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Starch Content, Antioxidant Activity and Inhibition of Starch Hydrolyzing Enzymes by Unripe *Musa paradisiaca* and *Musa acuminata*

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Abstract

Research on natural products has gained worldwide attention due to the promising prospect of discovering plants that can inhibit starch hydrolyzing activity in the management of diabetes. In this study, *Musa paradisiaca* flour (MAF) and flour pastry (MPP), and *Musa acuminata* flour (MAF) and flour pastry (MAP) were prepared from fresh unripe *Musa* species. The corresponding flour and flour pastry (mixture of flour in hot water to form a gel) of the samples were investigated to estimate their starch contents, antioxidant activities [DPPH (1,1-diphenyl-2-picrylhydrazyl), and hydroxyl (OH) radical scavenging ability], total phenolic contents, total flavonoid contents, and alpha-amylase and alpha-glucosidase inhibitory activity. The results revealed that the starch contents showed no significant difference in their values as the flour and the flour pastry samples of *Musa* spp. exhibited a low level of sugar release and glycaemic index. The antioxidant potentials of both samples were also not significantly different. The inhibitory activities of the *Musa* spp. on starch hydrolyzing enzymes were higher in *Musa paradisiaca* than in *Musa acuminata*. The antioxidant properties and starch hydrolyzing inhibition tendencies exhibited by unripe *Musa acuminata* in comparison with *Musa paradisiaca* show that both species can be an economical source of a natural scavenger of free radicals in the body to fortify the prevention and management of blood-glucose-related diseases.

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Introduction

Plantain (*Musa paradisiaca*) is a perennial tree crop that belongs to the kingdom Plantae and the family *Musaceae*. Its production due to low input of labor has immensely boosted the economy in Nigeria and has reportedly doubled in the last two decades to position it the third among starchy food before the year 2010 [1]. Apart from processing the pulp of its unripe fruit into products such as chips, plantain shelf life can be further extended by processing into flour to prepare pastry also known as 'amala' by mixing in the right quantity of boiling water [2]. Banana (*Musa acuminata*) is popularly identified as a menu of everyday nutrition. Its unripe form can only be awaited to be consumed as a ready-to-eat fruit after ripening [3]. *Musa* spp. are generally fruit sources that provide instant energy and are a very good source of dietary potassium intake [4]. Starch is an important carbohydrate polymer that is the basic carbohydrate constituent of widely consumed diets including but not limited to cereals, roots, and fruits. The unripe pulp of banana contains about 70-80% starch on a dry weight basis, which is comparable to the endosperm of white potato [5]. A new hygienic approach that involves a reduction of the consumption of glycaemic carbohydrates and an increase in the level of indigestible carbohydrates in food products are increasingly gaining recognition [6]. Starch contains two kinds of polysaccharide molecules; amylose, a linear polymer that is usually about 20-30%, and amylopectin, a branched polymer that normally ranges between 70 – 80% of the starch molecule [7, 8]. Despite controversial beginnings, the glycaemic index has steadily evolved into a standard and highly effective classification of foods according to their post-prandial glycaemic effect [9].

Musa spp. possesses some antioxidant properties, which are essential components that play a vital role in sustaining good health. Different type of phytochemicals, which includes flavonoid and phenolic can be found in the variety of *Musa* spp. (plantain and banana). Flavonoids potently scavenge a wide range of oxidizing molecules likewise phenolic acids in plants [10]. Amylases are enzymes that catalyze the hydrolysis of internal 1, 4-glycosidic bonds in large carbohydrate molecules and yield small molecular weight products while α-glucosidase, on the other hand, catalyzes the terminal step of carbohydrate digestion by hydrolyzing 1, 4-alpha linkages and producing glucose as the final product [11]. Small molecular weight compounds that inhibit α-amylase and α-glucosidase slow down the hydrolysis of carbohydrates in the gastrointestinal tract and can also decrease the postprandial blood glucose levels in persons living with diabetes [12].

