Trop J Nat Prod Res, February 2022; 6(2):227-235 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Free Polyphenol Contents, Antioxidant Activity and Inhibition of Enzymes Linked with Type-2-Diabetes of Bread Produced from Cocoa Powder Flavoured Improved Variety Cassava-Wheat Composite Flours

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ARTICLE INFO

Article history:
Received 10 September 2021
Revised 25 January 2022
Accepted 10 February 2022
Published online 06 March 2022

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ABSTRACT

Bread is one of the commonest staple foods across the globe. This study sought to investigate the phenolic constituents, antioxidant potentials and *in vitro* inhibition of enzymes linked with Type-2-diabetes of the bread produced from the composite of an improved variety cassava flour (CF) and wheat flour (WF) prepared in various proportion of 100:0 (100% CF), 50:50 (50% CF:50% WF), 20:80 (20% CF:80% WF), 10:90 (10% CF:90% WF) and 0:100 (100% WF) flavoured with 2g of cocoa powder (FL). Free phenolic extraction was carried out by homogenizing 50g of the composite bread sample with 80% acetone (1:4 w/v) and evaporating the filtrate to dryness. The dried extract was dissolved in water and used for subsequent analysis. The results revealed that there were significant ($p<0.05$) elevations in the total phenol (5.40 ± 0.09 mg/GAE/g) and Total flavonoid (1.98 ± 0.06 mg/QE/g) contents of 100% CF bread + FL as compared to 100%WF (control); total flavonoid (3.65 ± 0.09 mg/GAE/g) and total flavonoid (1.23 ± 0.06 mg/QE/g) respectively. The results also revealed significant ($p<0.05$) increase in the values of DPPH (1, 1-diphenyl-2 picrylhydrazyl) free radical, Fe^{2+} chelating ability, ferric reducing property (FRAP), 2,2-azinobis (3-ethylbenzo-thiazoline-6-sulfonate) ABTS radical scavenging ability, inhibition of Fe^{2+} induced Lipid peroxidation, α-amylase and α-glucosidase of the composite bread blend in comparison to their controls. The result suggests that an improved variety cassava flour bread blends could serve as cheap functional food recipe with high phenolic and antioxidant qualities, and nutritional intervention in the management and control of type-2-diabetes.

***Keywords*:** An improved variety cassava, Composite bread, Antioxidant, Wheat, Flavonoid.

Introduction

Nature has endowed medicinal plants with the ability to cure a large number of diseases in the human race.[1,2] Natural product use in disease prevention and treatment is increasing on a daily basis around the world.[3] Some studies have linked the ageing process to the harmful effects of free radicals, which are produced naturally in the body system as a result of chemical interactions during cellular metabolism or as a response to environmental changes.[4,5] Phenolic compounds have been confirmed to have numerous biological significances, including antioxidant property, antimutagenic, antitumor, and antibacterial activities.[6] Antioxidant properties of natural polyphenols stimulate antioxidant enzymes, reduce-tocopherol radicals, and block oxidases, resulting in positive health consequences.[7,8] They have been discovered to play a role in mediating *α*-amylase inhibition, and hence may play a role in Type-2 diabetes prevention and control.[9]
Antioxidants are powerful free radical scavengers in the body. Free radicals are extremely reactive chemical entities like superoxide, hydroxyl radicals, and singlet oxygen.[7,8,10] Antioxidant enzymes (produced by the body) and antioxidant nutrients (found in foods) scavenge all of the excess energy held by these free radicals, converting them to harmless particles and waste products; as a result, antioxidant nutrients are functional components of food with additional health benefits.[11] By blocking peroxidation chain reactions, dietary antioxidants have been discovered to protect against the onset of diabetes.[12] Diabetes mellitus is a collection of metabolic illnesses marked by hyperglycemia which is associated with long-term consequences such as microvascular, macrovascular, and neuropathic problems. Insulin secretion, action, or both can be affected by hyperglycemia.[13] Diabetes can cause problems such as neuropathy, retinopathy, and nephropathy. Diabetic complications are more likely in all types of diabetes. Diabetic retinopathy is visual loss caused by damage to the retina's blood vessels, which can lead to total blindness.[14] Diabetic patients are more likely to develop cataracts, glaucoma, and other eye conditions. A visit to an eye doctor once a year is recommended for diabetic patients.[15] Diabetic nephropathy, or kidney damage, can cause tissue scarring, protein loss in the urine, and finally chronic kidney disease, which may necessitate dialysis or kidney transplantation.[14] Cassava (*Manihot esculenta* Crantz) is one of Africa's most widely grown crops, with Nigeria leading the world in production. It belongs to the Malpighiales order, the Crotonoideae subfamily, the Manihot genus, and the Manihot esculenta species.[16] In many parts of tropical Africa, cassava is an important source of nutritional energy for low-income earners.[17] The Federal Government of Nigeria recently introduced a new improved variety of cassava (vitamin-A enriched cassava or yellow cassava) that could provide vitamin-A in the diet in the form of β-carotene (a carotenoid and precursor of vitamin A), an antioxidant that helps prevent heart disease, cancer, lower blood glucose levels, cataract risks, and muscular disorders, as well as boost the immune system.[18,19] Bread is a

