fingers because of its light skin. *Dioscorea bulbifera* belongs to the Order Dioscoreal, Family Dioscoreaceae, and Genus Dioscorea [4]. It is a vigorously twining, long-stemmed herbaceous vine which may arise from an underground tuber, although often tubers are inconspicuous or absent. Conspicuous aerial tubers (called bulbils) are pale, round to globose in shape, up to 13 cm wide and are formed in leaf axils [5].

The nutritional value of these crops lies in their potential ability to provide one of the cheapest sources of dietary energy in the form of carbohydrates which accounts for approximately 70-90% w/w of the tubers [6].

With the prevalent rate of consumption of these indigenous tubers by both the young and old, which are high in carbohydrate contents and most often without protein accompaniment. It is necessary to analyse these indigenous tubers to ascertain their nutritional and *in vitro* glycemic properties so as to have good control of glycemic response which plays a role in preventing a varied disease indirectly including diabetes, obesity and heart disease [7].

This work focuses on the macronutrient and mineral contents of the tubers. Furthermore the properties of the tubers that influence glycemic response such as the amount of carbohydrate, the amylopectin content, food processing and cooking methods of the tubers.

2. MATERIALS AND METHODS

2.1 Sample Collection

The tubers were collected from a farm at km 5 away from Buruku river Ugba road, in Binev council ward, Tombo mbalagh in Buruku local government area of Benue state, Nigeria. Buruku Local Government has a landmark of about 1,246 square kilometre. On the globe, lies on the geographical coordinates of 10° 37′ 0″ N, 7° 14′ 0″ E; for the latitude and longitude lines respectively, taking equator as the reference point.

2.2 Sample Preparation

2.2.1 Preparation *Tacca involuncrata* into raw flour sample

Tacca involuncrata roots were washed to remove farm dirt, it was peeled by scrubbing with hard sponge to remove the peels, then washed with clean portable water, then grated to obtain slurry. Water was added to the slurry then sieved with a muslin cloth, the slurry was allowed to settle, and then the water was decanted. The slurry was reconstituted with water and decanted the second time, this was repeated for the third time and checked orally for bitterness. The starch was allowed to stand then drained with a sack cloth. The starch was dried and then packaged in polyethylene bags.

2.2.2 Preparation of aerial yam and angawa into raw flour sample

The tubers were washed to remove dirt, tubers were peeled using a clean stainless steel knife, then sliced into thin slices, then dried using hot air oven 60°C for 48 h then milled into flour, then packaged using polyethylene bags.

2.2.3 Preparation of tacca involuncrata, aerial yam and angawa into cooked flour sample

Freshly harvested tubers were Washed, Peeled, washed again with clean portable water and diced. The tubers were cooked separately for 30 min and also for 1 h for each of the tubers. Then dried at 60°C for 48 h. The samples were milled into flour using a blender (dry mill), then sieved and packaged using polyethylene bags.

2.2.4 Determination of nutritional composition of tubers

The moisture content, Ash, Crude fat, Crude fiber, Protein of the 3 tubers were determined by the method of AOAC [8] Carbohydrate content was determined by difference. The minerals (Zn, Fe, Mg, Ca, K, Na) were determined according to the standard method of AOAC [9] using an Atomic Absorption Spectrophotometer (Varian Spectr AA. 20 plus). Phosphorous was determined by the colorimetric method using ammoniaum molybdate [8].

2.3 Alpha Amylase and Alpha Glucosidase

2.3.1 Alpha amylase

The activity of α-amylase was determined as described by Hassan HS, and West [9]. Soluble starch of 1% was prepared in 0.02 M sodium phosphate of pH 6.9 containing 0.006 M NaCl. Enzyme solution of 0.2 ml was added to 0.2 ml of substrate and incubated at 25°C for 3 min then 1 ml of 3.5 DNSA was added. The mixture was then heated in water bath (100°C) for 5 min. After heating, the mixture was cooled and 10 ml of distilled water was added, and then read in a colourimeter at 540 nm against a blank containing buffer without enzyme. A calibration curve was made with maltose, to convert colourimeter reading to unit of activity.

2.3.2 Alpha glucosidase

The activity of α-glucosidase was determined as described by Constantino et al. [10] A typical reaction mixture consisted of, in final concentration, 2.0 mM PNP α-G (dissolved in 10 mM sodium acetate buffer, pH 5.0 containing 1 mM EDTA) and an appropriate amount of enzyme solution. The total reaction mixture was 3 ml and was incubated at 40°C for 30 min. After incubation, an aliquot of 0.5 ml was added to 5 ml of 0.1 M sodium carbonate (to stop the reaction) and the absorbance of the mixture was read at 400 nm. The enzyme activity was expressed as the number of micromole of p-nitrophenol formed at 40°C per minute using the extinction coefficient of $18.8 \times 10-3\ M-1\ cm-1$.

