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ESTIMATED GLYCEMIC INDICES AND INHIBITORY ACTION OF SOME YAM (*DIOSCOREA SPP.*) PRODUCTS ON KEY ENZYMES LINKED WITH TYPE–2 DIABETES

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ABSTRACT

Postprandial glycemic control is important in the prevention and therapy of type-2 diabetes and related diseases. Carbohydrate is the main proportion of food consumed daily and the main energy source, but proper management of type-2 diabetes requires an adequate and proper selection of carbohydrates. Therefore, this study sought to assess the effect of processing of yam flour [white yam (*Dioscorea rotundata* Poir); water yam (*Dioscorea alata* Lam) and yellow yam (*Dioscorea cayenensis* Poir)] to paste on the glycemic indices. The yam varieties were prepared into flour and paste from which; aqueous extracts were subsequently produced. The glycemic index, starch, sugar, amylose, amylopectin contents and amylose/amylopectin ratio were determined. Inhibitory action of the yam products on key enzymes linked to type-2 diabetes (α-amylase and α-glucosidase) was determined. The pasting process caused a significant ($P < 0.05$) decrease in the starch, sugar and amylose contents, and a significant ($P < 0.05$) increase in amylopectin and glycemic index of all the yam varieties except in water yam. The sugar contents of the yams ranged from 4.36% (yellow yam) to 6.44% (white yam) for flour and 3.72% (yellow yam) to 5.07% (white yam) for paste. Also, the amylose/amylopectin ratio ranged from 0.54 (water yam) to 0.80 (white yam) for flour and 0.33 (water yam) to 0.53 (white and yellow yams) for paste. The yam extracts inhibited α-amylase and α-glucosidase activities *in vitro* in a dose-dependent pattern (1- 4mg/mL); however, the pasting process caused significant ($P < 0.05$) increase in the α-amylase and α-glucosidase inhibitory activities. Therefore, it thus appears that processing of yam varieties into paste (browned) would decrease the starch, sugar, amylose contents and amylose/amylopectin ratio while inhibition of α-amylase and α-glucosidase activities; and glycemic index increase (except water yam). In conclusion, water yam seems to be a better carbohydrate source for diabetic patients of all the yams studied.

Keywords: Yam, *Dioscorea* spp., Glycemic index, Amylose, Amylopectin, inhibitory

INTRODUCTION

Non-insulin dependent diabetes mellitus (Type 2 diabetes) is one of the commonest lifestyle-related diseases (Probst-Hensch, 2010) and clinical management of this disease requires an adequate selection of carbohydrates (Ferrer-Mairal *et al.*, 2011). Carbohydrate is the main proportion of food consumed daily and the main energy source that provides approximately 40-80% of total daily energy requirement in human (FAO/WHO, 1998). However, starch is the main carbohydrate source in a variety of diets (Chung *et al.*, 2006). According to NCCFN (2005), carbohydrate comprises 55-70% of daily energy intake. Thus, it is of importance to know more on this macronutrient especially the role played by good control of glycemic response in preventing a varied disease indirectly. Glycemic index is a system that ranks foods, particularly carbohydrate-based, on their actual postprandial

blood glucose response, compared to a reference food (Shanita *et al.*, 2011).

