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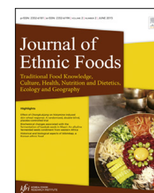

Original article

Starch composition, glycemic indices, phenolic constituents, and antioxidative and antidiabetic properties of some common tropical fruits

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ABSTRACT

Background: Studies have supported the protective effect of high fruit consumption in the management of chronic diseases such as diabetes.
Methods: Thirteen fresh tropical fruits were sourced for and the fruits juices were extracted, freeze dried, and then reconstituted for analysis. The sugar, starch, amylose, and amylopectin contents as well as glycemic indices, antioxidant properties, and the ability of the fruits to inhibit starch-hydrolyzing enzymes were determined. Also, the phenolic constituents of the fruits were characterized using high-performance liquid chromatography coupled with diode array detector.
Results: The starch, sugar, amylase, and amylopectin contents were 3.01–3.89 g/100 g, 35.34–60.91 g/100 g, 0.84–1.46 g/100 g, and 1.68–2.86 g/100 g, respectively, while the glycemic indices were 28.01–68.34, with African star apple (28.01) having the lowest and watermelon (68.34) the highest. Furthermore, the fruits exhibited high antioxidant properties as exemplified by their DPPH, ABTS+, •OH, and NO radical scavenging abilities. Likewise, the fruits also demonstrated α-amylase and α-glucosidase inhibitory property with Soursop (IC_{50} = 18.52 μg/mL), guava (IC_{50} = 19.77 μg/mL), and African star apple (IC_{50} = 20.86 μg/mL) showing the highest inhibitory potential among the 13 fruits. Similarly, the same trend was followed for α-glucosidase inhibitory activity.
Conclusion: The fruits' low glycemic indices, strong antioxidant properties, and inhibition of α-amylase and α-glucosidase activities could be possible mechanisms for their use in the management and prevention of type-2 diabetes.
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1. Introduction

Fruits are the edible part of flowering plants commercially available as human food, which is also given as gifts during traditional marriage in Nigeria. Fruits are eaten raw or processed into fruit juices such as orange juice or pineapple juice, or alcoholic beverages such as wine [1,2]. Fruits, however, are enjoyed all year-round, as a large part of Nigeria lies in the tropics, where many fruits are available. Popular fruits consumed in Nigeria include: orange, watermelon, mango, banana, soursop, African star apple, cashew, carrot, breadfruit, pawpaw, and pineapple to mention a few [1] (Figure 1). African star apple (*Chrysophyllum albidum*) is distributed throughout the southern part of Nigeria, Uganda, Niger Republic, Cameroon, and Ivory Coast [3]. In southwestern Nigeria, the fruit is called *agbalumo* and popularly referred to as *udara* in southeastern Nigeria. It is a popular tropical fruit tree found mostly in villages and picked by farmers on their way to farm. Pawpaw is the fruit of the plant *Carica papaya* native to the tropics. It is eaten as a food or cooking aid and in traditional medicine as it is a significant source of vitamin C and other polyphenols [4]. *Annona muricata* L. commonly known as Graviola or soursop is a typical tropical tree with heart shaped edible fruits and widely distributed in most tropical countries [5]. Graviola fruits have been widely consumed in Nigeria in fresh or processed forms for centuries. Cashew (*Anacardium occidentale*) is a soft fruit widely grown in

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Nigeria that is rich in nutrients with high vitamin C content [6]. Breadfruit (*Artocarpus altilis*) is a species of the mulberry family flowering tree grown in many parts of the world including Central and West Africa. It is a staple food in Nigeria that is rich in starch and tastes potato-like when cooked, fried, roasted, or baked [7].

Fruits have been identified as a highly nutritious naturally occurring food and in recognition of this national and international agencies recommended the consumption of fruit. Studies have shown that fruits are rich sources of antioxidants such as flavonoids, carotenoids, hydroxycinnamic acids, etc. These antioxidants may help the human body to protect against functional damage caused by reactive oxygen species, which are highly reactive pro-oxidants and toxins [8]. Previous work by Oboh et al. [9] reported correlation between the radical scavenging ability of antioxidant rich foods with its potentiality for the management of degenerative diseases such as hypertension and diabetes. A sudden rise in blood glucose levels (hyperglycemia) in type-2 diabetes patients is due to hydrolysis of starch by pancreatic α-amylase and catabolism of polysaccharides to glucose in the small intestine by α-glucosidase [10]. Inhibition of these enzymes involved in the breakdown of starch can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in the management of type-2 diabetes [11] (see Figure. 3)

