

Nutritional, antioxidant and inhibitory properties of cocoa powder enriched wheat-plantain biscuits on key enzymes linked to type 2 diabetes

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Article history

Received: 14 December 2016

Received in revised form:

31 January 2017

Accepted: 2 February 2017

Abstract

This study evaluated the nutritional, antioxidant and inhibitory effect of five blends cocoa powder enriched wheat-plantain biscuits on key enzymes linked to type 2 diabetes. The composite mixture of whole wheat flour (WWF) and unripe plantain flour (UPF) in the proportion (w/w) of 100:0, 75:25, 50:50, 25:75 and 0:100 as 100:0, WWF, UPF-25, UPF-50, UPF-75 and UPF-100 were prepared respectively. Aspartame served as sweetening agent (2%), cocoa butter (5%) with egg albumen (mixed to taste) as emulsifiers, and cocoa powder (3%) was used as flavoring agent for the production of the five different biscuits. The nutritional, antioxidant and inhibitory properties of the biscuits on key enzymes linked to type-2 diabetes (α -amylase and α -glucosidase) were evaluated. The result revealed a significant difference ($p < 0.05$) of the biscuits produced from UPF-100, UPF-75 and UPF-50 when compared with that of WWF and UPF-25 in their general acceptability and taste. The protein content of the biscuits produced decreased gradually with replacement of UPF. Conversely, the crude fibre of the biscuit samples was observed to increase with increased the percentage (%) inclusion of UPF in the composite mixture. The phenolic (total phenol and flavonoid) contents, antioxidant properties as typified by 2,2-azinobis-3-ethylbenzo-thiazoline-6-sulfonate radical (ABTS*) scavenging ability, reducing property and Fe^{2+} -chelating ability and inhibition of Fe^{2+} lipid peroxidation in pancreas- in vitro of the aqueous extracts of the biscuits increased with increase in UPF replacement. Furthermore, the biscuit extracts inhibited α -amylase and α -glucosidase activities significantly in dose-dependent manner. From the observed results, the biscuits may represent good sources of nutrients that may prevent type-2 diabetes and promote sound health.

Keywords

Diabetes
Wheat flour
Unripe plantain
 α -amylase
 α -glucosidase

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Introduction

Diabetes mellitus and cardiovascular diseases are the leading causes of death globally. Diabetes mellitus affects about 135 million people in the world and is projected to affect about 300 million individuals by the year 2025 (Wild *et al.*, 2004). The term diabetes mellitus is a condition in which the body either does not produce enough, or does not properly respond to insulin, a hormone which is produced in the pancreas (WHO, 1999). However, of the several types of diabetes mellitus, non-insulin dependent diabetes mellitus (NIDDM) is the most common form of diabetes, accounting for 90% of all cases. It is a metabolic disorder primarily characterized by insulin resistance, relative insulin deficiency and abnormal rise in blood sugar, right after meal, called postprandial hyperglycemia (Kwon *et al.*, 2007).

The inhibition of enzymes involved in the

breakdown of starch (α -amylase) and uptake of glucose (α -glucosidase) have been suggested to be useful approach to the management and prevention of Type-2 diabetes (Kwon *et al.*, 2007). Drugs such as acarbose and viglibose are currently used as α -amylase and α -glucosidase inhibitors but they exhibit side effects such as abdominal distension, bloating, flatulence, and diarrhea (Bischoff, 1994; Vinchayanrat *et al.*, 2002). Some of these side effects are possibly caused by excessive inhibition of the pancreatic α -amylase leading to bacterial fermentation of undigested carbohydrate in the colon (Bischoff, 1994). Foods of plant origin usually contain natural antioxidants such as phenolic compounds that can scavenge free radicals (Sun *et al.*, 2002; Alia *et al.*, 2003; Oboh *et al.*, 2010; Adefegha and Oboh, 2011).

Phenolic compounds are important group of secondary metabolites, which are synthesized by plants because of plant adaptation to biotic and

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abiotic stress condition such as infection, water stress and cold stress (Obboh and Rocha, 2007). In recent years, phenolic compounds have attracted the interest of researchers because of their antioxidants capacity; they can protect the human body from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells (Obboh *et al.*, 2012). The antiradical activity of flavonoids and phenols is principally based on the structural relationship between different parts of their chemical structure (Rice-Evans *et al.*, 1996). Polyphenols are common constituents of the human diet, present in most foods and beverages of plant origin. They are considered to contribute to the prevention of various degenerative diseases (Adefegha and Obboh, 2013). This assumption originally came from *in vitro* studies, showing the antioxidant properties of several polyphenols and their ability to modulate the activity of various enzymes (Adefegha and Obboh, 2013; Obboh *et al.*, 2013). Research suggests that many flavonoids are more potent antioxidant than vitamins C and E (Obboh and Rocha, 2007; Adefegha and Obboh, 2013).