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Citation: Ajani RA, Oboh G, Adefegha SA, Akindahunsi AA. Free Polyphenol Contents, Antioxidant Activity and Inhibition of Enzymes Linked with Type-2-Diabetes of Bread Produced from Cocoa Powder Flavoured Improved Variety Cassava-Wheat Composite Flours. Trop J Nat Prod Res. 2022; 6(2):227-235. doi.org/10.26538/tjnpr/v6i2.10

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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globally acknowledged and practical sort of staple meal that is necessary for everyone. It contains vital elements such as macronutrients (carbohydrates, proteins, and fats) and micronutrients (minerals and vitamins).[20] Composite flour has been manufactured using legumes, nuts, root and stem tubers like yam, cassava, and sweet potatoes, according to reports,[21] substances that could be utilized to make functional food confectionary.

This study could provide insight into possible ways of harnessing free phenolics from an improved cassava/ wheat flour bread blend as a strategy for scavenging radicals and controlling life-threatening pathologies like diabetes, cardiovascular challenges, and non-communicable diseases that pose a threat to human well-being and existence. The goal of this study is to look at total phenol, total flavonoid, DPPH radical scavenging ability, $ABTS^{+}$ scavenging ability, ferric reducing property (FRAP), Fe^{2+} chelation ability, inhibition of lipid peroxidation, and critical enzymes linked to type-2 diabetes (α-amylase and α-glucosidase) in the free phenolic extracts of composite bread made (*in-vitro*) from cocoa powder (WF).

Materials and Methods

Materials

Cassava (vitamin-A Cassava or Yellow Cassava) was obtained from the International Institute of Tropical Agriculture (IITA)-trained farmer, Mr. Adegbola at Agbelere farm in Ogbomoso, Oyo State, Nigeria in march 2016, while wheat flour (Golden Penny), cocoa powder, yeast, and eggs were acquired from Waso, a large market in Ogbomoso, Oyo State, Nigeria. A chemical supplier in Ilorin, Kwara State, was also contacted for aspartame. The water was glass distilled, and all of the compounds were of analytical quality.

Production of vitamin-A enriched cassava- wheat composite flour breads blend

Breads were made from an improved variety cassava-wheat composite flour. Cassava from an improved variety was peeled, cleaned, cut into smaller pieces, and dried for 72 hours in a cabinet dryer at 45^0C. An improved cassava flour was made from the dried cassava (CF). The composite mixture of an improved variety cassava flour (CF) and wheat flour (WF) in formulations of 100:0 (100% Cassava flour (CF) and 0% Wheat flour (WF)), 50:50 (50% CF and 50% WF), 20:80 (20% CF and 80% WF), 10:90 (10% CF and 90% WF), and 0:100 (0% CF and 100% WF)

In the manufacturing of composite breads, Aspartame was used as a sweetener, Egg Albumin as an emulsifier, cocoa powder as a flavouring agent and a source of phenolic chemical, yeast, and water to taste. Baking took place in a gas oven at 250^oC for 30 minutes, or until the desired brown colour was achieved (Figure 1).