A good number of earlier research works have revealed the antihyperglycemic potentials of unripe plantain [12-14]. Normally, the unripe samples of bananas do not necessarily need to be converted to the flour before being reconstituted in boiling water to prepare the pastry. However, the processing of unripe banana into flour is gradually becoming a popular trend among local farmers, especially in the Southwestern part of Nigeria which are falsely sold as regular unripe plantain flour. The reason for this unusual practice no doubt cannot be farfetched from the economic point of view. In light of the foregoing coupled with the dearth of scientific information on the consumption of pastry prepared from unripe bananas, the present study, therefore, sought to evaluate and compare the amylose, amylopectin, and antioxidant property as well as the inhibitory potential of starch hydrolyzing enzyme in *Musa paradisiaca* and *Musa acuminata.*

Materials and Methods

Sample collection

Two different varieties of matured unripe *Musaceae* family; plantain (*Musa paradisiaca*) and banana (*Musa acuminata*) were obtained from a local market at Ilara-Mokin, Ondo State. Nigeria [7.3497°N, 5.1067°E]. The authenticity of the samples was validated at the Department of Biology, Federal University of Technology, Akure, Nigeria.

Reagents/chemicals

Folin–Ciocalteu reagents, were procured from Sigma Aldrich, Inc. (St. Louis, MO, USA), Porcine pancreatic α-amylase (EC 3.2.1.1), α-glucosidase (EC 3.2.1.20), p-nitrophenyl- α-D-glucopyranoside, gallic acid, quercetin were purchased from Sigma-Aldrich Labor GmbH (Seelze, Germany). Trichloroacetic acid, malondialdehyde (MDA), and 3,5-dinitrosalicylic acid were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals used were of analytical grade, and the water used

was glass distilled. Optical absorbance was measured with a UV-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom).

Preparation of samples

The plantain (*Musa paradisiaca*) and banana (*Musa acuminata*) samples were peeled and sliced into tiny pieces and oven-dried at $40°C$. Later, both samples were milled into flour using a blender. Also, a sizeable portion of the raw flour of both species was prepared to make the thick elastic paste colloquially known as 'Amala'. To achieve this, the flour was transferred into boiling water and the mixture was stirred over a long period until it is well cooked, and a brown thick elastic paste was formed. The elastic paste was later oven-dried at $40°C$ and then milled again using a blender. Meanwhile, 5g of each of both raw and pastry flour samples of both species under study were transferred to a tube and 50 mL of distilled water was added. The samples were then mixed for 4 hours using an electronic laboratory shaker. Subsequently, filtration was done using a silk sieve.

Starch and sugar content assays

The estimation of the amount of starch and sugar present in the samples was carried out by initially extracting the sugar and starch content of the samples using hot 80% ethanol. The mixtures were then centrifuged at a speed of 2000 rpm for 10 min. After centrifugation, the supernatants of the samples were decanted and stored in another tube for sugar analysis, and the residues were stored for starch analysis [15]. To estimate the sugar content, the supernatant was initially diluted, and 0.2 ml of the resulting solution was mixed with 0.5 ml of phenol solution. The solution was conditioned to $25°C$ and the absorbance was measured subsequently in the spectrophotometer at 490 nm. To estimate the starch content of the samples, hydrolysis was carried out on the residues for 1 hr and 30 minutes using 9 ml of perchloride acid and the solution was subsequently made up to 50 ml by adding distilled water and filtering through glass wool equivalent. Then 0.05 ml of the filtrate was mixed with 0.5 ml of phenol solution (5%) and 2.5 mL H_2SO_4 (absolute). The mixture was allowed to cool to room temperature and the absorbance was read at 490 nm.