Whole wheat flour is full-flavored flour containing vitamins, minerals and protein. Whole-grain whole wheat flour is more nutritious than refined white flour, although white flour may, in a process called food fortification, have some micronutrients lost in processing added back to the white flour (Whole Grains Council, 2007). Fortified white wheat flour does not, however, contain the macronutrients of the wheat's bran and germ (especially fiber and protein) like whole-grain flour does, and is notably lacking in fiber. Whole grain is a good source of calcium, iron, fiber, and other minerals like selenium (Whole Grains Council, 2007). However, whole-wheat flour has a shorter shelf life than white flour because of its higher oil content which can lead to rancidity if not stored properly. Plantains and bananas are a good source of vitamin A (carotene), vitamin B complex (thiamin, niacin riboflavin and B6) and vitamin C (ascorbic acid). In comparison with other starchy staples; vitamin C content is similar to those of sweet potato, cassava and potato.

Plantains provide a better source of vitamin A than most other staples (Aurand *et al.*, 1987; Kirk and Sawyer, 1991). Although plantains do not provide a particularly good source of several important minerals in human nutrition, such as calcium, iron and iodine, they are notably high in potassium and low in sodium (Marriott *et al.*, 1983; Stover and Simmonds, 1987). Unripe plantain pulp has a total of 3.5% dry matter as cellulose and hemicellulose and therefore constitutes a good source of dietary fiber (Kirk and Sawyer, 1991). Cocoa and its derived

products, such as chocolate, represent a very rich source of dietary flavonoids, which contain a higher content per serving than tea or red wine (Lee *et al.*, 2003). The health benefits associated with cocoa consumption have been related to their capacity to improve the lipid profile and insulin sensitivity, diminish blood pressure, reduce platelet activity and function, and ameliorate endothelial dysfunction (Lamuela-Ravento's *et al.*, 2005; Ding *et al.*, 2006; Cooper *et al.*, 2008). Although, there are several studies reporting the nutritional qualities and antioxidant capacities of individual food and isolated foods or whole meals (Obboh and Rocha, 2007, 2008; Obboh and Adefegha 2010; Adefegha and Obboh, 2011, 2012, 2013). Food scientists are now keen to harness the nutritional and antioxidant properties of plant foods in the management of free radical-mediated chronic diseases, including type 2 diabetes, obesity, cancer, and cardiovascular diseases. Information also abound on the inhibitory action of individual plants and plant foods on key enzymes relevant to type 2 diabetes (McCue *et al.*, 2005; McDougall *et al.*, 2005; Cheplick *et al.*, 2007; Ranilla *et al.*, 2010; Adefegha and Obboh, 2012; Adefegha *et al.*, 2014). However, there is dearth of information on the effect of whole meals fortified with cocoa powder on the nutritional, antioxidant and antidiabetic effect of the food products. Thus, this study sought to produce wheat-unripe plantain flour blend biscuits, assess their nutritional, antioxidant and inhibitory effects on key enzymes linked to type-2 diabetes (α -amylase and α -glucosidase).

Materials and Methods

Chemicals and reagents

Chemicals and reagents used such as thiobarbituric acid (TBA), 1,10-phenanthroline, gallic acid, quercetin, ascorbic acid, Folin-Ciocalteu's reagent were procured from Sigma-Aldrich, Inc., (St Louis, MO), trichloroacetic acid (TCA), malondialdehyde (MDA) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were sourced from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany), hydrogen peroxide, methanol, acetic acid and HCl were sourced from BDH Chemicals Ltd., (Poole, England), sodium carbonate, AlCl₃, potassium acetate, Tris-HCl buffer, sodium dodecyl sulphate (SDS), FeSO₄, potassium ferricyanide and ferric chloride were of analytical grade while the water was glass distilled.

Sample collection

Whole wheat seeds and unripe plantain fruits were purchased from Erekesan market, Akure, Ondo

State, Nigeria while cocoa powder and butter were obtained from COOP Cocoa Limited, Akure-Owo Expressway, Akure, Ondo State, Nigeria.

Sample preparation

The unripe plantain fruits were cleaned, peeled and rinsed with tap water, and manually sliced (thickness 0.5 inches). The sliced edible part was immediately air-dried; the dried products were pulverized and passed through a 100-mesh sieve, producing a free-flowing powder as plantain flour. The whole wheat seeds were handpicked to remove the dirt and unwanted materials and ground into free flowing powder as wheat flour. Five blends were prepared from the composite mixture of whole wheat flour (WWF) and unripe plantain flour (UPF) in the proportion (w/w) of 100:0, 75:25, 50:50, 25:75 and 0:100 as 100:0, WWF, UPF-25, UPF-50, UPF-75 and UPF-100 respectively. Aspartame served as sweetening agent (2%), cocoa butter (5%) with egg albumen (mixed to taste) as emulsifiers, and cocoa powder (3%) was used as flavoring agent for the production of the five different biscuit samples. Baking was carried out in an electric oven (MVH-33).

