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Comparative Study of Starch Characteristics, *In-Vitro* Starch Digestibility and Glycemic Index of Some Starchy Foods Consumed in Nigeria

Israel Olusegun Otemuyiwa[1*], Adedayo Muideen Sanni[1] & Emmanuel Ayorinde Oyewumi[1]

[1] Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

[*] Israel Olusegun Otemuyiwa, E-mail: otemuyisegun@oauife.edu.ng

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Abstract

The study investigated the carbohydrate characteristics and in-vitro starch digestibility of some starchy food consumed in Nigeria. Ten foods samples (cassava, yam, red and white sorghum, rice, plantain, banana, semovita, noodles and bread) were selected. The content of starch, amylose and sugar were determined by colorimetric method, in vitro rate of starch hydrolysis was evaluated by multi-enzyme digestion method over a period of two hours, the glucose released was estimated by colorimetric method and was compared to the reference food (bread). The result showed that the percentage moisture content and total starch ranged from 9.8 to 15.3% and 236 to 248 mg/g, while amylose, Rapidly Digestible Starch (RDS) and Resistance Starch (RS) ranged from 8.41 to 19.2%, 30.8 to 51% and 7.8 to 37.4%, respectively. The in-vitro digestibility study indicated that the equilibrium Concentration (Cα), Kinetic constant (K), Hydrolysis Index (HI) and Glycemic Index (GI) ranged from 34 to 64.9, and 0.02 to 0.07, 56.6 to 104 and 71 to 96.8, respectively. Positive correlations ($P < 0.05$) exist between RDS and GI ($r = 0.700$) and RS and amylose ($r = 0.899$) The study revealed that, structure of dietary carbohydrate could greatly influenced the Glycemic Index, plantain and noodles with low RDS and low hydrolysis constant may be beneficial in management of diabetes whereas sorghum, semovita, cassava and bread with high RDS, and high GI should be taken sparingly or combined with high protein and low glycemic load foods.

Keywords

Glycemic Index, Resistant Starch, amylose, in vitro digestion, Nigeria

1. Introduction

In recent years it has become evident that significant health risks and benefits are associated with dietary food choice. A particular disease has been associated with carbohydrate foods with high Glycemic Index

and high digestibility rate. About 104 millions of people worldwide are being afflicted with the perfect epidemic known as the Diabetes. This figure is increasing daily. Diabetes meets all criteria for a public health disorder (Seal et al., 2003). Nutrition is a significant cornerstone of diabetes care as described in intensive management. The main focus in nutritional management of diabetes is to improve glycemic control by balancing food intake with endogenous and/or exogenous insulin level (Heacock et al., 2004). Historically, attempt has been made to control the glycemic response to food, particularly carbohydrate-containing foods, including use of very low carbohydrate and starvation diets, artificial sweeteners and pharmacological preparations such as fast acting insulin and inhibitors of carbohydrate absorption (Heacock et al., 2004).

In Nigeria, the diet of the people is predominantly carbohydrate obtained from either root tuber or cereal grains. There are varieties of food that were consumed which complement one another without the empirical knowledge of their digestion rate and optimum intake that will give sufficient nutrient intake. Foods like carbohydrates for instance should be monitored carefully. There is the need to consider the rate at which these foods digest and be able to predict their Glycemic Index in order to prevent glucose induced ailment.

One way to classify the glycemic response of various carbohydrate-containing foods is Glycemic Index (GI). The Glycemic Index (GI) is an *in-vitro* measurement based on glycemic response to carbohydrate-containing foods. The index allows ranking of carbohydrate foods on the basis of the rate of digestion and absorption (Jenkins et al., 1981; Englyst et al., 1992). *In-vitro* method has also been used to classify foods based on their digestion characteristics similar to the in vivo situation, and to identify slow release of carbohydrate in foods (Jenkins et al., 1984). The foods with GI values more than 70%, between 56% and 69% and lower than 55% were classified as high, medium, and low GI foods, respectively (Brand-Miller et al., 2003).

The study carried out using human subject by Asinobi et al. (2016) to determine the blood sugar response of some traditional fortified staple meals in Nigeria concluded that unripe plantain had the lowest Glycemic Index value with lowest postprandial glucose response. Also Fasanmade and Anyakudo (2007) concluded that yam based food product should be generously used by diabetes patient because of its low Glycemic Index. These experiments were carried out under *in-vivo* conditions as such none of the researcher addressed the nature and characteristics of the starch present in foods analysed.