Determination of amylose and amylopectin content

Amylose standard and samples (0.1 g) were weighed, transferred into a centrifuge tube and a solution containing 1 ml of 95% alcohol and 9 ml of 1N sodium hydroxide was added. The resulting solution was then mixed using a vortex mixer. The solution was incubated at 100°C for 10 minutes and subsequently cooled down to room temperature. Thereafter, 1 ml of the solution was transferred into a new tube and 9 ml of distilled water was added. Again, 0.5 ml of the diluted solution was transferred into another fresh tube followed by the addition of 0.1 ml acetic acid and 0.2 ml iodine solution. Similarly, the solution was made up to the 10 ml mark by adding a complementary volume of distilled water. Subsequently, a color-dependent reaction was allowed to occur for 10 minutes at room temperature and the absorbance of the vortexed mixture was read at 620 nm [16].

The concentration of amylose in the sample was then calculated using the following equation.

Amylose (%) = (amylose content of the standard × absorbance of sample) / absorbance of standard

Amylopectin concentration (%) of the sample was estimated by using the following formula [17].

Amylopectin (%) = 100% − amylose (%)

Glycaemic index profiling

50 mg of the sample was weighed and transferred into a beaker and a solution containing 1 mg of pepsin in 10 ml hydrochloric acid and KCl buffer was added, and the resulting solution was subsequently incubated at $40°C$ for 60 min. The pH of the solution was conditioned using phosphate buffer followed by the addition of α-amylase solution. The resulting solution was incubated at 37ºC and 200 μL of the solution was transferred into a fresh test tube at 30 minutes intervals for 2 hours. The solutions were subsequently boiled for 15 minutes, which precedes the addition of sodium acetate (500 μl, pH 4.75) and α-glucosidase (5 μl). Before the addition of DNSA (200 μl), the resulting solution was incubated at 60ºC for 45 min followed by a swift (5 min) incubation at 100ºC. Thereafter, 2 ml of distilled water was added, and the solution was centrifuged for 5 minutes at a speed of 3000 rpm. The supernatant was transferred to the spectrophotometer and the

absorbance was read at 540 nm. The glycaemic index was calculated as follows [18].

Glycemic index = (sum of area under the curve of sample / sum of areas under the curve for standard) ×100

Aqueous extract preparation

Ten g of each sample was soaked in 100 ml of distilled water and allowed to dissolve for about a day at 25°C. The mixture was then filtered, and the obtained filtrate was subsequently centrifuged at high speed for 10 minutes. The supernatant was then stored at 4°C for subsequent analysis [19].

Determination of total phenol and total flavonoid

A proportion of the diluted aqueous extract was mixed with 2.5 ml of 10% Folin-Ciocalteau's reagent to enhance oxidation. Swiftly, 2.0 ml of sodium carbonate (7.5%) was added to neutralize the reaction. The resulting solution was incubated for 40 min at 45℃ and the absorbance was determined at 765 nm [20]. Furthermore, as described by Meda et al. [21], 0.5 ml of the rightly diluted extract was mixed with an equal volume of methanol, 50 μl of aluminum chloride (10%), 50 μl of potassium acetate (1M), and 1.4 ml of distilled water. The resulting solution was allowed to incubate at room temperature for 30 minutes. The absorbance of the solution was then read at 415 nm. The total flavonoid content was calculated as quercetin equivalent.

Determination of ferric reducing activity

As described by Pulido et al. [22], 2.5 ml of the extract was mixed with an equal volume of 200 mM phosphate buffer (pH 6.6) and an equal volume of potassium ferric cyanide (1%). The resulting solution was then incubated for 20 minutes at 50℃. 2.5 ml of 10% TCA was subsequently added and the mixture was centrifuged for 10 min. Thereafter, 5 ml of the supernatant was transferred to a fresh tube and mixed with 5 ml of distilled water and 1 ml of 0.1% $FeCl_3$. The absorbance was then read at 700 nm.

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical assay

The diluted extract (0.1 ml) was mixed with 3.9 ml of a solution containing 60 μM DPPH in methanol. The mixture was incubated at room temperature in a dark environment for 2 hours. The absorbance of the mixture was then measured at 515 nm [23].