2.4 Amylose and Amylopectin

2.4.1 Determination of amylose

This was estimated by the iodine colorimetric method of Mohanna et al. [11].

The flour samples (100 mg) were dissolved in ethanol (1 ml, 95% and NaOH (1 N, 92 ml) and left overnight and made to 100 ml in volumetric flask.

An aliquot (5 ml) of this solution was then added with acetic acid (1 N, 1 ml) and iodine solution (2 ml, 0.2% I2 in 2%KL) and the volume was made up to 100 ml with distilled water and mixed. After 20 min, absorbance measurement was taken at 620 nm using blank with 5 ml 0.09 N NaOH, 1 ml acetic acid and 2 ml iodine solution and was made to 100 ml in total volume in triplicates (Juliano et al. 1981).

A standard curve was plotted for amylose and amylopectin eg aerial yam containing 0, 10, 25, 50, 75, and 100% amylose (McGrance et al. [12]).

2.5 Determination of Amylopectin

Amylopectin in tested food was calculated by difference using the following method [13].

$$Amylopectin\ (\%) = 100\% - amylose\ (\%)$$

2.6 Statistical Analysis

All the analyses reported in this study were carried out in triplicates. In each case, a mean value and standard deviation were calculated. Analysis of variance (ANOVA) was also performed and separation of the mean values was carried out using Duncan Multiple Range Test at p 0.05. Pearson correlation coefficients (r) for relationships between various flour properties were calculated.

3. RESULTS AND DISCUSSION

The results of the proximate composition of the tubers (flour samples) are presented in Fig. 1. It showed that protein, fat, crude fibre, Ash and moisture were significantly higher ($p<0.05$) in the raw tubers than in the 30 min and 1 h boiled tubers respectively while carbohydrate increased after boiling for 30 min and 1 h respectively. This increase in carbohydrate after boiling of tubers is also observed in the work of Adepoju [14].

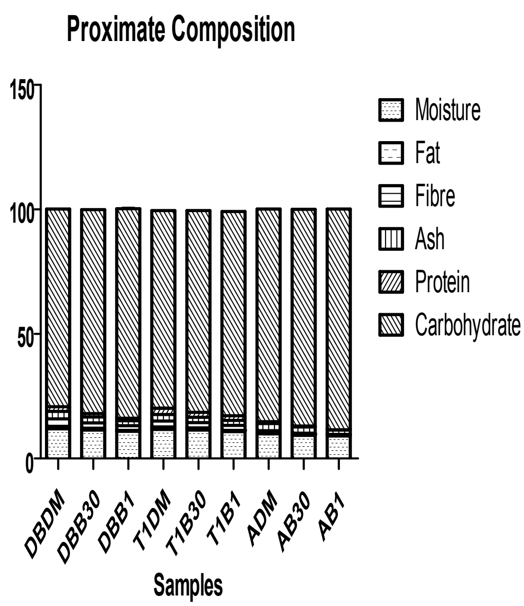

Fig. 1. Proximate composition of the flour samples of the tubers

Key: DBDM Dioscorea bulbifera Dried Milled, Dioscorea bulbifera boiled for 30 mins, DBB1 Dioscorea bulbifera boiled for, TIDM Tacca involuncrata Dried Milled, Tacca involuncrata Tacca involuncrata boiled for 1 h. Dioscorea angawa dried milled, Dioscorea angawa boiled for 30 mins, Dioscorea angawa boiled for 1 h

The moisture content values obtained which were in the range of (9.10 to 11.94%) could be said to have good storage stability. This is because, a moisture content of 10% is generally specified for flours and other related products. It should be pointed out that when these products are allowed to equilibrate for periods of more than one week at 60% relative humidity and at room temperature (25 to 27°C), moisture content might increase.

The starch contents of the flours ranged between 79.38 to 88.61%. This is an expected range for starch staples. The increase in carbohydrate content observed in boiled samples maybe due to the fact that boiling increase the rate at which starches get converted into sugars and also they undergo gelatinization and swelling when heated.

Fibre is the sum of the plant polysaccharides and lignin that are not digested by the enzymes of the gastrointestinal tract. The fibre content were in the range of 0.28 to 2.96%. The fibre content of the flour samples of *Dioscorea bulbifera* and *Tacca involucrata* are higher in crude fibre than those of *Dioscorea angawa* indicating their usefulness in the production of roughage and bulk and in contributing to a healthy condition of the intestine when consumed [15].

The ash content in the flour samples is an indication of the levels of minerals or inorganic component of the sample. Ash content varied in the tubers. The contents reduced significantly ($p<0.05$) with increase in the boiling time. The proportion of variation is down about 5%. This agrees with the work of Bell [16] who also found a slight decrease in ash content during the cooking of water yam tubers. The decrease of the ash content in tubers, during the cooking with water could be as a result of leaching of the minerals into the boiling water. The observed decrease in ash content after cooking implies that the potential ability of the tuber to supply essential minerals has been reduced.