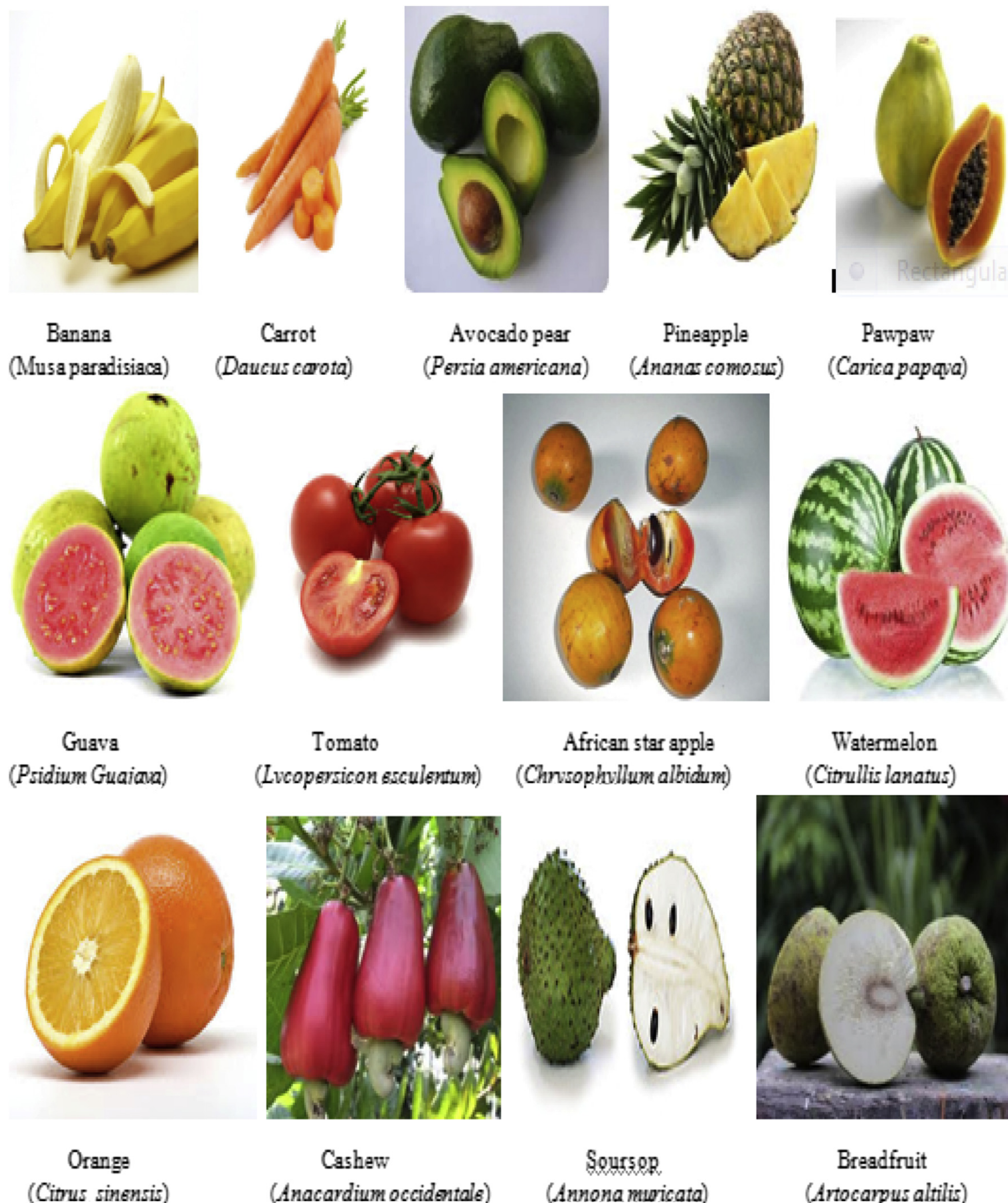

Figure 1. Some common tropical fruits in Nigeria.

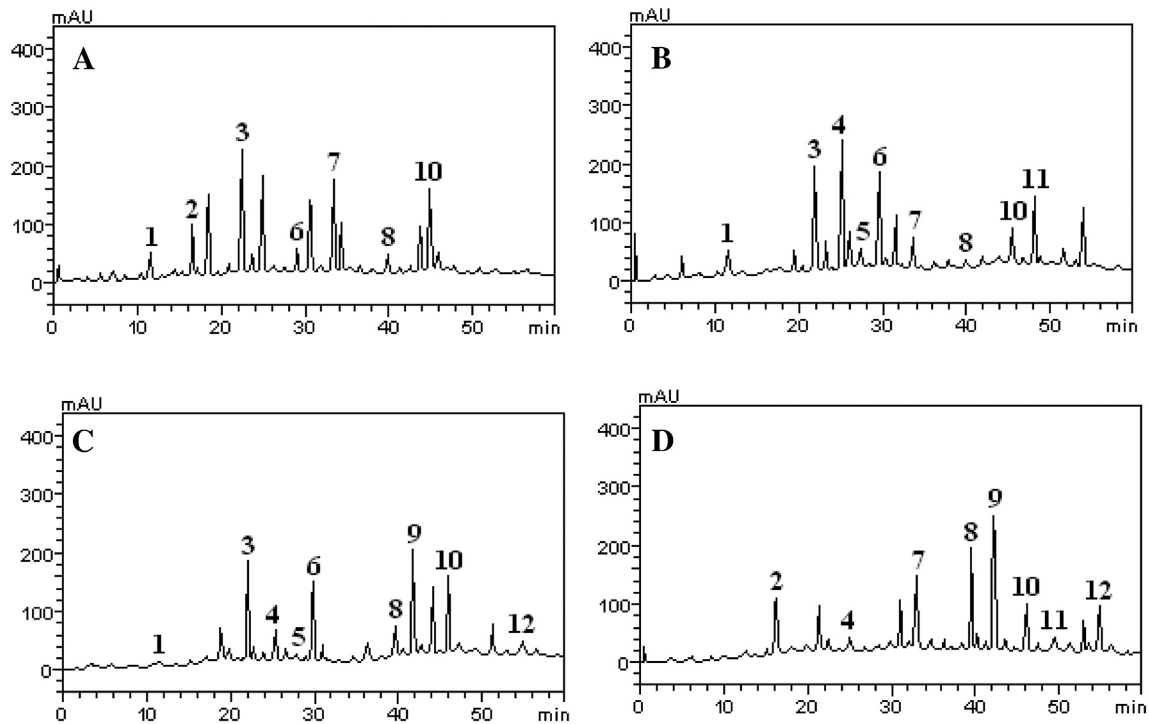

Figure 2. Representative HPLC-DAD profile of freeze-dried (A) avocado, (B) soursop, (C) pineapple, and (D) orange fruits extracts.
[Peak assignment:gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), p-coumaric acid (peak 6), epicatechin (peak 7), rutin (peak 8), quercitrin (peak 9), quercetin (peak 10), kaempferol (peak 11) and luteolin (peak 12)].