Postprandial hyperglycemia induces the non-enzymatic glycosylation of various proteins and biomolecules; resulting in the development of chronic complications. Therefore, control of postprandial plasma glucose levels is critical in the early treatment or management of diabetes mellitus, in particular type-2 diabetes, and in reducing chronic vascular complications (Ortiz-Andrade *et al*., 2007). According to Augustin *et al*., (2002), many factors influence postprandial blood glucose response, these factors include the food itself and individual physiological factors (Kirwan *et al*., 2001), while factors affecting postprandial glycemic response include the amount of carbohydrate, natural monosaccharide components, natural starch, food processing and cooking method and the presence of other food components (Sacks *et al*., 2014). Yam is the common name for some species in the genus *Dioscorea*; they are perennial herbaceous vines cultivated for the consumption of their starchy tubers in Africa, Latin America and Oceania. The consumption of yam and its products is distributed throughout the tropics and few temperate regions of the world. These tubers constitute the highest energy sources in Western Africa, South Asia (China, Japan, and Oceania) and the caribbean countries. Yam products generally have lower glycemic index than potato products (Brand-Millar *et al*., 2003) which means that they will provide more sustained form of energy, and give better protection against obesity and diabetes (Brand-Millar *et al*., 2003). Therefore, this study aimed to investigate the effect of processing of yam flour [white yam (*Dioscorea rotundata*); water yam (*Dioscorea alata*) and yellow yam (*Dioscorea cayenensis*)] to paste on the glycemic indices and the inhibitory action of the yam products on key enzymes linked to type-2 diabetes (α-amylase and α-glucosidase) so as to know the safest of the yam products for diabetic patients.

MATERIALS AND METHODS

Materials

Sample collection

Three popularly consumed yam tubers namely; water yam (*Dioscorea alata* L.), yellow yam (*Disocorea cayanensis* Lam) and white yam (*Dioscorea rotundata* Poir) in Nigeria were sourced from a market at Ilara- Mokin, Ondo State, Nigeria. The identification and authentication of the samples was carried out at the Department of Crop, Soil, and Pest Management (CSP), Federal University of Technology, Akure, Nigeria.

Sample Preparation

The yam samples were washed, peeled and sliced into about 2 cm diameter slices with 2 mm thickness and sun dried for 3 days. The sun dried samples were ground to flour and kept dry before analysis. About 500 g of the flour were further processed into yam paste by stirring in 1 litre of boiling water. The resulting paste (browned yam flour) was dried, powdered and then kept in an air tight container for further analysis.

Methods

Starch and Free Sugar Determination

The method described by Dubois *et al*., (1956) was used. This involves weighing 0.020 g finely ground sample into centrifuge tubes and wetted with 1 ml of ethanol. About 2 ml of distilled water was added, followed by 10 ml hot ethanol, the mixture was vortexed and centrifuged at 2000 rpm for ten minutes. The supernatant was collected and used for free sugar analysis, while the residue was used for starch analysis, 7.5 ml of perchloric acid was added to the residue and allowed to hydrolyze for 1 hour. It was diluted to 25 ml with distilled water and filtered through whatman no 2 filter papers. From the filtrate 0.05 ml was taken, made up to 1 ml with distilled water, vortexed and immediately analysed for reducing sugar using 3, 5-dinitrosalicylic acid. Also, the supernatant was made up to 20 ml with distilled water, and then 0.2 ml of the sample was taken. 0.5 ml (5% phenol) and 2.5 ml concentrated sulphuric acid was added. The sample was allowed to cool and the absorbance read on a UV/Visible at 490 nm wavelength.

Amylose Determination

Briefly, 0.1 g of flour sample or standard was weighed into a centrifuge tube and 1 ml of 95% ethanol and 9 ml 1N NaOH were carefully added, the test was covered and the content was mixed very well on a vortex mixer according to Williams *et al*., 1958. Thereafter, the samples

were heated for 10 minutes in a boiling water bath to gelatinize the starch, and then allowed to cool to room temperature, 1 ml of the extract of each sample was made up to 10 ml with distilled water, 0.5 ml of the diluted sample was mixed with 0.1 ml of Acetic acid and 0.2 ml of iodine solution respectively. The volume was made up to 10 ml with distilled water. Then the test mixture was left for 20 min for colour development after which it was vortexed and the absorbance was read at 620 nm.