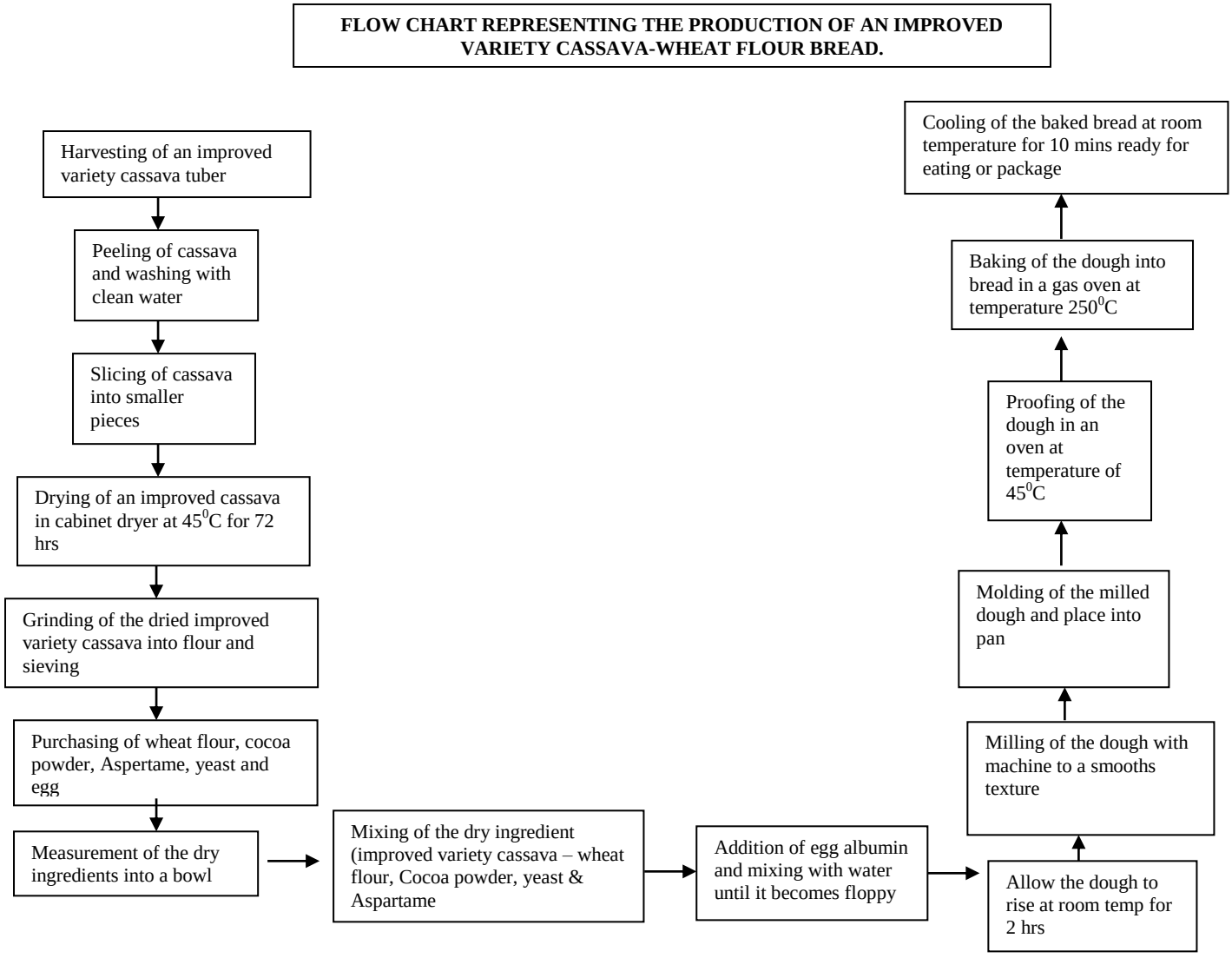

Figure 1: Flow Chart representing the production of an improved variety cassava-wheat flour composite breads