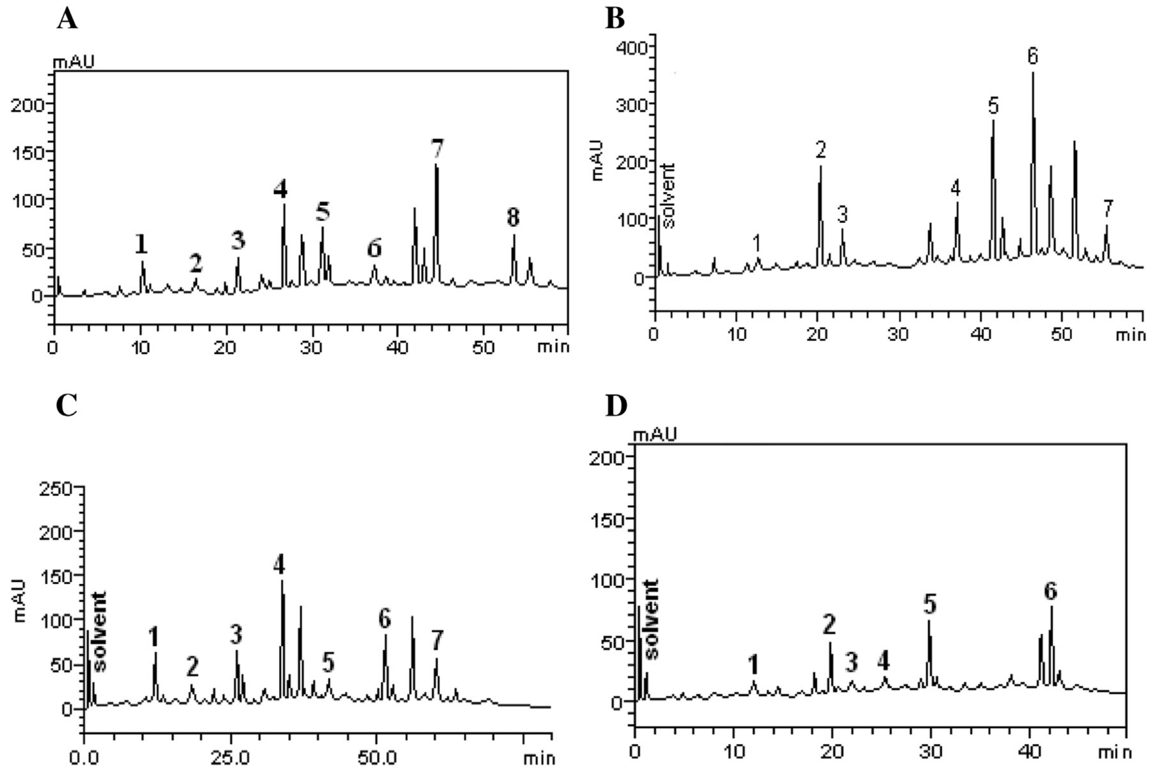

Figure 3. Representative HPLC-DAD profile of freeze dried (A) cashew, (B) carrot, (C) banana and (D) breadfruit extracts.
(A) cashew [peak assignment: gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), ellagic acid (peak 4), epicatechin (peak 5), rutin (peak 6), quercetin (peak 7) and kaempferol (peak 8).]; (B) carrot [peak assignment: gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), isoquercitrin (peak 4), quercetin (peak 5), luteolin (peak 6) and apigenin (peak 7).]; (C) banana [peak assignment: gallic acid (peak 1), catechin (peak 2), caffeic acid (peak 3), p-coumaric acid (peak 4), epicatechin (peak 5), quercetin (peak 6) and apigenin (peak 7).] and (D) breadfruit [peak assignment: gallic acid (peak 1), caffeic acid (peak 2), ellagic acid (peak 3), p-coumaric acid (peak 4), resveratrol (peak 5) and quercetin (peak 6)].

A major therapeutic target in diabetic patients is the improvement of postprandial hyperglycemic elevations. A report has shown increased risk of type-2 diabetes following high starch consumption [12]. Starch is a naturally occurring, biodegradable, and abundantly available polysaccharide molecule. It is widely distributed in the form of tiny granules in stems, roots, grains, and fruits of all form of green plants. Starch is composed of a mixture of two polymers called amylose and amylopectin. Amylose is a linear chain starch while amylopectin is a highly branched starch polymer [13]. In most plants especially fruits, the total native starch consists of 20–30% amylose and 70–80% amylopectin [14]. The ratio of amylose to amylopectin and dietary fiber content of foods are major factors that determine the glycemic index (GI) of the food [15]. The concept of GI was first developed by Jenkins et al. [16]; they described it as indexing of carbohydrate foods based on postprandial blood glucose responses dependent upon the nature of the food and type and extent of food processing [16]. The principle is that the slower the rate of carbohydrate absorption, the lower the rise of blood glucose level and the lower the GI value [17]. Consuming a low-GI diet compared to a high-GI diet has been shown to offer a number of health benefits including lowering of blood glucose and insulin levels, improving blood lipids, inflammatory markers, and coagulation factors. Similarly, the regular consumption of foods with a high GI has been associated as a risk factor for diseases such as diabetes, obesity, and cardiovascular disease and a low GI foods are recommended as treatment/management to such diseases [18]. A GI value of $\geq$70 is considered high, while 50–70 is medium, and $\leq$50 is low, where glucose is 100 [19] (see Figure. 4)

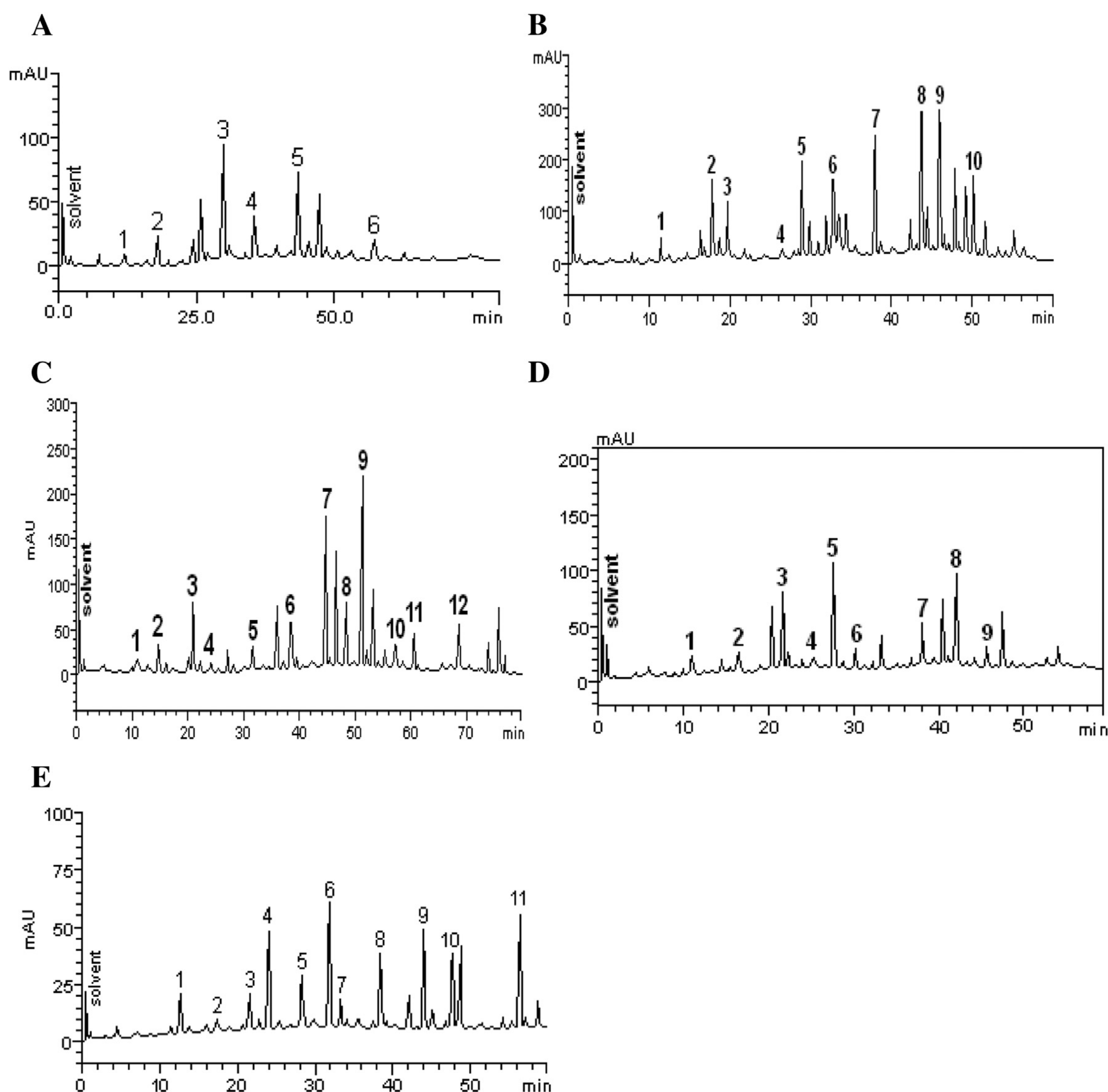

Figure 4. Representative HPLC-DAD profile of freeze dried (A) pawpaw, (B) African star apple, (C) guava, (D) watermelon and (E) tomato extracts.
(A) pawpaw [peak assignment: (1) gallic acid, (2) catechin, (3) p-coumaric acid, (4) epicatechin, (5) procyanidin and (6) quercetin.]; (B) African star apple [peak assignment: catechin (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), epicatechin acid (peak 4), cyanidin-3-0-glycoside (peak 5), cyanidin (peak 6), rutin (peak 7), quercitrin (peak 8), quercetin (peak 9) and kaempferol (peak 10).]; (C) guava [peak assignment: gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), epicatechin (peak 5), rutin (peak 6), orientin (peak 7), quercitrin (peak 8), quercetin (peak 9), kaempferol (peak 10), luteolin (peak 11) and apigenin (peak 12).]; (D) watermelon [peak assignment: gallic acid (peak 1), catechin (peak 2), caffeic acid (peak 3), ellagic acid (peak 4), p-coumaric acid (peak 5), epicatechin (peak 6), rutin (peak 7), quercetin (peak 8) and kaempferol (peak 9).]; (E) tomato [peak assignment: gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), p-coumaric acid (peak 6), epicatechin (peak 7), rutin (peak 8), quercetin (peak 9), kaempferol (peak 10) and apigenin (peak 11)].