α-Amylase and α-glucosidase inhibition assay

Fifty μl of the sample was mixed with 500 μl of phosphate buffer (20 mM, pH 6.9, 6 mM Sodium chloride), which contains pig pancreatic α amylase (0.5 mg/ml). The mixture was incubated at 250°C for 10 min. Subsequently, a solution containing 500 μl of starch solution (1%) mixed with 20 nM sodium phosphate (pH 6.9, 6 mM NaCl) was added. The reaction mixture was again incubated at 25°C for 10 minutes and the reaction was terminated with 1.0 ml of 3, 5-dinitrosalicylic acid. Later, the mixture was incubated at 100°C for 5 minutes and subsequently cooled to room temperature. Thereafter, 10 ml of distilled water was added to the mixture and the absorbance was read at 540 nm [24]. In the same vein, 50 μl of the sample was mixed with a solution containing 100 μl of α-glucosidase solution (1.0 U/ml) and 0.1 M phosphate buffer (pH, 6.9), and the resulting mixture was incubated for 5 minutes at 250°C. The absorbance was determined at 405 nm.

Results

Sugar content in *Musa* spp.

Fig 1. shows the sugar content in two cultivars of *Musa* spp. varieties. The findings revealed that *M. paradisiaca* flour (MPF) has a lower concentration of sugar relative to its pastry form (MPP) and the sugar content of *M. acuminata* flour (MAF) was similarly lower than its pastry form.

Amylose, amylopectin and amylose / amylopectin ratio

The amylose content of the samples presented in Fig. 2 ranges from 0.079%-0.163%. The amylose content of MPP is higher (0.163%) than its raw MPF (0.123%) while that of raw MAF is not significantly different (0.085%) from its pastry flour (0.079%). Starch is a polysaccharide composed of amylose and amylopectin. The concentration of the components is presented in Fig. 3. The amylopectin content of the samples as shown below revealed that there was no significant difference between the flour and pastry samples of *M. acuminata*. MPP contained a higher amylose content compared to MPF while amylose/amylopectin ratio content in MAF and MAP were lower in comparison to *M. paradisiaca* products (Fig. 4).
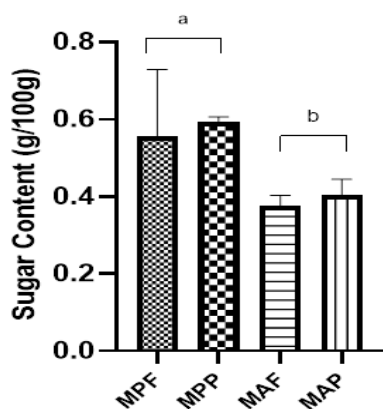
Fig 1. Graphical representation of sugar content in *Musa paradisiaca* and *Musa acuminata*.
MPF: *Musa paradisiaca* flour, MPP: *Musa paradisiaca* pastry, MAF: *Musa acuminata* flour, MAP: *Musa acuminata* pastry. Groups compared with a or b are statistically different at $P<0.05$.
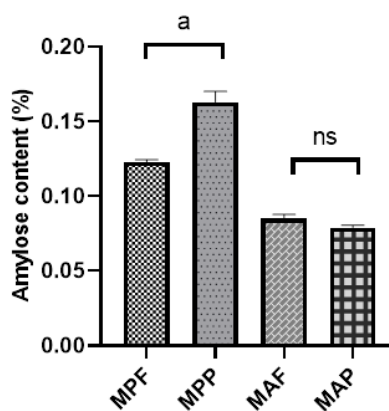

Fig 2 Graphical representation of amylose content in *Musa paradisiaca* and *Musa acuminata*.
MPF: *Musa paradisiaca* flour, MPP: *Musa paradisiaca* pastry, MAF: *Musa acuminata* flour, MAP: *Musa acuminata* pastry. Groups compared with a or b are statistically different at $P<0.05$. ns: no significant difference.
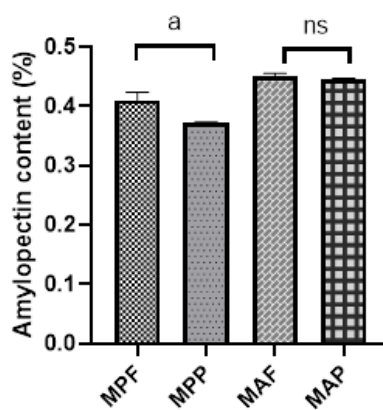

Fig 3 Amylopectin concentration of *Musa* species products.
MPF: *Musa paradisiaca* flour, MPP: *Musa paradisiaca* pastry, MAF: *Musa acuminata* flour, MAP: *Musa acuminata* pastry. Groups compared with a or b are statistically different at $P<0.05$. ns: no significant difference.