The digestibility of starch in foods may vary widely (Björck et al., 1994). Hence, a nutritional classification of dietary starch has been proposed, which takes into account both the kinetic component and the completeness of its digestibility, thus comprising Rapidly Digestible (RDS), Slowly Digestible (SDS), and indigestible or resistant fractions (RS) (Englyst et al., 1992).

The objectives of this study were to carry out *in-vitro* digestibility studies of some starchy staple diets consumed in Nigeria, determine the rate of hydrolysis and the starch content characteristics, and also predict the Glycemic Index. The study would provide an insight into the basic cause of epidemics associated with elevated glucose induced type 2-diabetes among Nigerians.

2. Materials and Methods

2.1 Sample and Sample Preparation

The samples selected for this study are yam flour, cassava flour, unripe plantain flour, unripe banana flour, flour of white and red sorghum, semovita, rice, noodles and bread. The samples were dried and milled using a locally fabricated mill (Lawood Metals, Osogbo, Nigeria). The milled samples were sieved using a local sieve (aperture size of 0.6 mm) to remove the coarser fragments. All the samples were milled as one batch, mixed thoroughly and sub-samples randomly taken from different parts of each milled sample, mixed together and stored in the freezer until analyzed.

2.2 Analysis of Proximate Composition

The proximate composition of the samples (moisture, ash, Crude fibre) were determined by the method of AOAC (2000).

2.3 Determination of Total and Reducing Sugar Content

Soluble sugar was extracted from 2.0 g sample with 85% ethanol using soxhlet extractor and refluxed for 2 h as described by Bambridge et al. (1996) Reducing sugar and total sugar were determined from the ethanolic extract by the ferricyanide method (AOAC, 1984). Glucose was used as a standard and the glucose content of the sample was calculated using a linear equation $y = 1.6216 - 0.001x$ ($R^2 = 0.972$).

2.4 Determination of Total Starch

The total starch content of the samples was determined on the residue obtained after ethanolic extraction of sugar. Residue (200 mg) was refluxed with 0.7 M HCl for 2.5 h. The acid hydrolysate was neutralized to pH 7.0 using 5.0 M NaOH, pour into 500 mL standard flask and made up to volume with distilled water. The hydrolysate was filtered through a Whatman no. 541 filter paper and the starch was determined as the reducing sugar using the ferricyanide method (Bainbridge et al., 1996). The glucose content was calculated using a glucose standard linear equation and then converted to starch content using the AOAC (1984) equation.

2.5 Determination of Amylose Content

Amylose content in rice samples were determined based on the Iodine-binding procedure as described by Thomas et al. (2013). The sample (100 mg) was measured into 100 mL standard flask, 1.0 ml of ethanol (95%) and 9.0 ml of 1.0 M NaOH were added, the mixture was heated on a boiling water bath for 10 min to gelatinize the starch. 5.0 ml of the gelatinized starch solution was transferred to a 100 ml standard flask, 1.0 mL of 1.0 M acetic acid and 2.0 ml of iodine solution were added and made up to volume with distilled water. All the contents were thoroughly vortex mixed and allowed to stand for 20 min. The absorbance was measured at 620 nm using a UV-Spectrophotometer (Model AA-6650, Shimadzu Co. Japan). The amylose content was calculated from the standard curve of potato amylose using the linear equation ($R^2 = 0.899$).

2.6 In-Vitro Starch Hydrolysis

The *in-vitro* starch digestibility was determined by multi-enzyme procedure described by Deepa et al.