Fruits are commonly consumed in various part of the world and are recommended for those with degenerative conditions such as type-2 diabetes. However, there is little information on the effect of fruits on the blood glucose levels and the possible biochemical mechanism that certifies its suitability as a functional food for the management of type-2 diabetes. This study therefore, seeks to investigate some tropical fruits' GIs, phenolic constituents, antioxidant properties, and inhibition of key enzymes linked with type-2 diabetes.

2. Materials and methods

2.1. Sample collection

Thirteen varieties of fruit: banana (*Musa paradisaca*), carrot (*Daucus carota*), avocado (*Persia americana*), pineapple (*Ananas comosus*), pawpaw (*Carica papaya*), guava (*Psidium Guajava*), tomato (*Lycopersicon esculentum*), African star apple (*Chrysophyllum albidum*), watermelon (*Citrullis lanatus*), orange (*Citrus sinensis*), cashew (*Anacardium occidentale*), soursop (*Annona muricata*), and breadfruit (*Artocarpus altilis*) were purchased at Owena market, Ondo, South West, Nigeria [7.2500°N, 5.1950°E]. Authentication of the samples was carried out at the Department of Crop, Soil, and Pest Management, Federal University of Technology, Akure, Nigeria.

2.2. Sample preparation

Fruits were washed with distilled water, the peel and seeds were removed from fruits where necessary, and the juices were extracted and freeze dried. The freeze-dried juice extract was later reconstituted for further analysis.

2.3. Chemicals and reagents

Chemicals and reagents used such as dinitrosalicylic acid color reagent, *p*-nitrophenyl-α-D-glucopyranoside, datechin, epicatechin, quercetin, rutin, and kaempferol were procured from Sigma–Aldrich, Inc. (St Louis, MO, USA). Methanol, acetic acid, sulfuric acid, sodium carbonate, potassium acetate, ethanol, perchloric acid, phenol, and sodium hydroxide were sourced from BDH Chemicals Ltd., (Poole, Dorset, UK). Gallic acid, caffeic acid, ellagic acid, and *p*-coumaric acid were purchased from Merck (Darmstadt, Germany). High-performance liquid chromatography–diode array detector (HPLC-DAD) was performed with a Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software (Shimadzu, Kyoto, Japan). All other chemicals and reagents were of analytical grades and the water used was glass distilled.

2.4. Determination of soluble sugars and starch

Aqueous solution of each fruit sample (100 mg) was weighed into a 50 mL centrifuge tube and 1.0 mL 80% ethanol added. Distilled water (20 mL) was added and mixed thoroughly. Then, 10 mL hot 80% ethanol was added and mixed thoroughly. The fruit samples were centrifuged at 1400 ×g for 5 minutes. Then, the supernatant was carefully decanted into 100 mL volumetric flask, followed by addition of 10 mL hot 80% ethanol to the residue. The mixture was mixed thoroughly and centrifuge 1400 ×g for 5 minutes, and the supernatant decanted into the same flask. The extraction with hot ethanol was repeated and the flask was made up to volume with distilled water while the residue was kept for starch determination. An aliquot of 1.0 mL of the supernatant was pipetted into a test tube and diluted to 2.0 mL with distilled water. Thereafter 5% phenol was added and mixed thoroughly. Then, 5.0 mL concentrated sulfuric acid was directly added to the liquid surface and not to the sides of the tube in order to obtain good mixing. The tubes was allowed to stand for 10 minutes and shaken thoroughly for proper mixing. The test tube was place in water bath for 10–20 minutes at 25–30°C and the absorbance was measured thereafter at 490 nm using a Jenway 6315 UV/Visible spectrophotometer (Bibby Scientific Ltd, Stone, Staffordshire, UK). The blank was prepared by substituting distilled water for the sugar extract solution. Perchloric acid (7.5 mL) was added to the residue and allowed to hydrolyze for 1 hour. It was then diluted to 25 mL with distilled water and filtered through Whatman No. 2 filter paper. A 0.2 mL aliquot was taken from the filtrate and made up to 2.0 mL with distilled water and vortexed, and ready for color development as was described for standard glucose curve preparation [20].

2.5. Determination of amylose and amylopectin content

A 100 mg sample of each fruit was weighed into a 100-mL volumetric flask. Then 1 mL of 95% ethanol and 9 mL of 1N NaOH were carefully added and samples were heated for 10 minutes in a boiling water bath to gelatinize the starch, the mixture was cooled and made up to volume with water. A 5-mL portion of the starch solution was pipetted into a 100-mL volumetric flask, 1 mL of 1N acetic acid (to acidify the solution) and 2 mL of Iodine Solution (0.20% or 2.0 mg/ml) were added. This was then made up to volume with distilled water. Thereafter, the mixture was shaken and absorbance was determined at 620 nm using spectrophotometer after 20 minutes. Amylopectin content was derived from starch and amylose content gotten to difference [21,22].

2.6. In vitro starch hydrolysis rate and GI

In vitro starch hydrolysis rate and GI were determined according to Goni et al. [23]. Fruit samples (50 mg) were incubated with 1 mg, of pepsin in 10 mL HCl-KCl buffer (pH 1.5) at 40°C for 60 minutes in a shaking water bath. The digest was diluted to 25 mL by adding phosphate buffer (pH 6.9), and then 5 mL of α-amylase solution containing 0.005 g of α-amylase in 10 mL of buffer was added. The fruit samples were incubated at 37°C in a shaking water bath. A 0.1-mL sample was taken from each flask every 30 minutes from 0 hours to 3 hours and boiled for 15 minutes to inactivate the enzyme. Sodium acetate buffer (1 mL 0.4M, pH 4.75) was added and the residual starch digested to glucose by adding 30 mL amyloglucosidase and incubating at 60°C for 45 minutes. Glucose concentration was determined by adding 200 μL of dinitrosalicylic acid color reagent. The reaction mixtures was stopped by placing the tubes in a water bath at 100°C for 5 minutes and then cooled to room temperature. The reaction mixture was then diluted by adding 5 mL of distilled water and the mixture was centrifuged 1200 xg. The supernatant was collected and the absorbance measured at 540 nm using spectrophotometer. The rate of starch digestion was expressed as the percentage of starch hydrolyzed per time. A 50-mg sample of glucose was used as the standard.