Glycaemic index

The glycaemic index (GI) is usually classified into low GI (0–55), medium GI (56–69), and high GI (≥70) using glucose as a reference [25]. The result of *M. paradisiaca* products and *M. acuminata* products followed the same trend as there was no significant difference in the observed glycaemic index. The studied *Musa* spp. were within the low range of classified GI (Fig. 5).

Anti-oxidative activity

In Fig. 6, the results of the analyses showed that radical scavenging ability increased in *M. paradisiaca* on pasting but significantly reduced in *M. acuninata*. The ferric reducing antioxidant activity power analysis showed that pasting reduced the ferric reducing power of both species (Fig. 7). Meanwhile, *M. paradisiaca* pastry (MPP) had higher phenolic content (Fig. 8) than *M. paradisiaca* flour (MPF). Similarly, *M. acuminata* flour (MAF) is reduced after processing. In Fig 9, it is evident that the *M. acuminata* has a significantly higher flavonoid content compared to *M. paradisiaca*. Pasting does not affect the antioxidant content of the two flours.

α-Amylase and α-glucosidase Inhibition assay

The results in Fig. 10 show the inhibitory properties of both unripe *M. paradisiaca* and *M. acuminata* aqueous extracts (flour and pastry). *M. paradisiaca* flour (MPF) had a significantly higher alpha-amylase inhibitory property compared to *M. acuminata* flour (MPP). Conclusively, pasting significantly reduces the amylase inhibitory property of *M. paradisiaca* flour while the amylase inhibitory property of *M. acuminata* flour is improved by pasting. Fig. 11 presents the inhibitory properties of α-glucosidase of both unripe *M. paradisiaca* and *M. acuminata* extract (flour and pastry). In Fig 11, unripe *M. paradisiaca* flour had a significantly higher amylase inhibitory property compared to unripe *M. acuminata* flour. However, pasting significantly increased α-amylase inhibition property of the two flours.

Data analysis

All laboratory experiments were performed in duplicates. The results were presented as mean ± standard error. Student's t-test and one-way analysis of variance (ANOVA) were carried out and significance was accepted at $P<0.05$.
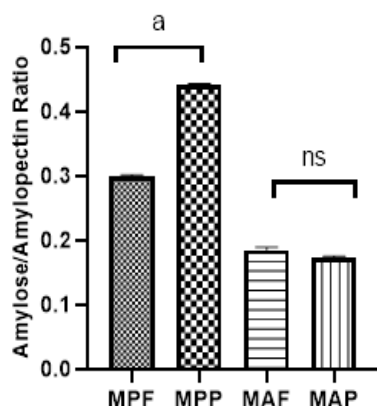
Fig 4 Graphical representation of Amylopectin ratio in *Musa paradisiaca* and *Musa acuminata.*
MPF: *Musa paradisiaca* flour, MPP: *Musa paradisiaca* pastry, MAF: *Musa acuminata* flour, MAP: *Musa acuminata* pastry. Groups compared with a or b are statistically different at *P*<0.05. ns: no significant difference.
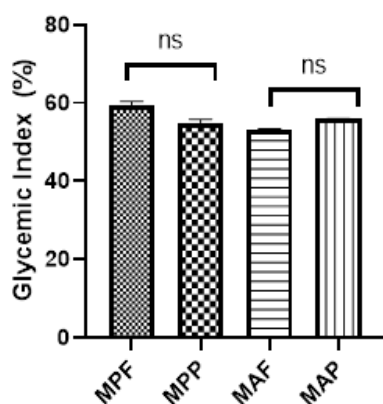