Amylopectin Determination

Amylopectin in tested food was calculated by difference (Juan *et al*. 2006) using following formula:

Amylopectin (%) = 100% – amylose (%)

***In vitro* Starch Hydrolysis and Estimated Glycemic Index Determination**

A previously reported *in vitro* method (Goni *et al*., 1997) with slight modifications was used. The aim of the *in vitro* starch hydrolysis was to simulate the gastrointestinal tract (GIT). The oral phase was simulated by means of mechanical disaggregation of 50 mg food portions. The gastric phase was developed for 1 h at 40 ^{o}C with 10 ml of HCl-KCl buffer pH = l.5 and pepsin (Sigma P-7000). The intestinal phase was carried out in sodium potassium phosphate buffer 0.05 M pH 6.9 containing pancreatic amylase (Sigma A3176). Samples were then incubated at 37 ^{o}C in a shaking water bath. Aliquot samples (0.2 mL) aliquot samples were taken from each tube at 0, 30, 60, 90 and 120 min and then immediately analyzed for reducing sugars. This was done using 3, 5-dinitrosalicylic acid method using a glucose standard curve. The glucose was converted into starch by multiplying by 0.9. Standard glucose was also analyzed as reference product. A non-linear model established by Goni *et al*. (1997) was applied to describe the kinetics of starch hydrolysis. The area under the hydrolysis curve (AUC) was calculated. The calculated hydrolysis index was obtained by dividing the area under the hydrolysis curve of the sample by the area obtained for standard glucose. The expected glycemic index (GI) was calculated using the equation described by Granfeldt (1994).

Preparation of Aqueous Extracts of the Yam Products

Briefly, 1 g of the powdered samples were soaked in 20 ml of distilled water overnight and centrifuged at 3000 rpm for 10 min. The supernant was then kept at about 4 °C for further analysis.

α-Amylase Inhibition Assay

Ability of the aqueous extract to inhibit α-amylase was determined according to the method of Worthington Biochemical Corp, (1978). Briefly, appropriate dilution of the aqueous extract (100 µl) and 100 µl of 0.2 M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing Hog pancreatic α-amylase (0.5mg/ml) were incubated at 25^{o}C for 10 minutes. Then 50 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) was added to each tube. The reaction mixture was incubated at 25 ^{o}C for 10 minutes and stopped with 200 µl Dinitrosalicyclic acid colour reagent. Thereafter, the mixture was incubated in boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was then diluted with 2 ml of distilled water and absorbance measured at 540 nm.

α - Glucosidase Inhibition Assay

The ability of the aqueous extract to inhibit α-glucosidase was determined according to the method of Apostolidis *et al*, (2007). Briefly, appropriate dilution of the aqueous extract (50 µl), mixed with 15µl of α-glucosidase solution from the intestine of albino rat in 0.1 M sodium phosphate buffer (pH 6.9) and 15 µl, 3 mM reduced glutathione (GSH) in the sodium phosphate buffer solution was incubated at 37 ^{o}C for 10minutes.Then 40ml of 5mM p-nitrophenyl- α-D-glucopyranoside solution (PNP-Glu) in 0.1M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 37 ^{o}C for 10 minutes then 2ml of Na_2CO_3 was added. The absorbance at 405 nm was measured with the spectrophotometer. The α-glucosidase inhibitory activity was expresses as percentage inhibition.

Data Analysis

The results of replicate readings were pooled and expressed as mean ± standard deviation. One way analysis of variance was used to

analyze the results and Duncan's New Multiple Range Test was used for the post hoc (Zar, 1984). Statistical package for Social Science (SPSS) 15.0 for Windows was used for the analysis. The IC_{50} was calculated using non-linear regression analysis.