(2010). The sample (250 mg) was gelatinized in 10 mL distilled water on a hot plate. The gelatinized sample was homogenized with 10 mL of HCl-KCl buffer (pH 1.5) using a basic homogenizer (Kika Labortechnik 725, Janke and Kukel GmbH & Co., Stanfen Germany) at 9500 rpm for 1 min and the homogenate was then digested with 20 mg of pepsin (Sigma; CAS 2001/75-6, code 10132561, 666 iu/mg, porcine gastric mucosa) solution (prepared by adding 1.0 g of pepsin/10 mL of HCl-KCl buffer) for 1 h in a shaking water bath at 37°C. The pH of the digestate was adjusted to 6.9 and the volume made to 25 ml using Tris-maleate buffer (pH 6.9). Then 5.0 mL of α-amylase (2.6 IU in 5 ml buffer pH 6.9) was added to the digestate which was incubated at 37°C in a shaking water bath. One ml of sample aliquots was collected at intervals of 30 min for 180 min, the enzyme activity in the aliquot withdrawn was inactivated by immediately placing the tube in a boiling water bath maintained at 100°C for 5 min and then refrigerated till the end of the incubation period, To these aliquots, 3 ml of 0.4 M sodium acetate buffer (pH 4.75) and 60 µl amyloglucosidase (Sigma, No;10105-5GF,70 ui/mg. *Aspegilius niger*) were added and incubated at 60°C for 5 min to hydrolyse the starch to glucose.

The glucose released was determined using dinitrosalicylic acid (Miller, 1959). The concentration of glucose was calculated from the linear equation of glucose standard (R^2 = 0.980) and glucose was converted into starch by multiplying with 0.9.

All the experiments were conducted thrice and with triplicate analysis each.

The rate of starch digestion was expressed as the percentage of TS hydrolyzed at different times. The digestibility curve for each food sample was fitted into the first-order equation (Grandfeidt et al., 1992).

$$C_t = C_\infty(1 - e^{-kt})$$

where C_t is the percentage of starch hydrolyzed at time t (min),

C_∞ is the equilibrium starch hydrolysis after 180 min,

k is a pseudo-first order rate constant.

The parameters, k and Cα were estimated for each sample based on the data obtained from starch hydrolysis procedure using Microsoft Excel Software.

Hydrolysis Index (HI) was obtained by dividing the area under the hydrolysis curve of the sample by the corresponding area of a reference food (white bread) expressed as a percentage (Grandfeidt et al., 1992).

Glycemic Index (GI) was estimated using the equation of Goni et al. (1997).

$$GI = 39.71 + (0.549 \times HI)$$

2.7 Determination of Rapidly Digestible, Slowly Digestible and Resistance Starch

The method of Han et al. (2007) was used to estimate the rapidly digestible, slowly digestible and resistance starch. The Rapidly Digestible Starch (RDS) is the fraction of starch hydrolyzed within 30 min of incubation, Slowly Digestible Starch (SDS) is the fraction hydrolyzed between 30 to 180 min and the fraction that remained un-hydrolyzed after 180 min is regarded as the Resistant Starch.

2.8 Statistical Analysis

Analyses were carried out in triplicate for each determination and the results were expressed as mean

and standard deviation. The data were subjected to analysis of variance, and pearson correlation coefficient and the levels of significant difference was performed. GraphPad InStat version 3.06 for Windows 2003 was used for statistical analysis.

3. Results and Discussion

The results of starch and sugar and chemical characteristics of the starch were presented in Table 1. The moisture content of the local foods ranged from 8.2 to 15.3%, the highest value was found in yam flour, these values compares with 12 to 14% predicted as optimum moisture content for storage of flour foods and for obtaining quality product during milling (Souilah et al., 2014).

The fibre content ranged from 0.48% in rice to 4.9% in noodles. The fibre content of rice is expectedly low compared to others, this is because it rice has been subjected to the process of milling and polishing in which the outer layer (bran) containing fibre has been completely removed. Though flours of yam, cassava, plantain and sorghum were sieved during processing but still contain high fibre content which could be adduced to pore size of the sieve.

Diets with a high content of fiber, have a positive effect on health since their consumption has been related to a decreased incidence of several types of diseases as due to its beneficial effects like increasing the volume of fecal bulk, decreasing the time of intestinal transit, lowering cholesterol and glycemic levels, and stimulating the proliferation of the intestinal flora (Dingra et al., 2012; Souilah et al., 2014).

The total and reducing sugar content (Table 1) ranged from 5.8 to 25.6 mg/g and 1.2 to 12.1 mg/g, respectively. The sugar content of banana flour was higher (total and reducing sugar) and this could be attributed to glucose release resulting from the activity of endogenous enzymes during processing of the flour The presence of sugar will help improve taste of the food products.