Sensory evaluation

Evaluation of the biscuit was carried out after cooling to room temperature. Sensory evaluation was performed by twelve trained respondents who were final year students of the Department of Biochemistry, Federal University of Technology, Akure, Nigeria. The mixed flour was randomly assigned to each respondent, after 30 minutes of briefing. The respondents were asked to evaluate the flour mixed for aroma, taste, texture, color, and general acceptability on 7-points Hedonic scale (7 = excellent, 6 = very good, 5 = good, 4 = average, 3 = fair, 2 = poor and 1 = very poor) using a modified method described by Potter (1968) and the attribute mean score was calculated.

Proximate analysis

The proximate composition (ash, fat and crude fiber) of the biscuits was determined using the standard AOAC (1990) method and the protein content was determined using the micro-Kjeldahl method.

Minerals analysis

The mineral composition of the biscuits was determined using colorimetric method according to AOAC (1990)

Preparation of aqueous extract of the flour mixed

Aqueous extracts of the wheat-plantain flour mixed (biscuit) were prepared by soaking 10 g of the milled biscuit in 100 ml of distilled water for about 24 h at 37°C; the mixtures were filtered, centrifuged and the supernatants were stored in the refrigerator for subsequent analysis (Adefegha and Oboh, 2012).

Determination of total phenol content

The total phenol content was determined according to the method of Singleton *et al.* (1999). Briefly, appropriate dilutions of the wheat and plantain flour mixture were oxidized with 2.5 ml 10% Folin-Ciocalteu's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm in the JENWAY UV-Visible spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by Meda *et al.* (2005). Briefly, 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 µl 10% aluminium chloride (AlCl₃), 50 µl 1M Potassium acetate and 1.4 ml water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm in the JENWAY UV-Visible spectrophotometer; the total flavonoid content was subsequently calculated using quercetin as standard.

Determination of vitamin C

Vitamin C content of the biscuit extracts was determined using the method of Benderitter *et al.* (1998). Briefly, 75 µl of the solution (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg Copper(II) sulphate pentahydrate in 100 ml of 5 M sulphuric acid) were added to 500 µl reaction mixture [300 µl of appropriate dilution of the mixed flour with 100 µl 13.3% trichloroacetic acid and water]. The reaction mixture was subsequently incubated for 3 h at 37°C, then 0.5 ml of 65% sulphuric acid (v/v) was added to the medium and the absorbance was measured at 520 nm in the JENWAY UV-Visible spectrophotometer. The vitamin C content was subsequently calculated using ascorbic acid as standard.

Determination of ferric-reducing antioxidant power (FRAP)

The reducing property of the extracts was determined by assessing the ability of the flour

mixture to reduce ferric chloride solution as described by Oyaizu (1986). 2.5 ml aliquot was mixed with 2.5 ml 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. and then 2.5 ml 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml 0.1% ferric chloride. The absorbance was measured at 700 nm in the JENWAY UV-Visible spectrophotometer. The ferric reducing antioxidant property was subsequently calculated as ascorbic acid equivalent.

2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging ability

The ABTS* scavenging ability was determined according to the method described by Re *et al.* (1999). The ABTS* was generated by reacting an (7 mmol/l) ABTS aqueous solution with K₂S₂O₈ (2.45 mmol/l, final concentration) in the dark for 16 h and adjusting the Absorbance 734 nm to 0.700 with ethanol. 0.2 ml of appropriate dilution of the extract was added to 2.0 ml ABTS* solution and the absorbance were measured at 734 nm after 15 min in the JENWAY UV-Visible spectrophotometer. The trolox equivalent antioxidant capacity was subsequently calculated.

Fe²⁺ chelation assay

The Fe²⁺ chelating ability of the extracts were determined using a modified method of Minotti and Aust (1987) with a slight modification by Puntel *et al.* (2005). Freshly prepared 500µM iron (II) sulphate (150 µl) was added to a reaction mixture containing 168 µl 0.1M Tris-HCl (pH 7.4), 218 µl saline and the biscuit extract (0 – 25 µl). The reaction mixture was incubated for 5 min, before the addition of 13 µl 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in the JENWAY UV-Visible spectrophotometer. The Fe (II) chelating ability was subsequently calculated.

Preparation of tissue homogenates

The rats were decapitated under mild diethyl ether anaesthesia and the pancreas was rapidly isolated and placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1/10 w/v) with about 10-up-and-down strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenates were centrifuged for 10 min at 3000xg to yield a pellet that were discarded, and a low-speed supernatant (S1) were kept for lipid peroxidation assay (Belle *et al.*, 2004).