Fig 5 Graphical representation of glycaemic index in *Musa paradisiaca* and *Musa acuminata.*
MPF: *Musa paradisiaca* flour, MPP: *Musa paradisiaca* pastry, MAF: *Musa acuminata* flour, MAP: *Musa acuminata* pastry. ns: no significant difference.
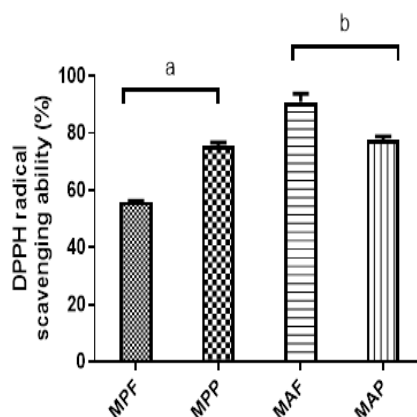

Fig 6 2,2-diphenyl-1-picrylhydrazyl-hydrate content in *Musa paradisiaca* and *Musa acuminata.*
MPF: *Musa paradisiaca* flour, MPP: *Musa paradisiaca* pastry, MAF: *Musa acuminata* flour, MAP: *Musa acuminata* pastry. Groups compared with a or b are statistically different at *P*<0.05.
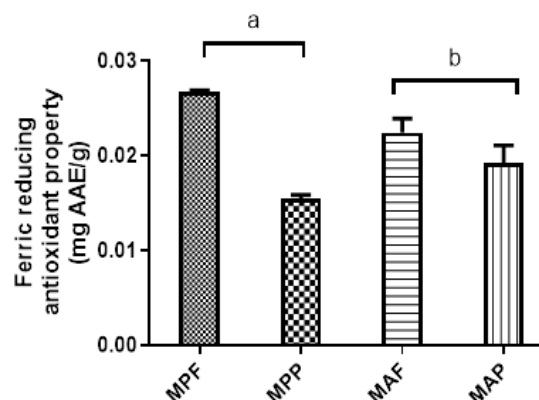

Fig 7 Ferric reducing antioxidant power content in *Musa paradisiaca* and *Musa acuminata.*
MPF: *Musa paradisiaca* flour, MPP: *Musa paradisiaca* pastry, MAF: *Musa acuminata* flour, MAP: *Musa acuminata* pastry. Groups compared with a or b are statistically different at *P*<0.05.
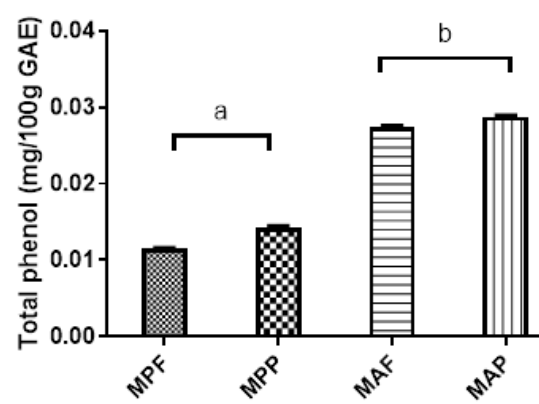

Fig 8 Total phenol content in *Musa paradisiaca* and *Musa acuminata.*
MPF: *Musa paradisiaca* flour, MPP: *Musa paradisiaca* pastry, MAF: *Musa acuminata* flour, MAP: *Musa acuminata* pastry. Groups compared with a or b are statistically different at *P*<0.05.

Fig 9 Total flavonoid content in *Musa paradisiaca* and *Musa acuminata.*
MPF: *Musa paradisiaca* flour, MPP: *Musa paradisiaca* pastry, MAF: *Musa acuminata* flour, MAP: *Musa acuminata* pastry. ns: no significant difference.