The total starch ranged from 222 to 293 mg/g, Plantain recorded the highest starch, the starch was not significantly different ($P < 0.05$) among banana, yam, sorghum and cassava flours. Amylose and amylopectin ranged from 8.41 to 26% and 74 to 92.4%, respectively. Sorghum and cassava flour recorded the least values for amylose though according to amylose classification in food (Juliano et al., 1981), these samples could be categorise as having intermediated amylose content. The implication of this amylose level is that these foods is expected to be soft and not sticky and will not become hard when cooling. Heating of starch in the presence of water will lead to gelatinisation that makes starch more easily digested, however after cooling amylose tend to recrystallise and form retrograded amylose which is inaccessible to enzymatic hydrolysis. Amylose and amylopectin are important in determining the structure of a carbohydrate food which may have a profound effect on starch digestibility. The mechanism of how an increased amylose/amylopectin ratio affect glycemic response is that linear amylose chain form a compact structure that limit enzyme accessibility and rate of amylosis (Halistrom, 2011). Amylopectin on the other hand with its branched structure is less ordered and therefore more easily digested.

The structural characteristics of digestible starch showed that Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS) and Resistant Starch (RS) ranged from 31.8 to 55.2%, 8.9 to 40.2% and 7.8 to 37.4%, respectively. Flours of cassava, banana, sorghum and bread were higher in RDS while low value was reported for plantain and noodles. Also the highest value for RS was found in rice (46.5%) followed by noodles and plantain and low values were found in white sorghum and yam flour though yam had high SDS. In foods, RS could corresponds to the physically inaccessible starches entrapped in cellular matrix or are native uncooked granules, the crystallinity of which makes them scarcely susceptible to hydrolysis or retrograded starch (Englist et al., 1999). RS has also been shown to have positive effects on colonic health by increasing faecal bulk and by generating Short Chain Fatty Acids (SCFAs) such as butyric acid, which is the main energy source for colonocytes and may therefore, have a protective role in inflammatory bowel diseases and colon cancer (Hallstrom et al., 2011).

Several factors can explain the difference found in the Resistant Starch quantities, some of which are: interaction of starch with different components present in the food system such as proteins, fats; botanical source of starch; and storage conditions (Perera et al., 2010).

From the results, it was observed that noodles recorded high value for fibre as well as Resistant Starch this could be from two sources; through enrichment of the ingredient with soluble dietary fibre or through heat processing of starch that lead to formation of retrograded starch which hinders enzymatic hydrolysis of starch (Englist et al., 1992).

Table 1. Total Starch, Rapidly Digested Starch (RDS), Slowly Digested Starch (SDS), Resistance Starch (RS), Amylose and Amylopectin Content of Starchy Food (Expressed as% of Total Starch)