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Plate 1: An improved variety cassava- wheat flours composite breads.
KEY:
A = 100% Improved variety cassava bread + cocoa powder.
B = 50% Improved variety cassava + 50% wheat flour bread + cocoa powder.
C = 20% Improved variety cassava + 80% wheat flour bread + cocoa powder.
D = 10% Improved variety cassava + 90% wheat flour bread + cocoa powder.
E = 100% Wheat flour bread + cocoa powder.
X = 100% Improved variety cassava bread without cocoa powder.
Y = 100% Wheat flour bread without cocoa powder.

Free polyphenol content determination
In a cold blender, 50 g of the composite bread sample was homogenized with 80% acetone (1:4 w/v) for around 10 minutes to obtain free phenolic extract. Under vacuum, the homogenate was filtered (with Whatman filter paper no. 2). Using a rotary evaporator and a vacuum at 45^0C, the filtrate was evaporated to dryness. The dried free phenolic extract was frozen and used for the analysis later.

Aqueous extract preparation
1 g of an improved variety cassava-wheat flour composite bread extract was dissolved in 100 ml of distilled water and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and kept at 4^0C for the analyses.[22]

Total phenol content determination
Singleton *et al*.,[23] method was used to determine the total phenol content of the extracts from the composite bread samples. 2.5 mL of 10% Folin-reagent Cioacalteu's (v/v) was used to oxidize the extract and 2.0 mL of 7.5 percent sodium carbonate was used to neutralize it. The content was incubated at 450^0C for 40 minutes, and the absorbance was measured using a spectrophotometer at 765 nm (Jenway 6405, Jenway Limited, Essex, CM6 3LB, Dunmow, England). Following that, using the gallic acid standard curve, the total phenol concentration was determined and reported as gallic acid equivalent.
Total Phenol (mg/g) = (Abs_{sam} * $Conc._{std}$) / (Abs_{std} * $Conc._{std}$).

Determination of total flavonoid contents
Meda *et al*.,[24] method was used to determine the total flavonoid content of the extracts of the composite bread samples. 0.5 mL of the diluted sample was mixed with 0.5 mL methanol, 50 µL of 10% AlCl3, 50 µL of 1 molL-1 potassium citrate, and 1.4 mL water, and incubated at room temperature for 30 minutes. After that, a spectrophotometer (Jenway 6405, Jenway Limited. Essex, CM6 3LB, Dunmow England) was used to measure the reaction's absorbance at 415 nm. Using the quercetin standard curve, total flavonoid content was determined and reported as quercetin equivalent.
Total flavonoid (mg/g) = (Abs_{sam} * $Conc._{std}$) / (Abs_{std} * $Conc._{sam}$).

Scavenging Ability of DPPH for Free Radicals
The extracts' ability to scavenge free radicals against DPPH (1, 1-diphenyl-2 picrylhydrazyl) was determined according to Gyamfi *et al*.,[25]. In a nutshell, 1ml of 0.4 mmol L-1 methanolic solution containing DPPH radicals was combined with 1 ml of adequate dilution of free polyphenol extracts (1 ml). After 30 minutes in the dark, the absorbance of the combination was measured at 516 nm with a spectrophotometer (Jenway 6405, Jenway Limited. Essex, CM6 3LB, Dunmow, England). Following that, the ability of DPPH to scavenge free radicals was determined in comparison to the standard (which contains all the reagents without the test sample).

The ability of $ABTS^+$ to scavenge free radicals was determination
Re *et al*.,[26] method was used in evaluating the ability of the composite bread extracts to scavenge $ABTS^+$ (2,2'-azobis-3-ethylbenzo-thiazoline-6-sulfonate). $ABTS^+$ was made by reacting an aqueous solution of ABTS (7 $mmolL^{-1}$) with $K_2S_2O_8$ (2.45 $mmolL^{-1}$, final concentration) in the dark for 16 hours and then adjusting the absorbance of 734 nm to 0.700 with ethanol. 0.2 mL of the extract combined with 2.0 mL $ABTS^+$ solution. After 15 minutes, the absorbance was measured at 734 nm with a spectrophotometer (Jenway 6405, Jenway Limited. Essex, CM6 3LB, Dunmow, England) after which the equivalent was determined.