RESULTS

The starch and sugar content (%) of the yam varieties is presented in Table 1. The results revealed that there was a significant ($P < 0.05$) decrease in the starch and sugar content of yam flour with processing into paste. However, yellow yam flour (90.41%) had the highest starch content and water yam paste (57.65%) had the least; whereas, for the sugar content white yam flour (6.45%) had the highest and yellow yam paste (3.73%) had the least. Furthermore, Table 2 depicts the amylose and amylopectin contents of the yam flour and paste. The table shows that there was a significant ($P < 0.05$) decrease in the amylose content with processing into paste, whereas there was a significant increase in the amylopectin with processing. Conversely, Tables 2 and 3 revealed the results of the amylose: amylopectin ratio and the glycemic index of the yam flour and paste. The results show that there was a significant ($P < 0.05$) decrease in the amylose: amylopectin ratio with pasting, however, white yam flour (0.80) had the highest and water yam paste (0.33) had the least value. The result of the glycemic index shows that there was also a significant ($P < 0.05$) increase with pasting, but with the exception of water yam in which there was a slight significant ($P < 0.05$) decrease in the glycemic index with processing into paste. The ability of the aqueous extracts of the three yam varieties (flour and paste) to inhibit α-amylase and α -glucosidase activity *in vitro* was investigated and the result is presented in Figures 1 and 2 respectively. The result revealed that the yam extracts inhibited α-amylase in a dose-dependent manner (1–4 mg /mL). However, as revealed by the IC_{50} values (Table 4), aqueous extract of white yam paste (2.12 mg/ mL) had the highest α-amylase inhibitory activity while aqueous extract of yellow yam flour (18.05mg/ mL) had the least. The result also revealed that pasting process caused a significant ($P < 0.05$) increase in the amylase inhibitory activity of the yam varieties. The result of the inhibition of α-glucosidase activity revealed that all the extracts inhibited α-glucosidase in a dose dependent manner (1–4 mg/ mL). However, as revealed by the IC_{50} values (Table 4), aqueous extract of white yam paste (1.60 mg/ mL) had the highest α-glucosidase inhibitory activity while aqueous extract of white yam flour (5.32 mg /mL) had the least. Pasting process caused a significant ($P < 0.05$) increase in the α-glucosidase inhibitory activity of all the yam varieties.

Table 1. Sugar and Starch contents of yam flour and paste

Sample	Sugar (%)	Starch (%)
WYF	$6.44^{a} \pm 0.07$	$77.04^{c} \pm 0.31$
WYP	$5.07^{b} \pm 0.10$	$71.07^{c} \pm 0.30$
HYF	$4.78^{c} \pm 0.07$	$79.80^{c} \pm 0.30$
HYP	$4.85^{c} \pm 0.07$	$57.05^{d} \pm 0.47$
YYF	$4.36^{d} \pm 0.03$	$90.40^{a} \pm 0.15$
YYP	$3.72^{e} \pm 0.03$	$86.23^{b} \pm 0.16$

Data represent the mean ± standard deviation of replicate readings.
Values with the same superscript letter along the same column are not significantly different ($P< 0.05$).

Keys

WYF- White yam flour WYP- White yam paste HYF- Water yam flour
HYP- Water yam paste YYF- Yellow yam flour YYP- Yellow yam paste

Table 2. **Amylose and Amylopectin contents and Amylose/Amylopectin ratio of yam flour and paste**

Sample	Amylose (%)	Amylopectin (%)	Amylose/Amylopectin ratio
WYF	$44.49^{a} \pm 0.06$	$55.51^{c} \pm 0.08$	$0.800^{a} \pm 0.003$
WYP	$34.68^{b} \pm 0.06$	$65.32^{b} \pm 0.08$	$0.530^{c} \pm 0.002$
HYF	$35.09^{b} \pm 0.14$	$64.91^{b} \pm 0.16$	$0.540^{c} \pm 0.004$
HYP	$24.99^{c} \pm 0.06$	$75.01^{a} \pm 0.08$	$0.330^{d} \pm 0.001$
YYF	$42.36^{a} \pm 0.47$	$57.64^{c} \pm 0.49$	$0.730^{b} \pm 0.015$
YYP	$34.62^{b} \pm 0.14$	$65.38^{b} \pm 0.16$	$0.530^{c} \pm 0.004$

Data represent the mean ± standard deviation of replicate readings.
Values with the same superscript letter along the same column are not significantly different ($P < 0.05$).