2.7. HPLC-DAD characterization of the phenolic constituents

Extract of freeze dried fruit juices and standards were injected onto reversed phase Phenomenex C_{18} column (4.6 mm × 250 mm) packed with 5 μm diameter particles. Mobile phases A and B were Milli-Q water, acidified to pH 3.0 with 2% of phosphoric acid and methanol, respectively, solvent gradient was used as follows: 0 minutes, 5% B; 0–5 minutes, 15% B; 5–10 minutes, 15% B; 10–25

minutes, 40%; 25–40 minutes 70% B; and 40–60 minutes, 100% B, following the method described by Boligon et al. [24] with slight modifications. Extract and mobile phase were filtered through 0.45 μm membrane filter (Millipore, Milford, MA, USA) and then degassed by ultrasonic bath prior to use, the extract was analyzed at a concentration of 15 mg/mL. The flow rate was 0.6 mL/min and the injection volume was 50 μL. The sample and mobile phase were filtered through 0.45-μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.030–0.500 mg/mL. Quantifications were carried out by integration of the peaks using the external standard method, at 254 nm for gallic and ellagic acids; 280 nm for catechin and epicatechin; 327 nm for caffeic acid and *p*-coumaric acid; and 366 nm for quercetin, rutin, and kaempferol. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200–600 nm).

2.8. Limits of detection and quantification

Limits of detection and quantification were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by Ademiluyi et al. [25]. Limits of detection and quantification were calculated as 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

2.9. Inhibition of Fenton reaction (degradation of deoxyribose)

The method of Halliwell and Gutteridge [26] was used to determine the ability of the fruits samples to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose. The fruits juice extract (0–200 μL) was added to a reaction mixture containing 120 μL 20mM deoxyribose, 400 μL 0.1M phosphate buffer, 40 μL 500μM $FeSO_4$, and the volume were made up to 800 μL with distilled water. The reaction mixture was incubated at 37°C for 30 minutes and the reaction was then stopped by the addition of 0.5 mL 2.8% trichloroacetic acid. This was followed by addition of 0.4 mL 0.6% thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 20 minutes and the absorbance was measured at 532 nm using spectrophotometer.

2.10. 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging ability

The 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical ($ABTS^+$) scavenging ability of the fruits was determined according to the method described by Re et al. [27] 1999. The $ABTS^+$ was generated by reacting 7mM ABTS aqueous solution with $K_2S_2O_8$ (2.45mM final concentration) in the dark for 16 hours and adjusting the absorbance at 734 nm to 0.700 with ethanol. Thereafter, 200 μL of appropriate dilution of the fruit juice extracts were added to 2.0 mL $ABTS^+$ solution and the absorbance was measured at 734 nm after 15 minutes using a spectrophotometer. The Trolox equivalent antioxidant capacity was subsequently calculated using Trolox as the standard.

2.11. Nitric oxide scavenging ability

Nitric oxide scavenging assay was performed using the Griess reagent method. Briefly, 0.3 mL of sodium nitroprusside (5mM) was added to 1 mL each of various concentrations of the fruits juice extracts. The test tubes were then incubated at 25°C for 150 minutes, after which, 0.5 mL of Griess reagent (equal volume of 1% sulfanilamide in 5% ortho-phosphoric acid and 0.01% naphthylethylenediamine in distilled water, used within 12 hours of preparation) was added [28]. The absorbance was measured at 546 nm using spectrophotometer.

2.12. 1,1-diphenyl-2 picrylhydrazyl free radical scavenging ability

The free radical scavenging ability of the fruits against 1,1-diphenyl-2 picrylhydrazyl (DPPH) free radical was evaluated as described by Gyamfi et al. [29]. Briefly, appropriate dilution of the fruits juice extracts (0–400 μL) was mixed with 1 mL 0.4mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 minutes and the absorbance was taken at 516 nm using a spectrophotometer. The DPPH free radical scavenging ability was subsequently calculated.

2.13. α-Amylase inhibition assay

Appropriate fruit juice extract dilution (0–200 μL) and 500 μL of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing porcine pancreatic α-amylase (EC 3.2.1.1; 0.5 mg/mL) was incubated at 25°C for 10 minutes. Then, 500 μL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) was added to each tube. The reaction mixtures was incubated at 25°C for 10 minutes and stopped with 1.0 mL of dinitrosalicylic acid color reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 minutes, and cooled to room temperature. The reaction mixture was then diluted by adding 10 mL of distilled water, and absorbance measured at 540 nm using a spectrophotometer. The α-amylase inhibitory activity was expressed as percentage inhibition [30].

2.14. α-Glucosidase inhibition assay

Briefly, appropriate dilution of the fruits juice extract (0–200 μL) and 100 μL of α-glucosidase (EC 3.2.1.20) solution in 0.1M phosphate buffer (pH 6.9) was incubated at 25°C for 10 minutes. Then, 50 μL of 5mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25°C for 5 minutes, before reading the absorbance at 405 nm in a spectrophotometer. The α-glucosidase inhibitory activity was expressed as percentage inhibition [31].

2.15. Determination of IC_{50} values

IC_{50} (effective concentration causing 50% enzyme inhibition/antioxidant ability) values for the enzyme inhibitory activity assays were calculated using nonlinear regression analysis.

2.16. Data analysis

The results of triplicate experiments were pooled and expressed as mean ± standard deviation. Means were compared by one way analysis of variance followed by Duncan's multiple range test and least significant differences were carried out and accepted at $p \le 0.05$ [32]. Differences between groups of HPLC were assessed by an analysis of variance model and Turkey's test. The level of significance for the analyses was set to $p < 0.05$. These analyses were performed by using the free software R version 3.1.1 [33].

3. Results

The free soluble starch contents of the 13 fruit samples were 3.01–3.89 g/100 g (Table 1). Breadfruit had significantly highest starch (3.89 g/100 g) content while pineapple (3.01 g/100 g) had the least starch content. Similarly, the results of the free soluble sugar

Table 1
Starch and sugar contents of some Nigerian fruits (g/100g).*

Sample	Sugar	Starch
Banana	44.55 ± 0.5[c]	3.83 ± 0.1[a]
Carrot	39.78 ± 0.3[e]	3.35 ± 0.13[b]
Avocado	45.21 ± 0.8[c]	3.23 ± 0.8[b]
Pineapple	51.52 ± 1.0[b]	3.01 ± 0.8[c]
Pawpaw	42.51 ± 0.6[d]	3.34 ± 0.13[b]
Guava	41.27 ± 0.8[d]	3.14 ± 0.1[b,c]
Tomatoes	39.28 ± 0.8[e]	3.08 ± 0.8[c]
African star apple	42.27 ± 1.8[d]	3.06 ± 0.09[a]
Water Melon	45.55 ± 0.5[c]	3.05 ± 0.06[c]
Orange	49.28 ± 1.1[b]	3.04 ± 0.08[c]
Cashew	35.34 ± 0.7[f]	3.27 ± 0.08[b]
Soursop	38.78 ± 0.8[e]	3.32 ± 0.09[b]
Breadfruit	60.91 ± 2.8[a]	3.89 ± 0.11[a]