Fig 10 Inhibitory activity of Alpha-amylase in *Musa paradisiaca* and *Musa acuminata*.
MPF: *Musa paradisiaca* flour, MPP: *Musa paradisiaca* pastry, MAF: *Musa acuminata* flour, MAP: *Musa acuminata* pastry. Groups compared with a or b are statistically different at $P<0.05$.
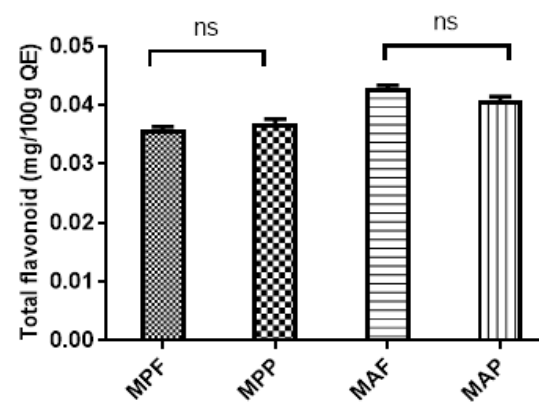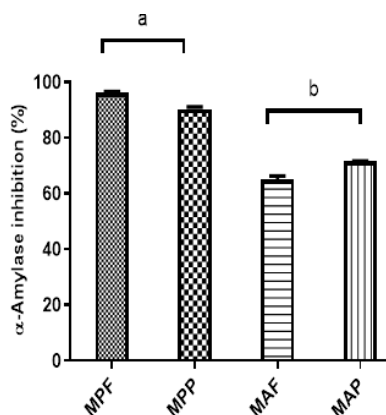

Fig 11 Inhibitory activity of Alpha-glucosidase in *Musa paradisiaca* and *Musa acuminata*.
MPF: *Musa paradisiaca* flour, MPP: *Musa paradisiaca* pastry, MAF: *Musa acuminata* flour, MAP: *Musa acuminata* pastry. Groups compared with a or b are statistically different at $P<0.05$.
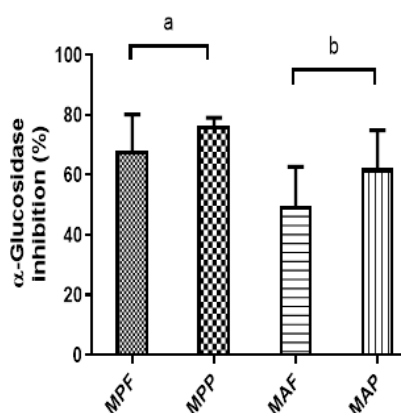
Discussion

The results showed that unripe plantain flour (*M. paradisiaca*) and unripe banana flour (*M. acuminata*) had a relatively low sugar content, which can be recommended for consumption. Our research further revealed that unripe plantain flour and unripe banana flour release reducing sugar gradually in small concentrations over some time. Flours from these two cultivars are among the best option for antidiabetic consumption because of their comparatively low release of reducing sugars [26]. In this study, there was a slight increase in the sugar concentration of both cultivars of flour

after undergoing a heat process. Amylose's positive association with indigestible carbohydrate content and its ratio to amylopectin indicate the pattern of crystallization during the starch composite making [27]. The ratio of amylose and amylopectin for unripe plantain flour and unripe banana flour showed statistically significant results. Plantain flour has a relatively low concentration of amylopectin ratio when compared to banana flour. The amylose amylopectin ratio of *M. paradisiaca* flour increases speedily when it undergoes the heat process while there was a slight decrease in the amylose/amylopectin ratio of *M. acuminata* flour after it passes through the heat process. The glycaemic indices of unripe plantain flour and unripe banana flour are shown in Fig 5. Carbohydrate foods that raise the blood glucose level quickly after a meal are regarded as high glycaemic index foods and have a value of 70 and above, whereas low glycaemic index foods which release glucose slowly into the bloodstream have a value of 55 and below [28]. *M. paradisiaca* flour reduces the glycaemic index after passing through the heat process, which makes it more consumable for diabetic patients. While there as a slight increment in glycaemic index of *M. acuminata* flour after it undergoes the heat process, but still in the range of low GI.