Ferric reducing Property (FRAP) determination
The ability of the extract to reduce $FeCl_3$ solution, as described by Oyaizu,[27] was used to determine the reducing property of the composite bread extracts. In a nutshell, a 2.5 mL aliquot was combined with 2.5 mL of 200 $mmolL^{-1}$ sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. After 20 minutes of incubation at 50^0C, 2.5 mL of 10% trichloroacetic acid (TCA) was added to the reaction mixture. This material was centrifuged at 650 rpm for 10 minutes. The supernatant was then mixed with 5 mL distilled water and 1 mL ferric chloride (0.1%). Using a spectrophotometer, the absorbance was determined at 700 nm (Jenway 6405, Jenway Limited. Essex, CM6 3LB, Dunmow, England). Following that, ascorbic acid and gallic acid were used as standards to calculate ferric reducing antioxidant capacity.

Fe^{2+} Chelation ability determination
The Fe^{2+} chelating ability of the composite bread extracts was determined using a variation of Minotti and Aust[28] approach, which was slightly modified by Pentuel *et al*.,[29]. In a reaction containing 168 µl of 0.1 mol L^{-1} Tris-HCl (pH 7.4), 218 µl saline, and the extracts (0-100 µl), freshly prepared 500 $\mu molL^{-1}$ $FeSO_4$ (150 µl) was combined with freshly prepared 500 µmolL-1 $FeSO_4$ (150 µl). After 5 minutes of incubation, 13 µL of 0.25 percent 1-phenanthroline (w/v) was added to the reaction mixture. Using a spectrophotometer, the absorbance was read at 510 nm (Jenway 6405, Jenway Limited. Essex, CM6 3LB, Dunmow, England). The ability to chelate Fe^{2+} was then determined.

The Thiobarbituric Acid Reaction and Lipid Peroxidation
The lipid peroxidation assay of the composite bread extracts was performed using Ohkawa *et al*.,[30] method, which was modified by Pentuel.[29] In a nutshell, 100 µl of the extract was mixed with 30 µl of 0.1 M pH 7.4 Tris-HCl buffer, bread extracts (0-100 µl), and 30 µl of the pro-oxidant (250 µM freshly manufactured $FeSO_4$), and the volume was built up to 300 µl by distilled water before incubation at 370C for 1 hour. The reaction color was developed by adding 300 µl 8.1% SDS (sodium dodecyl sulphate) to the mixture, this was subsequently followed by the addition of 600 µl of acetic acid/HCl (pH 3.4) and 600 µl 0.8% TBA (Thiobarbituric acid). For 1 hour, the mixture was incubated at 100^0C. The absorbance of TBARS (Thiobarbituric acid reactive species) was measured at 532 nm using a spectrophotometer (Jenway 6405, Jenway Limited. Essex, CM6 3LB,

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Dunmow, England) and compared to that of a standard curve using MDA (malondialdehyde). The amount of malondialdehyde (MDA) generated was measured in percentages.

α-Amylase Inhibition Assay
The method of Worthington Biochemical Corp.[31] was used to test the ability of the aqueous bread extracts of the composite bread to inhibit α- amylase. Briefly, 100 µl of suitable dilution of the aqueous extract and 100 µl of 0.2 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing Hog pancreatic-amylase (0.5 mg/ml) were incubated at 25^0C for 10 minutes. Each tube was then filled with 50 µl of a 1% starch solution in 0.0 2M sodium phosphate buffer (pH 6.9, 0.006 M NaCl). The action was halted with 200 µl Dinitrosalicyclic acid colour reagent after a 10-minute incubation period at 25^0C. The reaction mixture was then cooled to room temperature after being incubated for 5 minutes in a boiling water bath. After diluting the reaction mixture with 2 mL distilled water, the absorbance was measured at 540 nm.