*The starch and sugar contents of the fruits were determined as described and the absorbance measured at 490nm using Jenway 6315 UV/Visible spectrophotometer (Bibby Scientific Ltd, Stone, Staffordshire, UK). The starch and sugar contents was calculated and expressed in g/100 g. Values represent mean ± standard deviation ($n = 3$). Values with the same superscript letter within a column are not significantly ($p < 0.05$) different.

as presented in Table 1 range from 35.34–60.91 g/100 g with breadfruit having the significantly highest sugar (60.91 g/100 g) content while cashew (35.34 g/100 g) had the lowest. The amylose content of the fruits samples range from 0.84–1.46 g/100 g with breadfruit having the highest amylose content (1.46 g/100 g) among all the fruits while tomatoes (0.84 g/100 g) and watermelon (0.84 g/100 g) had the lowest (Table 2). The 13 fruits samples had glycemic indices ranging from 28.01 to 68.34 (Table 3), African star apple 28.10 had the lowest glycemic index and watermelon 68.34 had the highest.

Table 4 shows the HPLC-DAD characterization of constituent phenolic compounds present in the fruits. As revealed in Figures 2, 3 and 4, quercetin is present in all the fruits while gallic acid, catechin, epicatechin, rutin, and chlorogenic acid are other phenolic constituents in most of the fruits.

Table 5 shows the IC_{50} values of $\dot{O}H$, $ABTS^{+}$, NO, and DPPH radical scavenging ability of the fruits. The $\dot{O}H$ scavenging ability of the fruit juice extracts range from 16.76 μg/mL to 47.06 μg/mL with guava (IC_{50} = 16.76 ± 0.27μg/mL) having the highest scavenging ability while pineapple (IC_{50} = 47.06 ± 0.23 μg/mL) had the least. $ABTS^{+}$ scavenging ability of the fruits juice extracts presented as

Table 2
Amylose and amylopectin contents of some Nigerian fruits (g/100 g).*

Sample	Amylose	Amylopectin	Amylose/amylopectin
Banana	1.11 ± 0.08[b]	2.72 ± 0.10[a]	0.41
Carrot	1.06 ± 0.05[b]	2.29 ± 0.08[b]	0.46
Avocado	0.98 ± 0.03[b,c]	2.25 ± 0.05[b]	0.43
Pineapple	1.05 ± 0.02[b]	1.96 ± 0.08[c]	0.53
Pawpaw	1.02 ± 0.04[b]	2.32 ± 0.09[b]	0.44
Guava	1.03 ± 0.02[b]	2.86 ± 0.12[a]	0.36
Tomatoes	0.84 ± 0.01[b]	2.24 ± 0.08[b]	0.37
African star apple	1.06 ± 0.06[b]	2.81 ± 0.18[a]	0.38
Watermelon	0.84 ± 0.02[b]	2.24 ± 0.11[b]	0.37
Orange	1.06 ± 0.04[b]	1.98 ± 0.07[c]	0.54
Cashew	0.91 ± 0.01[b,c]	2.36 ± 0.13[b]	0.39
Soursop	0.88 ± 0.01[b]	2.44 ± 0.06[b]	0.36
Breadfruit	1.46 ± 0.07[a]	1.68 ± 0.05[a]	0.86

*The amylose content of the fruits was determined as described and the absorbance measured at 620 nm using a Jenway 6315 UV/Visible spectrophotometer (Bibby Scientific Ltd, Stone, Staffordshire, UK) after 20 minutes. Amylopectin content was derived from starch and amylose content gotten to difference and then the amylose/amylopectin ratio calculated. Values represent mean ± standard deviation ($n = 3$). Values with the same superscript letter within a column are not significantly ($p < 0.05$) different.

Table 3
Glycemic indices of some Nigerian fruits (based on glucose = 100).*

Sample	Glycemic index
Banana	52.78 ± 0.81[g]
Carrot	35.86 ± 1.03[c]
Avocado	40.34 ± 0.72[e]
Pineapple	56.00 ± 1.12[h]
Pawpaw	55.29 ± 1.33[h]
Guava	32.25 ± 0.62[b,c]
Tomatoes	38.38 ± 1.42[d]
African star apple	28.01 ± 0.53[a]
Watermelon	68.34 ± 2.11[j]
Orange	42.68 ± 0.92[f]
Cashew	31.60 ± 0.61[b]
Soursop	30.33 ± 1.13[b]
Bread fruit	64.50 ± 1.23[i]

**In vitro* starch hydrolysis rate and hydrolysis index of the fruits were determined as described with the absorbance measured at 540 nm using Jenway 6315 UV/Visible spectrophotometer (Bibby Scientific Ltd, Stone, Staffordshire, UK). The rate of starch digestion was expressed as the percentage of starch hydrolyzed per time using glucose standard. Values represent mean ± standard deviation ($n = 3$). Values with the same superscript letter are not significantly ($p < 0.05$) different.

Trolox equivalent antioxidant capacity revealed that all the extracts scavenged $ABTS^{+}$. Guava (IC_{50} = 58.50 μg/mL) had the highest NO radical scavenging ability while the least scavenging ability was exhibited by avocado (IC_{50} = 133.88 μg/mL). Also, African star apple (IC_{50} = 76.51 μg/mL) had the highest DPPH free radical scavenging ability and pineapple (IC_{50} = 311.81 μg/mL) the least.

Table 6 shows that all the fruits samples inhibited α-amylase activity in a concentration dependent (0–40 μg/mL) pattern. Soursop and guava had the highest inhibitory activity on α-amylase exhibiting 50% inhibitory concentration (IC_{50}) value of (IC_{50} = 19.52 μg/mL) and (IC_{50} = 20.77 μg/mL), respectively ($p < 0.05$), compared to watermelon (IC_{50} = 43.76 μg/mL), which had the least.

Soursop had the highest inhibitory activity on α-glucosidase (IC_{50} = 17.93 μg/mL) while breadfruit had the least (IC_{50} = 41.60 μg/mL; Table 6).

4. Discussion

Dietary recommendation on the consumption of fruits and vegetables among diabetics has experienced low adherence in the developing countries as a result of inadequate awareness and research backing in the area [34]. Regular consumption of fruits is associated with reduced risks of cancer, cardiovascular disease, stroke, neurodegenerative disease, cataracts, and some of the functional declines associated with aging [35]. Fruits are generally high in fiber, water, vitamin C, phytochemicals, and sugars. The sugar content in most fruits increases upon ripening as a result of hydrolysis of starch to sugar in the course of the ripening process [36]. This may have accounted for the low starch content and high sugar content observed in the fruits used for this study. This is in agreement with previous study by Moneruzzaman et al. [37] who attributed this to the conversion of starch to reducing sugar upon the advancement of ripening of the fruits.