Sample	Moisture	Total Starch	RDS %	SDS %	RS %	Amylose %	Amylopectin %	Fiber %	Total sugar mg/g	Red sugar mg/g
Plaintain flour	11.3 ±0.1^{c}	239 ±13^{c}	31.8 ±1.0^{f}	19.2 ±0.4^{f}	31.4 ±0.5^{c}	17.1 ±0.3^{c}	82.9 ±0.3^{c}	1.3 ±0.02^{b}	10.5 ±0.5^{e}	8.45 ±1.4^{b}
Banana flour	9.8 ±0.2^{d}	242 ±0.9^{b}	53.7 ±0.4^{b}	8.9 ±0.5^{g}	37.4 ±1.2^{b}	17.7 ±0.3^{c}	82.3 ±0.3^{c}	1.4 ±0.05^{b}	24.6 ±0.6^{ab}	12.1 ±0.6^{a}
Yam Flour	15.3 ±0.3^{a}	248 ±1.0^{b}	44.3 ±0.2 ^{d}	47.9 ±1.6^{a}	7.8 ±0.1^{h}	14.5 ±0.2^{d}	85.5 ±0.2^{b}	3.22 ±0.8^{a}	21.1 ±1.4^{b}	1.45 ±0.5^{e}
Red Sorghu	12.7 ±1.1^{b}	246 ±0.9^{b}	51.6 ±0.6^{bc}	33.3 ±0.2^{c}	15.1 ±0.6^{f}	14.4 ±0.3^{d}	85.6 ±0.3^{b}	2.29 ±0.09^{a}	15.6 ±0.26^{d}	2.6 ±0.07^{d}
white Sorghum	13.1 ±0.6^{b}	241 ±10^{c}	49.4 ±0.2^{c}	40.2 ±1.5^{b}	10.4 ±0.2^{gh}	8.41 ±0.7^{e}	92.4 ±0.5^{a}	2.0 ±0.07^{ab}	17.1 ±0.22^{c}	2.2 ±0.07^{d}
Cassava flour	12.5 ±1.0^{b}	248 ±2.0^{b}	55.2 ±1.0^{a}	22.6 ±0.1^{e}	22.2 ±0.4^{e}	12.8 ±1.1^{d}	87.8 ±2.0^{b}	3.6 ±0.3^{a}	10.08 ±0.05^{e}	1.83 ±0.05^{e}
Rice	8.2 ±0.3^{e}	222 ±0.14^{c}	38.0 ±0.3^{e}	16.5 ±0.2^{f}	46.5 ±0.9^{a}	26.0 ±0.8^{a}	74.0 ±0.6^{e}	0.48 ±0.01^{c}	10.9 ±0.02^{e}	1.17 ±0.13^{e}
Semovita	10.2 ±1.2^{cd}	237 ±8.0^{c}	43.8 ±0.3^{d}	27.9 ±1.4^{d}	28.3 ±1.0^{d}	17.4 ±0.2^{c}	82.6 ±0.2^{c}	2.4 ±0.01^{a}	25.6 ±0.03^{a}	2.71 ±0.1^{d}
Noodles	12.4 ±0.8^{b}	236 ±5.0^{c}	30.8 ±0.8^{f}	35.4 ±0.8^{c}	33.8 ±0.6^{c}	19.2 ±0.6^{b}	80.8 ±4.0^{d}	4.9 ±0.9^{a}	5.8 ±0.62^{f}	5.3 ±0.007^{c}
Bread	10.6 ±0.3^{cd}	293 ±5.0^{a}	49.0 ±1.0^{c}	21.0 ±1.0^{ef}	30.0 ±2.0^{c}	21.0 ±0.2^{b}	79.0 ±0.8^{d}	1.2 ±0.02^{b}	17.8 ±0.67^{c}	6.53 ±0.5^{b}

Note. Mean ± SD—mean and standard deviation of triplicate analysis.

Values in the same column with same superscript are not significantly different $P \leq 0.05$.

[1] Rapidly digestible starch; [2] Slowly digestible starch; [3] Resistance starch.

The results of *in-vitro* digestibility and the kinetic parameter were presented in Figure 1 and Table 2, the results indicated that the equilibrium Concentration (Cα), kinetic constant (*K*), hydrolysis index (HI) and Glycemic Index (GI) ranged from 34 to 64.9, and 0.02 to 0.07, 56.6 to 104 and 71 to 96.8, respectively. With the exception of yam flour and noodles that recorded low hydrolysis index, all sample had both high hydrolysis and Glycemic Index. Correlation coefficient (Table 3) showed that there is a positive correlation HI and RDS (r = 0.700), GI and RDS (r = 0.701) whereas fibre was negatively correlated with GI (r = -0.624) and HI (r = -0.628).