Lipid peroxidation and thiobarbituric acid reactions

The lipid peroxidation assay was carried out using the modified method of Ohkawa *et al.* (1979), briefly 100 µl S1 fraction was mixed with a reaction mixture containing 30 µl of 0.1M pH 7.4 Tris-HCl buffer, extract (0 – 100 µl) and 30 µl of 250 µM freshly prepared iron (II) sulphate (the procedure was also carried out using 5 mM Sodium nitroprusside). The volume was made up to 300 µl by water before incubation at 37°C for 1 h. The colour reaction was developed by adding 300 µl 8.1% sodium dodecyl sulphate (SDS) to the reaction mixture containing S1, this was subsequently followed by the addition of 500 µl of acetic acid/HCl (pH 3.4) mixture and 500 µl 0.8% thiobarbituric acid (TBA). This mixture was incubated at 100°C for 1 h. TBARS (Thiobarbituric acid reactive species) produced were measured at 532 nm in the JENWAY UV-Visible spectrophotometer and the absorbance was compared with that of standard curve using MDA (Malondialdehyde).

α-Amylase inhibition assay

The α-Amylase inhibitory activity was determined according to the method of Bernfield (1951). The aqueous extracts dilution (500 µl) and 500 µl of 0.02 mol/l sodium phosphate buffer (pH 6.9 with 0.006 mol/l NaCl) containing Hog pancreatic α-amylase (EC 3.2.1.1) (0.5 mg/ml) were incubated at 25°C for 10 min. Then, 500 µl of 1% starch solution in 0.02 mol/l sodium phosphate buffer (pH 6.9 with 0.006 mol/l NaCl) was added to the reacting mixture. Thereafter, the reaction mixture was incubated at 25°C for 10 min and stopped with 1.0 ml of dinitrosalicylic acid (DNSA). The mixture was then incubated in a boiling water bath for 5 min, and cooled to room temperature. The reaction mixture was then diluted by adding 10 ml of distilled water, and absorbance measured at 540 nm in the JENWAY UV-Visible spectrophotometer. The α-amylase inhibitory activity was expressed as percentage inhibition.

$$\% \text{ Inhibition} = (\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}}) / \text{Abs}_{\text{ref}} * 100$$

α-Glucosidase inhibition assay

The α-Glucosidase inhibitory activity was determined according to the method of Apostolidis *et al.* (2007). Appropriate dilution of the aqueous extracts (50 µl) and 100 µl of α-glucosidase solution were incubated at 25°C for 10 min. Thereafter, 50 µl of 5 mmol/l p-nitrophenyl-α-D-glucopyranosidesolution in 0.1 mol/l phosphate buffer (pH 6.9) was added. The reacting mixture was then incubated at 25°C for 5 min, before reading the absorbance at 405 nm in the JENWAY UV-Visible spectrophotometer. The

α -glucosidase inhibitory activity was expressed as percentage inhibition.

$$\% \text{ Inhibition} = (\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}}) / \text{Abs}_{\text{ref}} * 100$$

Data analysis

The results of the sensory analyses are expressed as the mean \pm standard deviation with twelve replicates, while that of antioxidants and enzyme inhibitory properties were expressed as the mean \pm standard deviation of three replicates. A one way analysis of variance (ANOVA) was used to compare the treatments followed by Duncan's post hoc test. Microsoft excel and Graph Pad Prism 6 were used for the statistical analysis. Differences between the treatments were accepted at 5% level of significance ($p \leq 0.05$).

Results and Discussion

Sensory qualities (aroma, taste, colour, flavour, texture, and general acceptability) in food may represent the major determinant in consumer choice of food (Deliza and Macfie, 1996). The result of the sensory evaluation test of the formulated biscuits based on hedonic ratings for product (biscuit) attributes showed scores for aroma range from 5.6-6.0, colour (5.9-6.2), taste (5.3-6.3), texture (5.7-6.0), and for general acceptability (5.5-6.1). It is noteworthy that the cocoa powder (3%) was added to the developed products to enhance flavor and serve as additional phenolic source. Furthermore, inclusion of unripe plantain flour in wheat flour blend showed good desirability of aroma, color, and texture in biscuit production when compared with whole wheat flour biscuit. There was no significant ($p > 0.05$) difference between the whole wheat flour (WWF) and unripe plantain flour (UPF) in aroma, color, and texture. The non-significant ($p > 0.05$) differences in the texture, color, and flavor may have been resolved during baking where browning reactions took place. Significant ($p < 0.05$) differences were observed in the taste and general acceptability of the UPF-100, UPF-75, and UPF-50 compared to WWF, whereas there was no significant difference between UPF-25 and WWF in their general acceptability and taste. This could be an indication that substitution of wheat flour with plantain flour at 25% supplementation for biscuit production may be well acceptable by consumers. The above result agrees with the good quality biscuits produced from the mixtures of wheat flour and bread fruit flour (Omobuwajo 2003) and bread produced from waxy rice and wheat flour blends (Nakamura *et al.*, 2009). The proximate composition