High amylose products have been found to induce low blood glucose and insulin responses when compared with similar products high in amylopectin. In this study, the amylopectin content is higher than that of amylose yet the fruits sample displayed a good potential in hyperglycemic response. This could be attributed to other contents such as fiber and phenolic constituents, which have been confirmed to lower blood glucose in previous studies [9,38]. Interestingly, studies on the *in vitro* starch digestibility of rice

Table 4
Phenolic constituents of some tropical fruits.*

Fruits ► phenolics (mg/g) ▼	Banana	Carrot	Avocado	Pineapple	Pawpaw	Guava	Tomatoes	African star apple	Watermelon	Orange	Cashew	Sour sop	Breadfruit
Procyanidin	–	–	–	–	3.64	–	–	–	–	–	–	–	–
Gallic acid	2.76	0.31	1.58	0.31	0.37	1.34	2.56	–	0.84	–	1.73	1.46	0.71
Catechin	1.09	–	3.01	–	1.09	2.98	0.71	0.52	0.87	3.17	0.65	–	–
Caffeic acid	2.80	1.16	–	1.47	–	0.65	6.08	2.08	3.15	0.68	–	6.83	2.35
Ellagic acid	–	–	–	0.29	–	–	3.27	–	0.49	–	3.98	0.74	0.64
p-Coumaric acid	5.73	–	0.96	4.16	4.70	–	8.19	–	4.16	–	–	4.90	0.61
Epicatechin	1.12	–	4.71	–	1.85	2.71	1.62	0.41	0.79	4.09	2.65	1.51	–
Rutin	–	–	0.93	1.50	–	6.42	4.85	4.73	1.58	5.25	1.08	0.36	–
Quercetin	3.05	4.81	4.75	4.15	1.13	18.54	6.01	5.52	3.74	2.71	5.39	1.71	3.58
Kaempferol	–	–	–	–	–	2.73	4.78	2.63	0.81	0.69	2.68	3.25	–
Apigenin	2.46	1.19	–	–	–	4.95	8.03	–	–	–	–	–	–
Resveratrol	–	–	–	–	–	–	–	–	–	–	–	–	3.29
Chlorogenic acid	–	3.05	6.27	5.63	–	7.13	2.53	2.67	–	–	1.76	5.92	–
Isoquercitrin	–	2.37	–	–	–	–	–	–	–	–	–	–	–
Luteolin	–	7.25	–	0.72	–	3.06	–	–	–	2.73	–	–	–
Cyanidin-3-o glycoside	–	–	–	–	–	–	–	3.29	–	–	–	–	–
Cyanidin	–	–	–	–	–	–	–	2.65	–	–	–	–	–
Quercitrin	–	–	–	5.89	–	7.12	–	5.49	–	6.84	–	–	–
Orientin	–	–	–	–	–	15.09	–	–	–	–	–	–	–

*The phenolic phytoconstituents of the freeze dried fruits juices were identified and quantified with the aid of high performance liquid chromatography coupled diode array detector and the result expressed as mg/g. Calibration curve of catechin: y = 13682x + 1195.3 (r = 0.9998); epicatechin: y = 13185x + 1196.2 (r = 0.9997); gallic acid: y = 11762x + 1208.7 (r = 0.9999); caffeic acid: y = 12457x + 1239.5 (r = 0.9996); ellagic acid: y = 11965x + 1364.8 (r = 0.9994); *p*-coumaric acid: y = 13509x + 1287.6 (r = 0.9998); kaempferol: y = 12731x + 1179.5 (r = 0.9997); quercetin: y = 11964x + 1185.3 (r = 0.9999); and rutin: y = 11874x + 1308.9 (r = 0.9995). All chromatography operations were carried out at ambient temperature and in triplicate.

showed that rice cultivars with higher amylose content showed a lower digestibility than those with low amylose content [39,40]. This could be a major reason behind the use of fruits in aiding digestion.

The GI characterizes the carbohydrates consumed in different types of foods on the basis of postprandial level of blood glucose [16,41]. Dietary changes are often necessary to control type-2 diabetes, whether insulin is required or not. The GI was formulated in an attempt to aid diabetic populations in their food selection with the recommendation that diabetics select foods with a low GI [42]. Using glucose as the reference, foods are classified as having low (0–55), medium (55–69), or high (≥70) GI. The low GI of the fruits as shown in Table 3 has provided the basis for the recommendation of their consumption by the diabetics. This low GI could be due to the presence of polyphenols (responsible for the fruits' colored pigments), sucrose (fructose and glucose), and fibers. Viscous, soluble fibers transform intestinal contents into gel-like matter that slows down enzymatic activity on starch, which may result to a low GI [43]. Research has also suggested that low-GI diets improve glycemic control in individuals with impaired glucose tolerance and type-2 diabetes by lowering fasting blood glucose and glycated proteins and improving insulin sensitivity [44].

The fruits samples also demonstrated strong free radical scavenging activities as exemplified by their scavenging activity of moderately stable $ABTS^{+}$, NO, OH, and DPPH radicals *in vitro*. There is an agreement between the $ABTS^{+}$, NO, OH, and DPPH free radical scavenging ability and the phenolic content of the fruits samples, with African star apple, guava and cashew having the highest radical scavenging ability. The radical scavenging ability of the fruit samples may be linked to their total phenolic phytoconstituent. This finding agreed with earlier findings where plant antioxidant properties (free radical scavenging ability) correlates with their phenolic content [45,46]. Dietary use of plant or plant based food is the most practical approach to the prevention and management of chronic degenerative diseases such as diabetes and cardiovascular diseases. Hence, steady supply of dietary antioxidants to augment or boost the endogenous antioxidant defense mechanisms could be one practical approach through which free radical-mediated oxidative stress in type-2 diabetes may be curtailed. It has been demonstrated that elevated consumption of plant antioxidants is

Table 5
IC_{50} values of hydroxyl (OH), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), nitric oxide (NO) and 1,1-diphenyl-2 picrylhydrazyl (DPPH) radical scavenging abilities of some tropical fruits.

Sample	OH* (μg/mL)	ABTS* (mmol TEAC/μg)	NO*(μg/mL)	DPPH* (μg/mL)
Banana	21.27 ± 0.15^{b}	18.50 ± 0.15^{e}	63.68 ± 0.13^{b}	181.86 ± 0.151^{e}
Carrot	23.38 ± 0.12^{c}	11.53 ± 0.15^{g}	62.14 ± 0.15^{b}	228.05 ± 0.133^{g}
Avocado	42.22 ± 0.35^{g}	20.76 ± 0.12^{d}	133.88 ± 0.33^{l}	52.63 ± 0.375^{d}
Pineapple	47.06 ± 0.23^{h}	10.00 ± 0.18^{h}	82.34 ± 0.25^{e}	311.81 ± 0.255^{k}
Pawpaw	29.64 ± 0.37^{d}	6.92 ± 0.08^{i}	112.36 ± 0.37^{j}	251.51 ± 0.372^{h}
Guava	16.76 ± 0.27^{a}	41.54 ± 0.25^{a}	58.50 ± 0.28^{a}	105.17 ± 0.269^{c}
Tomatoes	42.75 ± 0.17^{g}	9.23 ± 0.14^{h}	125.33 ± 0.16^{k}	277.43 ± 0.176^{i}
African star apple	20.44 ± 0.28^{b}	24.62 ± 0.18^{c}	67.71 ± 0.27^{c}	76.51 ± 0.29^{a}
Watermelon	34.27 ± 0.19^{f}	27.69 ± 0.17^{b}	96.35 ± 0.15^{i}	217.56 ± 0.179^{g}
Orange	31.73 ± 0.37^{e}	16.15 ± 0.19^{f}	76.26 ± 0.37^{d}	187.30 ± 0.351^{f}
Cashew	24.94 ± 0.14^{c}	42.30 ± 0.40^{a}	64.99 ± 0.19^{bc}	89.24 ± 0.16^{b}
Soursop	25.65 ± 0.27^{c}	20.00 ± 0.22^{d}	66.86 ± 0.25^{c}	102.86 ± 0.215^{c}
Breadfruit	40.29 ± 0.35^{g}	16.92 ± 0.21^{f}	93.12 ± 0.35^{f}	288.39 ± 0.377^{j}