The result of the protein content of the tubers are generally low. The protein content ranged from 0.14 to 2.38%. The Protein contents reduced significantly ($p<0.05$) with increase in the boiling time. The decrease of the protein content in tubers, during the cooking with water, this may be as a result of protein denaturation by heat or the loss of the nitrogenous material by solubilisation.

The crude fat content found to be in the range of 0.33 to 1.0% in the flour samples is expected as tubers are generally low in fats. The decrease in crude fat content after boiling may be due to chemical changes such as oxidation and destruction by heat.

The result above compares favourably to Yam which is mainly composed of starch (75-84% of dry weight) with small amounts of proteins, lipids and rich minerals [17].

In general, boiling of tubers decreases water soluble content such as protein, ash, fiber and fat content due to some leaching out into the water during boiling.

3.1 Mineral Content

The result of mineral content of raw and boiled samples of the tubers are presented in Fig. 2. The highest values were obtained in the raw flour samples and its various contents decreased significantly p<0.05 as the flour samples were pretreated by boiling for 30 min and 1h respectively.The highest amounts of Sodium were observed in *Dioscorea bulbifera,* and lowest in *Dioscorea angawa.* The highest amounts of Potassium were obs*erved in Dioscorea angawa* and the lowest in *Dioscorea bulbifera.* The highest amounts of Calcium were observed in *Dioscorea bulbifera,* and the lowest in *Tacca involucrata.* All the samples recorded low amounts of Fe, Zn. The reduction of magnesium after boiling might be due to magnesium oxalate which is less soluble than the potassium and sodium salts [18], this may be the possible reason observed in the reduction of magnesium level upon boiling [19].

The loss of calcium from boiling may be due to leaching out of the calcium to the boiling water [20]. The losses of phosphorus content in tubers were due to leaching on boiling and this can occur up to 25% [21].

3.2 Alpha Amylase and Alpha Glucosidase

The result for the alpha amylase and alpha glucosidase activities are shown in Table 1. The results show that Alpha amylase activities increased significantly (p<0.05) as boiling time increased. Alpha amylase activity was highest in *Dioscorea bulbifera* and lowest in *Dioscorea Angawa.*

Alpha glucosidase activity also increased significantly (p<0.05) with increase in boiling time. Its activity was highest in samples boiled for 1 h. Alpha glucosidase and alpha amylase are the important enzymes involved in the digestion of carbohydrates [22]. Alpha Amylase is a carbohydrate splitting enzyme, which hydrolyzes starch to yield monomeric carbohydrates i.e. it is involved in the breakdown of long chain carbohydrates. Alpha glucosidase breaks down starch and disaccharides to glucose [23]. They serve as the major digestive enzymes and help in intestinal absorption. Enhancement of amylolytic activity in boiled samples may be attributed to swelling and rupturing of starch granules which facilitate more randomized configuration for alpha-amylase to affect hydrolysis [24].

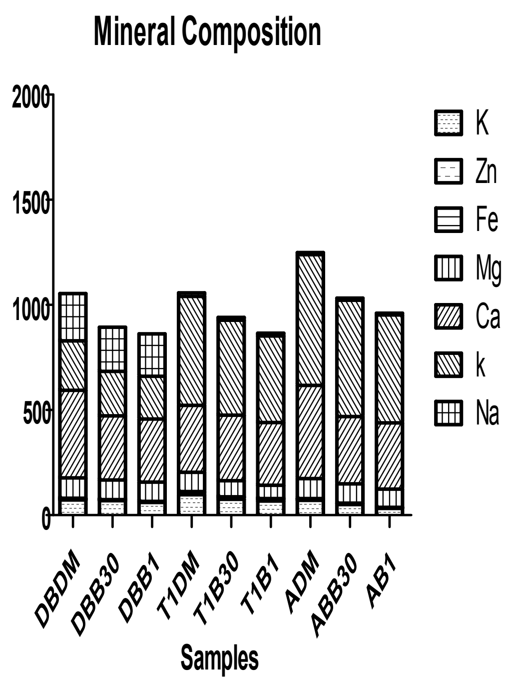

Fig. 2. Mineral composition of the flour samples of the tubers

Key: DBDM Dioscorea bulbifera dried milled, Dioscorea bulbifera doiled for 30 mins, DBB1 Dioscorea bulbifera boiled for, TIDM Tacca involuncrata Dried Milled, Tacca involuncrata Tacca involuncrata boiled for 1 h. Dioscorea angawa dried milled, Dioscorea angawa boiled for 30 mins, Dioscorea angawa boiled for 1 h

3.3 Amylose and Amylopectin Content

Results of the amylose and amylopectin contents of the tubers are presented in Table 2. All the flour samples had higher amylopectin than amylose. The amylose contents were highest in