DDPH radical scavenging assays are commonly used to quantify the potency of antioxidants in plants where they negate the formation of free radicals and destroy its progression by donating an electron or hydrogen atom [29, 30]. The DPPH free radical scavenging ability of the flour and pastry samples of unripe *M. paradisiaca* and *M. acuminata* aqueous extracts are presented in Fig 6. There was an observed increase in the inhibitory activity of the pastry of *M. paradisiaca* in comparison with the flour. This present finding is in contrast with a recent study conducted Shodehinde and Oboh [13], in which, unripe plantain flour was higher in DPPH radical scavenging ability than the pastry. Contrarily, *M. acuminata* is in line with that same study [13], which reported flour of unripe plantain to be higher in DPPH radical scavenging ability than the pastry form. Phenols have an arsenal that fights free radicals, which is a product of normal aerobic metabolism and is capable of protecting the body by eliminating the toxic radicals, iron chelation, activation of antioxidant enzymes, and inhibiting oxidases [31]. This present study as depicted in Fig. 8 and 9 revealed the total phenol and total

flavonoid content of *M. paradisiaca* and *M. acuminata*, respectively. The aqueous extracts (flour and pastry) from both species exhibited significant phenolic properties. This is in line with the earlier work research work of Shodehinde and Oboh [13], in which, unripe *M. paradisiaca* product exhibited phenolics compound. In this study, the total flavonoid contents of *M. paradisiaca* flour (MPF) are slightly lower than the pastry (MPP), which is in line with a previous study [13]. However, there was a decrease in the flavonoid contents after processing *M. acuminata* flour (MAF), which is in contrast with a previous study [13]. It was observed that the pastry of *M. paradisiaca* (MPP) had higher phenolic contents compared to flour (MPF). Likewise, in *M. acuminata*, the pastry (MAP) is slightly higher than the flour (MAF, which also validates the previous findings of Shodehinde and Oboh [13].

α-amylase, also known as endoamylase is an enzyme that cleaves α, 1-4 glycosidic bonds of starch such as amylose or amylopectin to disaccharide, which later breaks down to glucose [32]. The polyphenols present in unripe plantain and banana samples may play a crucial role in α-amylase inhibition. When this enzyme is inhibited, postprandial hyperglycaemia is reduced [33]. The present study shows that the flour and pastry samples of both *M. paradisiaca* and *M. acuminata* displayed high inhibitory activity against α-amylase. This result validates the reports of Shodehinde and Oboh [13]. This implies that both medicinal plants represent potential dietary means for the management of diabetes as inhibition of α-amylase activity will reduce glucose release. α-glucosidase is an enzyme that is used to break down alpha, 1-4 glycosidic bond of disaccharide to glucose. The inhibition of α-glucosidase, therefore, is a good way of controlling hyperglycaemia [14]. This present study revealed the α-glucosidase inhibitory properties of extracts of unripe *M. acuminata* and *M. paradisiaca*. The aqueous extracts (flour, pastry) from the two plants in line with the result of α-amylase also exhibited inhibitory potentials on the activities of α-glucosidase. This, however, corroborated the earlier work of Shodehinde and Oboh [13] in which unripe *M. paradisiaca* products inhibited starch hydrolyzing enzyme activities.

Conclusions

The present study has demonstrated that unripe plantain flour (*M. paradisiaca*) and unripe banana flour (*M. acuminata*) meals have a low sugar content and a range of low-medium glycaemic index content, which make them safe for consumption for everyone, including diabetic and obese patients. There is a need to encourage and also create awareness about the health impact of consuming unripe plantain flour and unripe banana flour, especially for those living with sugar-related chronic diseases. The high inhibitory effect of extracts of unripe plantain and unripe banana on key enzymes associated with type 2-diabetes could make them economical and good sources of nutraceuticals for the management of diabetes.

Conflict of interest

The authors declare no conflict of interest.

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