(protein, fat, ash, moisture, and crude fibre) of the biscuits produced showed highest protein content (%) of 9.48 recorded for WWF, and this value decreased gradually to the lowest one of 9.42 for UPF-100. The protein content increased with increase in the proportion of wheat flour; this could be attributed to the presence of gluten in the whole wheat flour blended biscuits. In a similar manner, the percentage fat content of WWF was reported to be the highest (12.43%) while UPF-100 had the lowest fat content (11.84%). The minimal fat contents obtained for the samples could be relevant in that fat plays a vital role in the determination of shelf-life of foods. Higher amount of fat is very undesirable in food items. This is mainly because such high levels can initiate and accelerate spoilage by promoting rancidity, leading to off flavours and odorous development (Porter, 1978; Ihekoronye and Ngoddy, 1985). Conversely, the crude fibre of the biscuit sample was observed to increase with increased level of PF in the composite flours used for production. For UPF-100, UPF-75, UPF-50, UPF-25 and WWF, the crude fibre (% values) were 14.27, 13.89, 13.65, 13.52 and 11.96, respectively. Crude fibre may promote health by binding to fat deposits in digestive tracts of humans, preventing several degenerative diseases, such as atherosclerosis, obesity and diabetes. This indicates that the UPFs could attract acceptability by many people, especially in Africa, where most of our diets are bulky, consisting mainly of carbohydrates and fat. Proximate contents of all the biscuits produced are significantly higher ($p < 0.05$) when compared to the proximate composition of bread prepared from plantain-wheat flour blends (Olaoye *et al.*, 2006), and the significant difference could be traced to involvement of other principal materials used in the biscuits production. The mineral concentrations in the biscuits produced and the mineral ratios revealed that plantain-wheat flour biscuits were rich in potassium [highest in UPF-100 (18256 ppm) and lowest in WWF (10818 ppm)]. This follows a similar trend (WWF < UPF-25 < UPF-50 < UPF-75 < UPF-100), reported for crude fibre contents in this study. The potassium content increased with increase in the proportion of plantain flour, and this agreed with the already established fact that plantain is very rich in potassium, magnesium and phosphates (Demming-Adams *et al.*, 1996). It has been established that high dietary potassium in humans plays a protective role against hypertension, stroke, cardiac dysfunctions, renal damage, hypercalciuria, kidney stones, and osteoporosis (Demigné *et al.*, 2004), especially if such diets are also low in sodium (Arbeit *et al.*, 1992). The K/Na ratio of the CO₄ (3.5) was slightly

Table 1. Total Phenol, Total Flavonoid, Vitamin C, FRAP contents and ABTS radical scavenging ability.

Groups	Total phenol mg/g GAE	Total flavonoid mg/g QE	Vitamin C mg AAE/g	FRAP $\times 10^{-2}$ (mol. AAE/g)	ABTS (mmol. TEAC/g)
WWF	0.22±0.02 ^a	0.08±0.01 ^a	0.20±0.01 ^a	1.41±0.50 ^a	0.73±0.07 ^a
UPF-25	0.36±0.04 ^b	0.12±0.02 ^b	0.22±0.01 ^b	1.76±0.61 ^b	0.79±0.05 ^b
UPF-50	0.45±0.02 ^c	0.14±0.02 ^b	0.23±0.01 ^c	2.28±0.82 ^c	0.88±0.08 ^c
UPF-75	0.89±0.04 ^d	0.17±0.03 ^c	0.24±0.01 ^d	3.30±0.75 ^d	0.96±0.09 ^d
UPF-100	1.28±0.03 ^e	0.24±0.02 ^d	0.25±0.01 ^e	4.11±1.00 ^e	1.18±0.05 ^e

Values represent mean \pm S.D of three (3) replicates. Values with different superscripts along the column are statistically different ($p < .05$).