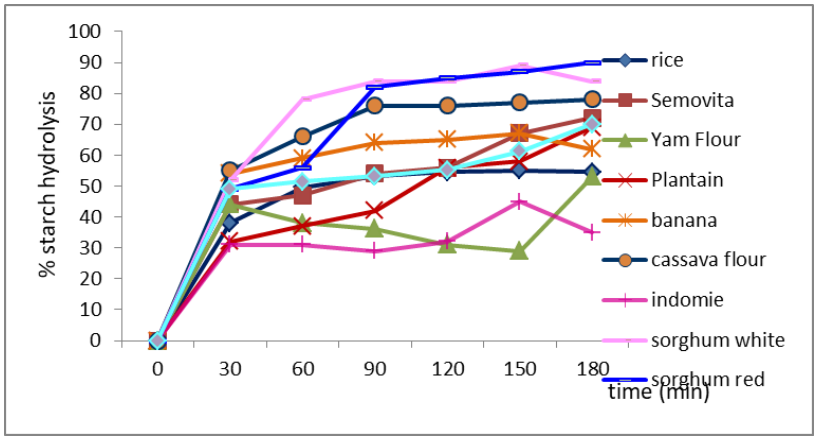

Figure 1. Starch Hydrolysis Curve of Some Starch Foods in Nigeria

Table 2. Kinetic Parameters of In-Vitro Starch Hydrolysis of Starchy Foods

Sample	Cα	*K*	AUC	HI(%)	GI (%)
Plaintain flour	44.7 ±1.0^{d}	0.03 ±0.001^{c}	6606 ±12^{c}	73.01 ±0.9^{d}	79.8 ±2.1^{c}
Banana flour	60.0 ±3.0^{b}	0.07 ±0.002^{a}	9442 ±22^{a}	104 ±10^{a}	96.8 ±1.1^{a}
Yam Flour	43.7 ±0.8 ^{d}	0.02 ±0.001^{d}	5911 ±10^{g}	65.32 ±0.8^{e}	75.6 ±1.2^{d}
Semovita	55.2 ±0.7^{c}	0.05 ±0.001^{b}	8800 ±2.9^{d}	97.2 ±0.5^{c}	93.0 ±2.8^{b}
White Sorghum flour	61.9 ±0.1^{ab}	0.07 ±0.001^{a}	9014 ±23^{c}	99.6 ±1.4^{b}	94.4 ±3.0^{ab}
Red Sorghum flour	64.9 ±2.0^{a}	0.07 ±0.004^{a}	9113 ±25^{b}	100 ±6.0^{b}	94.8 ±4.0^{a}
Cassava flour	60.0 ±0.6^{b}	0.07 ±0.009^{a}	9403 ±15^{a}	104 ±0.7^{a}	96.8 ±1.2^{a}
Rice	58.6 ±0.5^{c}	0.03 ±0.001^{c}	8603 ±9.0^{e}	95.1 ±2.6^{c}	91.9 ±0.5^{b}
Noodles	39.0 ±0.4^{e}	0.02 ±0.002^{d}	5123 ±8.5^{h}	56.6 ±0.8^{f}	71.0 ±0.8^{e}
Bread*	54.5 ±0.3^{c}	0.07 ±0.001^{a}	9048 ±12^{h}	100 ±0.0^{b}	94.6 ±0.3^{ab}

* white bread—control sample.

Mean and standard deviation of triplicate analysis.

Cα-equilibrium concentration, *K*-rate constant, AUC-Area Under Curve, HI-Hydrolysis Index, GI-Glycemic Index.

Values within the same column with different superscripts are significantly different at $P \leq 0.05$.

The kinetic constant K of amylolysis has been proposed as a reliable index of the inherent susceptibility of flour starches to amylase hydrolysis (Goni et al., 1997; Frei et al., 2003). From the results, the hydrolysis constant of plantain flour, yam flour and noodles is low (k = 0.02), the content of RSD was lower and RS was higher than in bread, this implied that the rate at which they will digest may take a longer time which may not adversely affect the blood sugar. This observation agrees with the report from *in-vivo* study that yam and unripe plantain had low Glycemic Index (Fasanmade & Anyakuro, 2007; Asinobi et al., 2016).

Banana flour unlike plantain recorded high hydrolysis constant (K = 0.07) which is the same as bread, the flour recoded high RDS and sugar content which may result from enzymatic degradation (endogenous enzymes) of starch that led to ripening. Sorghum flour (red and while cultivars) also recorded high hydrolysis rate which is higher than bread.

The hydrolysis constant in cassava flour also did not differ from that of bread, processing of cassava into flour involve grating, soaking and fermentation to make pulp free of cyanide (a toxic compound that is lethal), during fermentation starch is broken down by enzymes an aerobically to sugar which is rapidly released when it is consumed as food. The Glycemic Index is even higher than white bread (reference food)

From this study, it was discovered that the Glycemic Index of cassava flour, banana flour, semovita and sorghum flour (red and white cultivars) were higher whereas those of plantain flour, yam flour and noodles were lower compared to bread which was taking as standard high Glycemic Index food.

4. Conclusion

The study revealed that starchy foods which are staple diets among Nigerians are high glycemic load foods and that structure of dietary carbohydrate could greatly influenced the Glycemic Index of the foods, therefore, foods like plantain and noodles with low RDS and low hydrolysis constant may be beneficial in management of diabetes whereas sorghum, semovita, cassava and bread which contain high content of rapidly digestible starch should be taken sparingly. Hence, in order to effectively reduce the high incidence of type 2-diabetes, these foods should be complimented with high protein sources.

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