Keys

WYF- White yam flour WYP- White yam paste HYF- Water yam flour
HYP- Water yam paste YYF- Yellow yam flour YYP- Yellow yam paste

Table 3. **Glycemic index (GI) of yam flour and paste**

Sample	Glycemic index (%)
WYF	36.55^{b}
WYP	37.32^{b}
HYF	35.14^{b}
HYP	32.34^{b}
YYF	29.44^{c}
YYP	48.73^{a}

Data represent the mean ± standard deviation of replicate readings.
Values with the same superscript letter along the same column are not significantly different ($P < 0.05$).

Keys

WYF- White yam flour WYP- White yam paste HYF- Water yam flour
HYP- Water yam paste YYF- Yellow yam flour YYP- Yellow yam paste

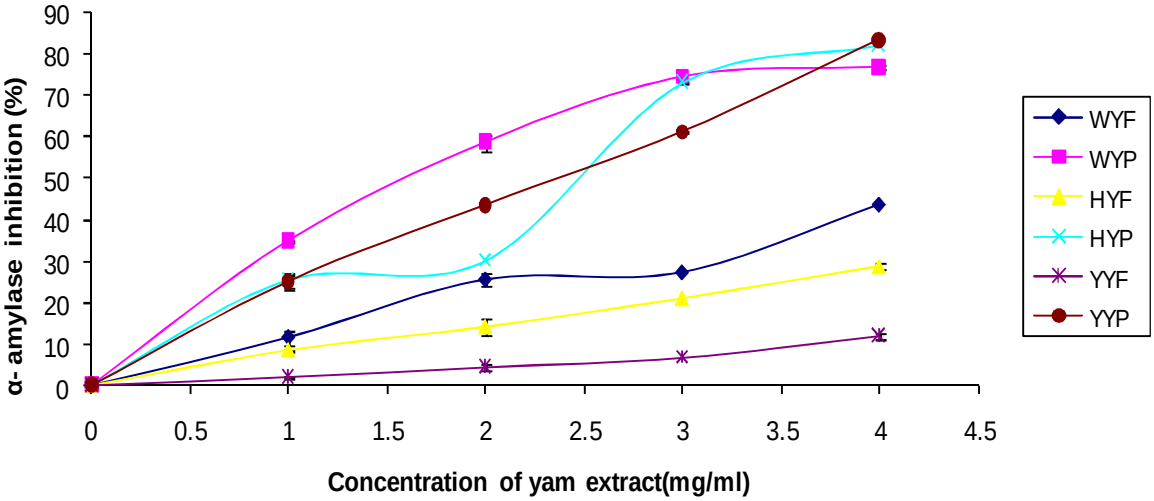

Fig. 1. **α- amylase inhibitory activity of aqueous extract of yam flour and paste *in vitro***
Values represent mean ± deviation of replicate readings

Keys

WYF- White yam flour	WYP- White yam paste	HYF- Water yam flour
HYP- Water yam paste	YYF- Yellow yam flour	YYP- Yellow yam paste