WWF – Biscuit produced from 100% whole wheat flour; UPF-25 – Biscuit produced from composite flours of 75% wheat flour/ 25% plantain flour; UPF-50 – Biscuit produced from 50% wheat flour/ 50% plantain flour; UPF-75 - Biscuit produced from 25% wheat flour/ 75% plantain flour UPF-100 – Biscuit produced from 100% plantain flour.

higher than the ideal ratio (3.1) as reported by Food and Nutrition Board (FNB) (Food and Nutrition Board 2001), but lower than those of other biscuits produced. This indicates that the consumption of the biscuit could be a dietary approach towards the management of some degenerative diseases, such as obesity, diabetes, hypertension and cancer. Phenolic antioxidants are the most abundant antioxidants in human diets. They exhibit a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and vasodilatory actions (Middleton *et al.*, 2000; Scalbert, and Williamson, 2000). They are capable of removing free radicals, chelate metal catalysts, activating antioxidant enzymes, reducing α -tocopherols, and inhibiting oxidases (Amic *et al.*, 2003). They are also important for improving the sensory and nutritional qualities, in that they impart colors, flavors and tastes (Kim *et al.*, 2002).

The results of the total phenol content of the biscuits are presented in Table 1, and total phenol content of the biscuits ranged from 1.28 mg/g (UPF-100) to 0.22 mg/g (WWF). Moreover, of all the biscuits analyzed, UPF-100 had the highest total phenol content. This reveals that much of the phenolic compounds were contributed by the unripe plantain flour. These findings agree with an earlier report that plantain is rich in carotenoids, phenols, provitamin A, vitamin C and potassium (Demming-Adams *et al.*, 1996). The total flavonoid, reported as quercetin equivalent antioxidant capacity (QEAC), is also presented in Table 1. Flavonoids have antioxidant activity and could, therefore, lower cellular oxidative stress, which has been implicated in the pathogenesis of various neurodegenerative diseases, including

Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Rice-Evans *et al.*, 1996; Amic *et al.*, 2003). The high flavonoid contents of plantain may have contributed to their medicinal properties. The flavonoid contents of the biscuits ranged from 0.24 mg/g (UPF-100) to 0.08 mg/g (WWF). This revealed that an agreement between the total phenol and total flavonoid contents of all the biscuits, thus agrees with many earlier reports where correlations were established between total phenol and total flavonoid contents (Melo *et al.*, 2006; Oboh *et al.*, 2007; Oboh and Rocha, 2008; Oboh *et al.*, 2013). Reducing power is an antioxidation defense mechanism; the two mechanisms that are available to affect this property are electron transfer and hydrogen atom transfer (Dastmalchi *et al.*, 2007). The reducing powers of the biscuit extracts were assessed based on their ability to reduce Fe (III) to Fe (II), and the results are also presented as ascorbic acid equivalent. The results revealed that all the biscuits had higher reducing power, with UPF-100 showing the highest reducing ability (4.11×10^{-2} mmole AAE g^{-1}) while WWF (1.41×10^{-2} mmole AAE g^{-1}) had the lowest reducing ability. The results of the reducing power agreed with the total phenols and total flavonoids as earlier discussed.

The ABTS radical-based model of free radical scavenging ability has the advantage of being more versatile as spectral interference is minimized at absorption maximum of 760 nm, a wavelength not normally encountered with natural products (Re *et al.*, 1999). ABTS[•] scavenging ability, reported as Trolox equivalent antioxidant capacity (TEAC), is presented in Table. 1. The results also revealed an increase in ABTS[•] scavenging ability of the biscuits with increase in proportion of the plantain

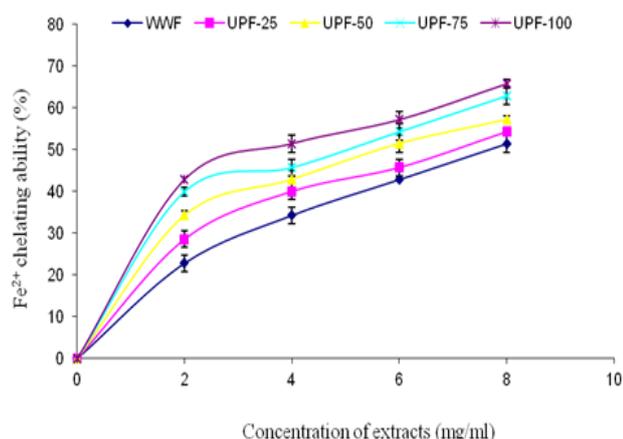


Figure 1. Fe²⁺ chelating ability of aqueous extracts of cocoa powder enriched plantain flour based biscuit.