α-Glucosidase Inhibition Assay
The effectiveness of aqueous bread extracts to inhibit α-glucosidase was determined using Apostolidis *et al*.,[32] technique. Briefly, an appropriate dilution of the aqueous extract (50 µl) was mixed with 15 µl of α-glucosidase solution from the albino rat's intestine in 0.1 M sodium phosphate buffer (pH 6.9) and 15 µl of 3 mM reduced glutathione (GSH) in the sodium phosphate buffer solution was incubated at 37^0C for 10 minutes. Following that, 40 ml of 5 mM p-nitrophenyl- α—D-glucopyranoside solution (PNP-Glu) in 0.1 M phosphate buffer (pH 6.9) was added. After a 10-minute incubation period at 37^0C, 2 mL Na_2CO_3 was added to the solutions. Using the spectrophotometer (Jenway 6405, Jenway Limited. Essex, CM6 3LB, Dunmow, England), the absorbance at 405 nm was determined. The % inhibition of α-glucosidase was calculated.

Statistical analysis
The findings of the experiment were expressed as the mean of three replicates ± standard deviation. One-way Analysis of Variance was used to statistically analyze the data acquired (ANOVA) and $p < 0.05$ was chosen as the level of significance. Duncan multiple range test was used to differentiate means.

Results and Discussion

Total flavonoid and phenol total
Phenolics have long been thought to play a significant role in food sensory qualities like taste, color, and flavor.[22] The growing desire for diets high in phenolic components has prompted new approaches to supplementing diets with phenolic antioxidants for improved and prospective health benefits. Cocoa powder is used as a flavouring agent as well as a phenolic source in this research. Due to their redox characteristics, phenolics are thought to be powerful antioxidants.[33] By preventing the creation of or deactivating the active species and precursors of free radicals, they suppress the evolution of free radicals, lowering the rate of oxidation.[34] Due to their redox characteristics, they are considered potent antioxidants.[33] Consumption of such foods is required for the prevention of a variety of degenerative disorders as well as the maintenance of good health. [35,22,36] Table 1 shows the total phenol of the composite bread extracts expressed in gallic acid equivalents. According to the table, the extract of 100% CF bread + FL had the highest total phenolic content (5.40 ± 0.09 mg/GAE/g), whereas commercial bread had the lowest (3.61 ± 0.19 mg/GAE/g). It is worth noting that when the percentage of wheat flour replaced with an improved variety of cassava flour increased, the total phenol content of the bread extracts increased. In addition, Table 1 shows the flavonoid content of the composite bread extract given as quercetin equivalent. The extract of 100% CF bread + FL (1.98 ± 0.06 mg/QE/g) had the highest flavonoid concentration, while commercial bread (0.95 ± 0.01 mg/QE/g) had the lowest flavonoid content. Flavonoids contain antioxidant properties, which could help to lower cellular oxidative stress, which has been linked to the development of neurodegenerative disorders such as amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease.[37] Vegetables' high flavonoid concentration could have aided their therapeutic effects. They are phytochemicals that have a variety of biological functions and are extensively dispersed. The amount of OH groups on the molecule and their agreement have been connected to flavonoids' antioxidant activity. Components of plant foods that have been linked to a reduction in the risk of cancer.[38] Some flavonoids have potent anti-inflammatory properties, such as preventing platelet stickiness and lowering the risk of edema.[39] Adedayo *et al*.,[40] found that the total phenol and flavonoid concentrations of various tropical Nigerian yam varieties (*Dioscorea spp*) were similar to those found in our study.

Scavenging Ability of DPPH for Free Radicals
Figure 2 depicts the DPPH free radical scavenging capability of the bread extracts. The DPPH method is commonly used to determine the free radical scavenging capacity of various food components as well as sample solubility.[41] As a result, the extracts' ability to scavenge free radicals was investigated further using ABTS radicals, a fairly stable nitrogen-centered radical species.[42] According to the findings, all extracts of the composite bread made from an improved variety of cassava/wheat flour scavenged DPPH radicals in a dose-dependent manner, with 100% CF bread + FL (IC50 = 2.51 ± 0.03 mg/ml) having the highest DPPH radical scavenging ability, followed by 100% WF bread (IC50 = 5.57 ± 0.13 mg/ml), and commercial bread (IC50 = 6.08 ± 0.11 mg/ml). The DPPH free radical scavenging ability of extracts from an improved variety cassava/wheat flour composite bread followed similar patterns as total phenol and flavonoid content.