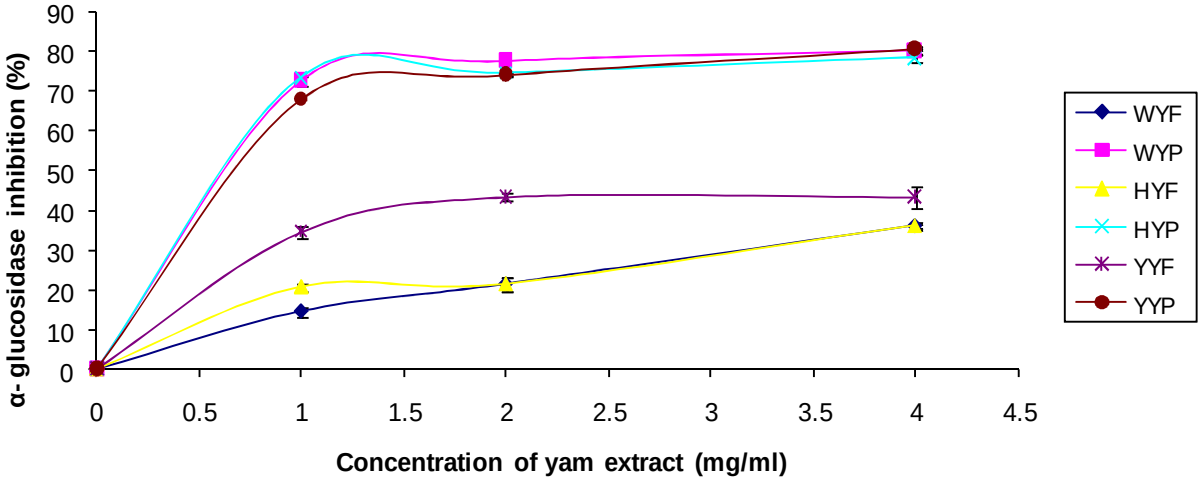

Fig. 2. **α – glucosidase inhibitory activity of aqueous extract of yam flour and paste *in vitro***
Values represent mean ± deviation of replicate readings

Key:

WYF- White yam flour	WYP- White yam paste	HYF- Water yam flour
HYP- Water yam paste	YYF- Yellow yam flour	YYP- Yellow yam paste

Table 4. IC_{50} (mg/ml) of the α-amylase and α-glucosidase inhibitory activities of aqueous extract of yam flour and paste.

Sample	α-amylase		α-glucosidase	
	Flour	Paste	Flour	Paste
White yam	4.72^{c}* ± 0.03	$2.12^{a\#}$ ± 0.10	5.32^{a}* ±0.17	$1.60^{a\#}$ ±0.44
Water yam	6.99^{b}* ± 0.07	$2.36^{a\#}$ ± 0.32	5.26^{a}* ± 0.00	$1.65^{a\#}$ ± 0.44
Yellow yam	18.05^{a}* ± 0.91	$2.37^{a\#}$ ± 0.19	3.69^{b}* ± 0.01	$1.68^{a\#}$ ± 0.38

Values represent mean ± deviation of replicate readings
Values with the same superscript letter along the same column are not significantly different ($P< 0.05$).
Values with the same superscript symbol along the same row are not significantly different ($P< 0.05$).

DISCUSSION

It has long been recognised that there are major differences between simple sugars and complex carbohydrates with regard to their effects on glucose metabolism and insulin action (Storlien *et al*., 1988; Higgins *et al*., 1996). It is further recognised that different complex carbohydrates may have different physiological effects. Foods that produce such high glycemic responses have been linked to diseases such as non-insulin-dependent or type 2 diabetes mellitus (Salmeron *et al*., 1997a, b; Morris and Zernal, 1999). In contrast, amylose may form helical inclusion complexes due to its structure and interaction with dietary lipids and is less accessible to digestive enzymes and is generally digested and absorbed more slowly. Digestion of high amylose starches therefore leads to reductions in postprandial glycemic and insulin digressions relative to high-amylopectin starches. There are many factors that influence postprandial blood glucose response (Augustin *et al*., 2002), the food itself and individual physiological factors (Kirwan *et al*., 2001). Factors affecting postprandial glycemic response also include the amount of carbohydrate, natural monosaccharide components, natural starch, food processing and cooking method and the presence of other food components (Sacks *et al*., 2014).

The results in Table 1 revealed that there was a significant ($P<0.05$) decrease in the starch and sugar contents of the yam varieties following processing into paste which implies that processing help reduce the harmful effect of high consumption of starchy and sugary products that is linked to hyperglycemia. Hyperglycemia is a condition associated with diabetes mellitus and is linked to most diabetes complications as their primary cause. Hyperglycemia is a condition of abnormal rise in plasma glucose level, and in type-2 diabetes is as a result of insulin resistance which may be due to a number of defects in signal transduction ranging from abnormal insulin or insulin receptors to defects in glucose transporters (Oritz-Andrade *et al*., 2007). Prolonged hyperglycemia leads to increased generation of reactive oxygen species (ROS) and alteration of

endogenous antioxidants (Oritz- Andrade *et al*., 2007).