Values represent mean \pm S.D of three (3) replicates

WWF – Biscuit produced from 100% whole wheat flour; UPF-25 – Biscuit produced from composite flours of 75% wheat flour/ 25% plantain flour; UPF-50 – Biscuit produced from 50% wheat flour/ 50% plantain flour; UPF-75 - Biscuit produced from 25% wheat flour/ 75% plantain flour UPF-100 – Biscuit produced from 100% plantain flour

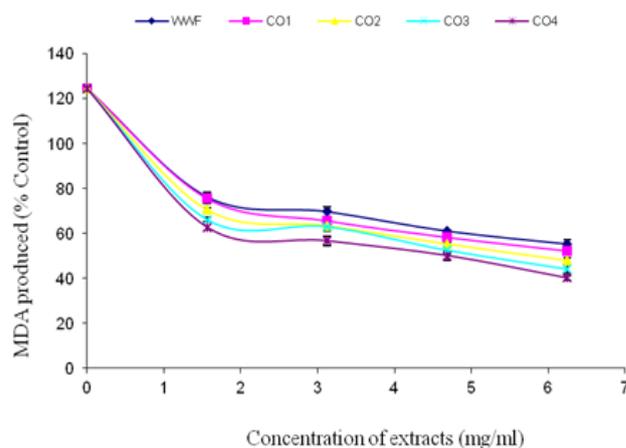


Figure 2. Lipid peroxidation scavenging ability of aqueous extracts of cocoa powder enriched plantain flour based biscuit.

Values represent mean \pm S.D of three (3) replicates

WWF – Biscuit produced from 100% whole wheat flour; UPF-25 – Biscuit produced from composite flours of 75% wheat flour/ 25% plantain flour; UPF-50 – Biscuit produced from 50% wheat flour/ 50% plantain flour; UPF-75 - Biscuit produced from 25% wheat flour/ 75% plantain flour UPF-100 – Biscuit produced from 100% plantain flour.

flour; UPF-100 (1.18×10^{-2} mmol TEAC g⁻¹) had the highest ABTS^{*} scavenging ability while the lowest activity (0.73×10^{-2} mmol TEAC g⁻¹) was observed in WWF. This trend in ABTS^{*} scavenging ability agrees with that of the reducing power in that substitution of wheat flour with plantain flour in the biscuit caused increase in the scavenging abilities of the biscuits against ABTS radicals. Fe (II)-chelating ability of the biscuit extracts were determined and the results are presented in Figure 1. The results revealed that all the extracts chelate Fe (II); however, all the plantain flour-substituted biscuit extracts had a significantly higher ($P < 0.05$) Fe (II)-chelating ability than the WWF extracts. The trend in Fe (II)-chelating ability is similar to that of ABTS^{*} scavenging ability and reducing power. The total antioxidant capacity is a combination of different antioxidant mechanisms, including free radical scavenging ability, reducing power and Fe (II)-chelating ability. The ability of biscuit extracts to inhibit Fe²⁺-induced lipid peroxidation was determined on rat's brain tissue in vitro and presented in Figure 2. However, all the biscuit extracts caused a significant decrease ($P < 0.05$) in the brain MDA levels at all extract concentrations in a dose-dependent manner. UPF-100 showed the highest inhibitory effect against Fe²⁺-induced lipid peroxidation in rat's brain homogenates when compared to other biscuit extracts while WWF had the lowest inhibitory effect. Inhibition of Fe²⁺-induced lipid peroxidation in rat's brain tissues follows a similar trend (WWF < UPF-25 < UPF-50 < UPF-75 < UPF-100) with the reducing power, Fe²⁺

chelating ability, and ABTS^{*} free radical scavenging ability. The results also revealed that increase in proportion of the plantain flour in biscuits produced, caused a decrease in the brain MDA levels. This decrease in Fe²⁺ - induced lipid peroxidation in rat's brain homogenates in the presence of the extracts could be a result of the ability of the antioxidant phytochemicals in the biscuit extracts to chelate Fe²⁺ and scavenge free radicals produced by Fe²⁺ catalyzed production of reactive oxygen species (ROS) in rat's brain homogenates (Materska, and Perucka, 2005; Podsedek, 2007). Inhibition of enzymes involved in retardation of glucose absorption (α -glucosidase) and reduction of starch hydrolysis (α -amylase), is considered a therapeutic approach in the control/management of type-2 diabetes (Apostolidis *et al.*, 2007; Kwon *et al.*, 2007; Adefegha and Oboh, 2012; Oboh *et al.*, 2013; Oboh *et al.*, 2014). Although, many foods and plant extracts have been reported as having hypoglycemic effect and could provide health benefits without the side effects presently encountered in the most available drugs such as abdominal distention, flatulence, and possibly diarrhea (McDougall *et al.*, 2005; Bhadari *et al.*, 2008). In this study, aqueous extracts of cocoa powder phenolic-enriched plantain-wheat biscuit were evaluated for its possible inhibition of α -amylase and α -glucosidase. The result of α -amylase inhibitory activity in Figure 3 showed that biscuits extract inhibited α -amylase activity in vitro in a dose-dependent manner (1.0–4.0 mg/ml) with UPF-100 (EC₅₀ = 2.48 mg/ml), UPF-75 (EC₅₀ = 3.33 mg/ml), UPF-50 (EC₅₀ = 4.50 mg/ml), UPF-25