$ABTS^+$ free radical scavenging ability determination
Because of various limitations of DPPH related to color interference and sample solution, the $ABTS^+$ (2,2-azinobis (3-ethylbenzo-thizoline-6-sulfonate radical) model was created to test for free radical scavenging capabilities.[26] By monitoring the reduction of the radical cation at 734 nm, the decolorization of ABTS by antioxidant is measured. Figure 3 depicts the $ABTS^+$ scavenging ability, which is expressed as mol TEAC. When compared to the 100% WF bread control (9.05 ± 0.00 µmol/TEAC/g), the extract of 100% CF bread + FL (14.55 ± 0.00 µmol/TEAC/g) had the highest value of ABTS free radical scavenging ability, while commercial bread (5.40 ± 0.10 µmol/TEAC/g) had the lowest.

Table 1: Determination of the Total phenol and Total flavonoid contents of free polyphenol extract of the bread samples

Sample	Total phenol (mg/GAE/g)	Total flavonoid (mg/QE/g)
COMMERCIAL BREAD	3.61^a ± 0.19	0.95^a ± 0.01
100% WF BREAD	3.65^a ± 0.09	1.23^b ± 0.06
100% CF BREAD	4.82^e ± 0.18	1.72^e ± 0.03
100% CF BREAD + FL	5.40^f ± 0.09	1.98^f ± 0.06
50% CF + 50% WF BREAD + FL	4.65^d ± 0.09	1.56^d ± 0.06
20% CF+ 80% WF BREAD + FL	3.90^c ± 0.12	1.40^c ± 0.03
10% CF+ 90% WF BREAD + FL	3.78^b ± 0.13	1.28^{bc} ± 0.01
100% WF BREAD + FL	3.70^{ab} ± 0.06	1.29^{bc} ± 0.06

Values are Mean plus or minus standard deviation (SD) with carrying the same superscripts in the same column are not significantly different ($p>0.05$). KEY: Commercial bread; 100% WF (100% Wheat flour bread without cocoa powder); 100% CF (100% improved variety cassava bread without cocoa powder); 100% CF bread + FL (100% improved variety cassava bread + cocoa powder); 50% CF + 50% WF bread + FL (50% improved variety cassava + 50% wheat flour bread + cocoa powder); 20% CF + 80% WF bread + FL (20% improved variety cassava + 80% wheat flour bread + cocoa powder); 10% CF + 90% WF bread + FL (10% improved variety cassava + 90% wheat flour bread + cocoa powder); 100% WF bread + FL (100% wheat flour bread + cocoa powder).

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

As a result of an increase in substitution of an improved variety cassava flour in the composite bread, the trend in the results of ABTS radical scavenging capacity of the extracts accords with that of DPPH free radical scavenging ability and phenolic content of the extracts of the composite bread.

Ferric reducing Property (FRAP) determination

Another antioxidant process that works by transferring electrons and hydrogen atoms is ferric reducing antioxidant power (FRAP).[43] The ability of an improved variety cassava/wheat flours composite bread extract to decrease Fe^{3+} to Fe^{2+} at OD700 was determined using FRAP, and the findings are shown in Figure 4 expressed as ascorbic acid equivalents. A higher reducing power is shown by a higher absorbance at 700 nm.[44] The results showed that all composite bread extracts have higher reducing properties (FRAP), with 100% CF bread + FL (7.45×10^{-2} mgAAE/g) having the lowest value of reducing property and commercial bread (3.30×10^{-2} mgAAE/g) having the highest. The elevated phenolic content of an improved variety of cassava employed in the bread formulation may have contributed to the increase in reducing power. Antioxidants' capacity to deactivate and chelate transition metals prevents metal-catalyzed reactions from causing oxidative stress and lipid peroxidation.[43] As a result, the extracts' capacity to chelate transition metals is thought to be owing to an antioxidant mechanism.[45,43]