The amylose content ranged between 24.99 and 44.50%; when compare with the result of the classification of rice reported by Juliano (1992), the amylose content of the yam varieties happens to fall within the highest amylose content. From the analysis, amylopectin was found to be greater than amylose in all the yam varieties tested. This observation is in agreement with the report of Yotsawjmonwat *et al*. (2008) that amylopectin is the major component in most starch. Also, there is a significant ($P<0.05$) decrease in the amylose content of the yam varieties with processing while; there is a significant ($P<0.05$) increase in the amylopectin content of the yam varieties. The reason for this cannot be categorically stated but it may be due to the fact that amylopectin content contributed immensely to the swelling power of starch when considering its solubility (Tester and Morrison, 1990).

The results in Tables 2 and 3 showed that processing caused a significant ($P<0.05$) decrease in the amylose/amylopectin ratio of the yam varieties. The starch in raw food is stored in compact granules that are difficult to digest (Brand-Miller *et al*., 1992). Also many factors have been ascribed to digestion and absorption of starch in the human small intestine these factors include the botanical source (Goni *et al*., 1997; Jenkins *et al*., 1984), food processing/preparation methods (Bravo *et al*., 1998; Jenkins *et al*., 1982; Sagum and Arcot, 2000), physiochemical properties (particularly gelatinization characteristics) (Panlasigui *et al*., 1991), particle size (Snow and O'Dea, 1981), amylose/amylopectin ratio (Goddard *et al*., 1984; Juliano and Goddard, 1986) and the presence of lipid-amylose complexes (Goddard *et al*., 1984; Guraya *et al*., 1997) and extrinsic factors like extent to which food is chewed, transit time through the gut and the degree of the insulin response (Urooj and Puttaraj, 2000, Leinonen *et al*., 1999).

Amylose and amylopectin content of a food are one of the factors that affect blood glucose response. It is inversely correlated to glycemic index (GI) (Behall and Howe 1995). The result of the glycemic index of the yam varieties revealed that the glycemic index of white and yellow yam significantly ($P<0.05$) increased with processing whereas, that of water yam significantly ($P<0.05$) decreased with processing which may be due to the various factors mentioned above. Cooking has a large impact on GI. Uncooked starches have a low GI because amylase does not readily attack it. In contrast, gelatinized starch is readily attacked by the amylase resulting in a higher GI than raw starches. Starch in food that has been heated and allowed to cool has a much lower GI than freshly gelatinized starches because starch chains line up and re-crystallize (retrograde) upon cooling (Björck *et al*., 1994 and Jenkins *et al*., 1987). The crystallized starch impedes amylase activity. Thus, a warm boiled potato has a much higher GI than the same potato eaten cold (Fernandes *et al*., 2005).

Inhibition of enzymes involved in the hydrolysis of carbohydrates such as α-amylase and α-glucosidase has been suggested as practical therapeutic approaches for reducing postprandial hyperglycaemia (Shim *et al*., 2003). Pancreatic α-amylase is the enzyme primary involved in the breakdown of starch into disaccharides and oligosaccharides before intestinal α- glucosidase catalyses the breakdown of disaccharides to liberate glucose which is later absorbed into the blood circulation. Inhibition of these enzymes has been suggested to slow down the breakdown of starch in the gastrointestinal tract, which reduces the amount of glucose absorbed into the blood circulation (Oboh *et al*., 2010; Shodehinde and Oboh, 2013). However, as shown in Figures 1 and 2, all the yam extracts inhibited both α-amylase and α-glucosidase activities *in vitro* with paste exhibiting stronger inhibitory activities than the flour.

CONCLUSION

In conclusion, the processing of various yam varieties flour into paste (browned) caused a significant ($P<0.05$) decrease in their starch, sugar, amylose and amylose/amylopectin ratio. Conversely, a significant ($P<0.05$) increase was observed in their inhibition of key enzymes linked to type-2 diabetes (α-amylase and α-

glucosidase) and glycemic index (except water yam); however, water yam seems to be a better dietary energy source in the regular meal of type-2 diabetes patients of all the yam varieties studied.

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