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, amyllopectin contents and amylose/amyllopectin 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 amyllopectin 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/amyllopectin 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/amyllopectin 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 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 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:

\[
\text{Amylopectin} (%) = 100\% - \text{amylose} (%)
\]

**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 °C with 10 ml of HCl-KCl buffer pH = 1.5 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 °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°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 °C for 10 minutes and stopped with 200 μl Dinitrosalicylic 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 °C for 10 minutes. 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 °C for 10 minutes then 2ml of Na2CO3 was added. The absorbance at 405 nm was measured with the spectrophotometer. The α-glucosidase inhibitory activity was expressed 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<sub>50</sub> 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 amyllose and amylpectin contents of the yam flour and paste. The table shows that there was a significant (P < 0.05) decrease in the amyllose content with processing into paste, whereas there was a significant increase in the amylpectin with processing. Conversely, Tables 2 and 3 revealed the results of the amyllose: amylpectin 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 amyllose: amylpectin 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<sub>50</sub> 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<sub>50</sub> 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>
<thead>
<tr>
<th>Sample</th>
<th>Sugar (%)</th>
<th>Starch (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WYF</td>
<td>6.44± 0.07</td>
<td>77.04± 0.31</td>
</tr>
<tr>
<td>WYP</td>
<td>5.07± 0.10</td>
<td>71.07± 0.30</td>
</tr>
<tr>
<td>HYF</td>
<td>4.78± 0.07</td>
<td>79.80± 0.30</td>
</tr>
<tr>
<td>HYP</td>
<td>4.85± 0.07</td>
<td>57.05± 0.47</td>
</tr>
<tr>
<td>YYF</td>
<td>4.36± 0.03</td>
<td>90.40± 0.15</td>
</tr>
<tr>
<td>YYP</td>
<td>3.72± 0.03</td>
<td>86.23± 0.16</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amylose (%)</th>
<th>Amylopectin (%)</th>
<th>Amylose/Amylopectin ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>WYF</td>
<td>44.49\textsuperscript{a} ± 0.06</td>
<td>55.51\textsuperscript{c} ± 0.08</td>
<td>0.800\textsuperscript{a} ± 0.003</td>
</tr>
<tr>
<td>WYP</td>
<td>34.68\textsuperscript{b} ± 0.06</td>
<td>65.32\textsuperscript{b} ± 0.08</td>
<td>0.530\textsuperscript{c} ± 0.002</td>
</tr>
<tr>
<td>HYF</td>
<td>35.09\textsuperscript{b} ± 0.14</td>
<td>64.91\textsuperscript{b} ± 0.16</td>
<td>0.540\textsuperscript{c} ± 0.004</td>
</tr>
<tr>
<td>HYP</td>
<td>24.99\textsuperscript{c} ± 0.06</td>
<td>75.01\textsuperscript{a} ± 0.08</td>
<td>0.330\textsuperscript{d} ± 0.001</td>
</tr>
<tr>
<td>YYF</td>
<td>42.36\textsuperscript{b} ± 0.47</td>
<td>57.64\textsuperscript{c} ± 0.49</td>
<td>0.730\textsuperscript{b} ± 0.015</td>
</tr>
<tr>
<td>YYP</td>
<td>34.62\textsuperscript{b} ± 0.14</td>
<td>65.38\textsuperscript{b} ± 0.16</td>
<td>0.530\textsuperscript{c} ± 0.004</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glycemic index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WYF</td>
<td>36.55\textsuperscript{b}</td>
</tr>
<tr>
<td>WYP</td>
<td>37.32\textsuperscript{b}</td>
</tr>
<tr>
<td>HYF</td>
<td>35.14\textsuperscript{b}</td>
</tr>
<tr>
<td>HYP</td>
<td>32.34\textsuperscript{b}</td>
</tr>
<tr>
<td>YYF</td>
<td>29.44\textsuperscript{c}</td>
</tr>
<tr>
<td>YYP</td>
<td>48.73\textsuperscript{a}</td>
</tr>
</tbody>
</table>

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.** $\alpha$-amylose 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.** $\alpha$-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 $\alpha$-amylase and $\alpha$-glucosidase inhibitory activities of aqueous extract of yam flour and paste.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\alpha$-amylase</th>
<th>$\alpha$-glucosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flour</td>
<td>Paste</td>
</tr>
<tr>
<td>White yam</td>
<td>4.72$^{c*} \pm 0.03$</td>
<td>2.12$^{a#} \pm 0.10$</td>
</tr>
<tr>
<td>Water yam</td>
<td>6.99$^{b*} \pm 0.07$</td>
<td>2.36$^{a#} \pm 0.32$</td>
</tr>
<tr>
<td>Yellow yam</td>
<td>18.05$^{a*} \pm 0.91$</td>
<td>2.37$^{a#} \pm 0.19$</td>
</tr>
</tbody>
</table>

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 compared 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/amyllopectin 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/amyllopectin 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, amylase 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.

REFERENCES