*The radical scavenging abilities of the fruits were determined as described and expressed as percentage. The IC_{50} (effective concentration causing 50% antioxidant ability) were calculated using nonlinear regression analysis. Values represent mean ± standard deviation ($n = 3$). Values with the same letter within a column are not significantly different ($p > 0.05$).

Table 6
IC_{50} values for the α-amylase, α-glucosidase inhibitory activities of some Nigerian fruits (μg/mL).*

Sample	IC_{50} for α-amylase and α-glucosidase (μg/mL)	
	α-Amylase	α-Glucosidase
Banana	39.12 ± 0.21[e]	31.89 ± 0.12[d]
Carrot	31.89 ± 0.53[b,c]	36.32 ± 0.31[f]
Avocado	37.20 ± 0.17[d]	29.51 ± 0.33[c]
Pineapple	42.31 ± 0.31[f]	34.25 ± 0.15[e]
Pawpaw	37.95 ± 0.16[d]	33.69 ± 0.24[e]
Guava	20.77 ± 0.17[a]	20.3 ± 0.35[a,b]
Tomatoes	34.50 ± 0.28[c]	28.00 ± 0.13[c]
African star apple	28.86 ± 0.62[b]	24.07 ± 0.31[b]
Watermelon	43.76 ± 0.31[f]	34.84 ± 0.15[e]
Orange	40.62 ± 0.24[e,f]	35.85 ± 0.13[e]
Cashew	28.78 ± 0.15[b]	25.74 ± 0.37[b]
Soursop	19.52 ± 0.35[a]	17.93 ± 0.15[a]
Breadfruit	42.71 ± 0.17[f]	41.60 ± 0.37[g]

*α-amylase inhibitory activity of the fruit extracts. The reaction media contained extracts (500 μL), 500 μL of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing hog pancreatic α-amylase (EC 3.2.1.1; 0.5 mg/mL) and then incubated at 25°C for 10 minutes. Thereafter, 500 μL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) was added to each tube. The reaction mixtures was incubated at 25°C for 10 minutes and stopped with 1.0 mL of dinitrosalicylic acid color reagent. α-Glucosidase inhibitory activity of the fruits extracts. Appropriate dilution of the (50 μL) and 100 μL of α-glucosidase solution (1.0 U/mL) in 0.1M phosphate buffer (pH 6.9) was incubated at 25°C for 10 min. Then, 50 Ìl of 5mM p-nitrophenyl-D-glucopyranoside solution in 0.1M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25°C for 5 minutes, before reading the absorbance at 405 nm in the spectrophotometer (Bibby Scientific Ltd, Stone, Staffordshire, UK). The IC_{50} (effective concentration causing 50% enzyme inhibitory ability) were calculated using nonlinear regression analysis. Values represent means ± standard deviation of triplicate readings. Values with the same letter within a column are not significantly different ($p > 0.05$).

accompanied by increased activity of extracellular antioxidant enzymes like glutathione peroxidase and superoxide dismutase [47].

The predominant presence of orientin in guava fruit may account for the highest NO and OH radical scavenging activity and antidiabetic property it posed among all the fruits, as previous studies have reported α-glucosidase inhibitory potential as well as potent antioxidant effect of orientin on aged mice [48,49]. These different phytochemicals identified in the fruits have various protective and therapeutic effects essentially to manage degenerative diseases such as type-2 diabetes [50]. Phenolics are capable of scavenging free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases [51,52]. Their potent antioxidant activity is due to the redox properties of their hydroxyl groups attached to the chemical structure of the phenolic compounds [53,54].

The fruits samples also demonstrated their ability to inhibit α-amylase and α-glucosidase activities *in vitro* (Table 6). α-Amylase and α-glucosidase are the key enzymes of dietary carbohydrate digestion and inhibitors of these enzymes may be effective in retarding glucose absorption [55]. The inhibition of the enzyme α-glucosidase slows down the breakdown of disaccharide to simple glucose, by so doing reducing the amount of glucose absorbed in the blood thus influencing the GI. This forms the basis for the hypothesized mechanism of action of α-amylase and α-glucosidase inhibitors in reducing the glycemic index. However, of all the 13 fruits, soursop, guava and African star apple showed the highest inhibitory activity on α-amylase exhibiting IC_{50} value of 18.52 μg/mL, 19.77 μg/mL, and 20.86 μg/mL respectively. Similarly, the same trend was followed for α-glucosidase inhibitory activity. Research has shown fruits to have many health benefits, including antidiabetic effect [56,57]. Phenolic fractions of plants generally have long been recognized to inhibit carbohydrate hydrolyzing enzymes in mammals. Phenolic compounds derived from red cabbage, strawberries, and raspberries have been identified to be inhibitors of α-amylase and α-glucosidase [56]. Although Jenkins et al. [41] affirmed that the concept of glycemic index is no longer novel as far as diabetes management is concerned, he further hypothesized that pharmacologic approaches to slowing carbohydrate absorption, notably the use of glycoside inhibitors, are now accepted in the management of diabetes.

It is worthy of note that this study has been able elucidate the GIs, antioxidant properties as well as α-amylase and α-glucosidase inhibitory properties of some tropical fruits. In view of this, we have been able to establish that there is a link between the GI and the hypoglycemic potential of foods hence, affirm that foods with low GI will be very good inhibitors of starch hydrolyzing enzymes.

In conclusion, the fruits used in this study exhibited antioxidant activities as typified by their radicals scavenging abilities. The low glycemic indices of the fruits generally, most especially in African star apple, guava, cashew, soursop, and carrot combined with their inhibition of α-amylase and α-glucosidase activities potentiates the biochemical justification for the recommendation and consumption by the diabetics. Hence, the health promoting potential of these fruits could be the reason behind its use in the management of diabetes in Africa. Although most of these fruits are indigenous to sub-Saharan Africa, most especially Nigeria, it should be pointed out that the phytoconstituents and overall activity might be different from those obtained in other continents.

Conflicts of interest

The authors declare that there is no funding from any organization and that there is no conflict of interest as far as this manuscript is concerned.

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