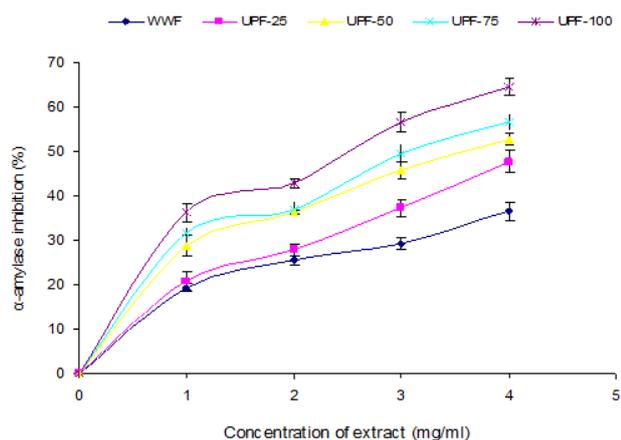


Figure 3. α -amylase inhibitory ability of aqueous extracts of cocoa powder enriched plantain flour based biscuit.

Values represent mean \pm S.D of three (3) replicates

WWF – Biscuit produced from 100% whole wheat flour; UPF-25 – Biscuit produced from composite flours of 75% wheat flour/ 25% plantain flour; UPF-50 – Biscuit produced from 50% wheat flour/ 50% plantain flour; UPF-75 – Biscuit produced from 25% wheat flour/ 75% plantain flour UPF-100 – Biscuit produced from 100% plantain flour.

($EC_{50} = 5.34$ mg/ml), and WWF ($EC_{50} = 7.25$ mg/ml) order of arrangement. Inhibition of α -amylase activity observed in the biscuit extracts may contribute to the management of hyperglycemia, by slowing down the breakdown of starch. Subsequently, the ability of α -glucosidase inhibitory activity (Figure 4) also revealed that aqueous extract inhibited α -glucosidase in vitro in a dose dependent manner (1.0–4.0 mg/ml) following a similar trend of inhibition in α -amylase activity considering their EC_{50} values (WWF = 3.33 < UPF-25 = 2.69 < UPF-50 = 2.13 < UPF-75 = 1.25 < UPF-100 = 0.72) measured in mg/ml. The inhibition of α -glucosidase slows down the breakdown of disaccharide to simple glucose, by so doing it reduces the amount of glucose that is absorbed in the blood (Apostolidis *et al.*, 2007). Furthermore, the result showed higher ($p < 0.05$) inhibition of α -glucosidase than that of α -amylase. This agrees with the assertion that dietary management of hyperglycemia linked to type-2 diabetes can be targeted through foods or plant extracts that possess high α -glucosidase and mild α -amylase inhibition (Kwon *et al.*, 2007; Adefegha *et al.*, 2014).

Conclusion

The high antioxidant properties of cocoa powder phenolics-enriched plantain-wheat biscuits, as typified by high free radical scavenging ability (ABTS), Fe^{2+} -chelating ability, ferric-reducing antioxidant power and inhibition of α -amylase and α -glucosidase showed a great potential of the replacement of wheat

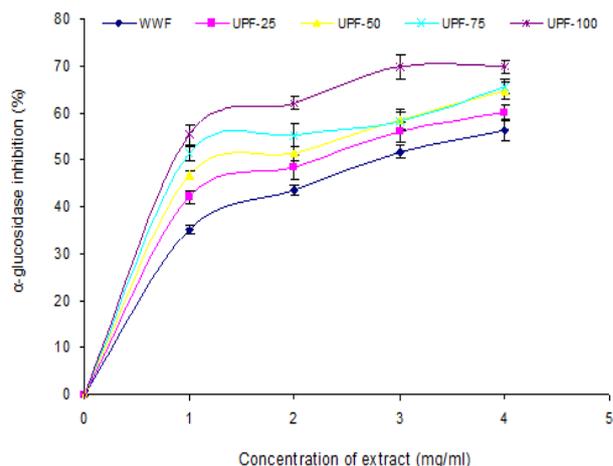


Figure 4. α -glucosidase inhibitory ability of aqueous extracts of cocoa powder enriched plantain flour based biscuit.

Values represent mean \pm S.D of three (3) replicates

WWF – Biscuit produced from 100% whole wheat flour; UPF-25 – Biscuit produced from composite flours of 75% wheat flour/ 25% plantain flour; UPF-50 – Biscuit produced from 50% wheat flour/ 50% plantain flour; UPF-75 – Biscuit produced from 25% wheat flour/ 75% plantain flour UPF-100 – Biscuit produced from 100% plantain flour.

with unripe plantain which could be a cheap and safe substitute for biscuit production and could also serve as functional food in the management/prevention of diabetes.

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