Dioscorea angawa and lowest in *Tacca involucrata* and amylopectin content were highest in *Tacca involucrata* and lowest in *Dioscorea angawa.* Generally, starch has higher amylopectin (70 to 80%) than amylose (20-30%) [25]. The amylose content decreased significantly ($p<0.05$) with increase in boiling time, while amylopectin content increased significantly ($p<0.05$) with boiling time. Heating breaks down starch granules to allow amylopectin and amylose to be more readily digested by pancreatic amylase, which theoretically should increase the glycemic index of tubers. Previous reports [6] has shown that the amylose content plays a key role in the digestion of starches, as starches with low amylose contents are more digestible than starches with high amylose content and starches that are high in amylopectin are digested and absorbed more quickly than starches with a high amylose content and produce larger postprandial glucose and insulin responses [26]. High amylose slows the digestion rate. There is greater hydrogen bonding between glucose units in amylose molecule than amylopectin thus less exposure to enzymatic digestion [27]. In addition, the larger size of amylopectin provides more open and wide surface for enzymatic attack as compared to smaller amylose [28,29].

Table 1. Alpha amylase and alpha glucosidase activities on the tubers

Samples	Alpha amylase (mg/g)	Alpha glucosidase (mg/g)
DBDM	$49.16^{h}\pm0.06$	$603.25^{h}\pm0.01$
DBB30	$114.24^{b}\pm0.01$	$1004.12^{b}\pm0.13$
DBB1	$118.76^{a}\pm0.03$	$1014.92^{a}\pm0.01$
T1DM	$53.51^{g}\pm0.01$	$644.43^{g}\pm0.03$
T1B30	$67.67^{d}\pm0.02$	$800.35^{e}\pm0.01$
T1B1	$70.29^{c}\pm0.05$	$822.03^{d}\pm0.07$
ADM	$38.27^{i}\pm0.01$	$566.11^{i}\pm0.01$
AB30	$56.67^{f}\pm0.04$	$800.03^{f}\pm0.02$
AB1	$65.93^{e}\pm0.01$	$992.14^{c}\pm0.01$

Means with the same superscript in the same column are not significantly different ($p<0.05$)
Key: DBDM Dioscorea bulbifera dried milled, Dioscorea bulbifera boiled for 30 mins, DBB1 Dioscorea bulbifera boiled for, TIDM Tacca involuncrata Dried Milled, Tacca involuncrata Tacca involuncrata boiled for 1 h. Dioscorea angawa dried milled, Dioscorea angawa boiled for 30 mins, Dioscorea angawa boiled for 1 h

Table 2. Percentage amylose and amylopectin content of the tubers

Samples	Amylose %	Amylopectin %
DBDM	$29.22^{b}\pm0.01$	$70.80^{h}\pm0.02$
DBB30	$24.56^{h}\pm0.06$	$75.49^{b}\pm0.01$
DBB1	$23.45^{i}\pm0.01$	$76.56^{a}\pm0.05$
T1DM	$28.08^{d}\pm0.04$	$71.95^{f}\pm0.01$
T1B30	$26.37^{f}\pm0.01$	$73.65^{d}\pm0.08$
T1B1	$25.52^{g}\pm0.03$	$74.50^{c}\pm0.01$
ADM	$30.65^{a}\pm0.01$	$69.38^{i}\pm0.02$
AB30	$28.19^{c}\pm0.01$	$71.82^{g}\pm0.01$
AB1	$26.97^{e}\pm0.02$	$73.32^{e}\pm0.01$

Means with the same superscript in the same column are not significantly different ($p<0.05$)
Key: DBDM Dioscorea bulbifera dried milled, Dioscorea bulbifera boiled for 30 mins, DBB1 Dioscorea bulbifera boiled for, TIDM Tacca involuncrata Dried Milled, Tacca involuncrata Tacca involuncrata boiled for 1 h. Dioscorea angawa dried milled, Dioscorea angawa boiled for 30 mins, Dioscorea angawa boiled for 1 h

4. CONCLUSION

In conclusion, *in vitro* alpha amylase and alpha glucosidase were significantly greater in boiled samples than in raw samples. Boiling for 1 h increased the carbohydrate content, amylopectin content, *in vitro* alpha amylase and alpha glucosidase activities of the tubers more than when it was boiled for 30 min. The effect of moist and dry heat on *in vitro* tuber starch digestibility showed that boiling tubers for a longer time will result in faster rates of digestion which theoretically should increase the glycemic response of these tubers. Among the three tubers, *Dioscorea angawa* had more amylose content than *Dioscorea bulbifera* and *Tacca involucrata* had the lowest amylose content. This implies that *Dioscorea angawa* will digest much slower than the corresponding tubers and will result in a much slower glycemic response.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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