Fe^{2+} chelating ability determination

Figure 5 shows the extract's Fe^{2+} chelating activity. The hydroxyl radical (OH*) is produced by the Fenton reaction when Fe^{2+} reacts with hydrogen peroxide (H_2O_2), whereas superoxide reacts with Fe^{3+} to form Fe^{2+} that can participate in the Fenton process.[39,46] Overproduction of reactive oxygen species (ROS) can destroy the polyunsaturated fatty acids (PUFA) in cell membranes, causing lipid peroxidation. All composite bread extracts chelated Fe^{2+} in a dose-dependent manner, according to the findings. However, 100% CF bread + FL had the most Fe^{2+} chelating ability (IC50 =1.00 ± 0.10 mg/ml), but 100% WF bread had the least Fe^{2+} chelating ability (IC50 = 2.67 ± 0.05 mg/ml).

Thiobarbituric Acid Reaction and Peroxidation of Lipids

Figure 6 shows the effects of inhibiting Fe^{2+}-induced lipid peroxidation in the extract of an improved cassava/wheat flour composite bread. Lipid peroxidation is an oxidative process in which free radicals steal electrons from lipids, resulting in cell membrane depletion. Reactive aldehydes like malondialdehyde (MDA) and 4-hydroxynonenal (HNE), which are important indicators, are the end products of lipid peroxidation.[47] Reactive oxygen species destroy polyunsaturated fatty acids, resulting in the formation of malondialdehyde. This substance is a reactive aldehyde and one of a number of reactive electrophile species that produce toxic stress in cells and create advanced glycation end products. This aldehyde's synthesis is utilized as a biomarker to determine an organism's level of oxidative stress.[48,49] Free radical-induced lipid peroxidation has been linked to cellular problems such as mutation and cell death via altering membrane structure and function.[50] Figure 6 shows the *in-vitro* capacity of an improved variety cassava/wheat flour composite bread extracts to reduce Fe^{2+}-induced lipid peroxidation. With Fe^{2+} chelating ability, ferric reducing property, DPPH and ABTS free radical scavenging abilities, the inhibition of Fe^{2+} induced lipid peroxidation *in vitro* in a dose response manner follows a similar trend (100% CF bread + FL > 100% CF bread > 50% CF + 50% WF bread +FL > 20% CF + 80% WF bread +FL > 10% CF + 90% WF bread + FL > 100% WF bread + FL > 100% WF bread > commercial bread). The findings demonstrated that using an improved variety cassava flour in composite bread resulted in a lower MDA level. This is consistent with Oboh and Adefegha[22] findings that biscuits made from unripe plantain flour inhibited MDA levels in the rats' brain, as well as Ajani *et al*.,[51] findings that edible tropical cereals inhibited MDA levels in the rats' brain.

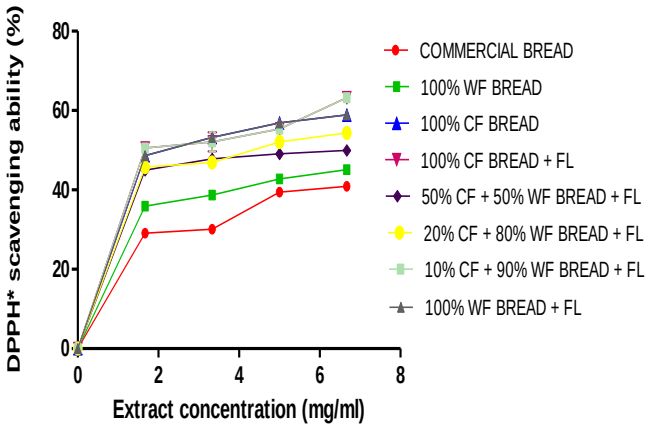

Figure 2: DPPH free radical scavenging ability of free polyphenol extracts of the bread samples.
Values represent mean ± deviation of the replicate reading.
KEY: Commercial bread; 100% WF (100% Wheat flour bread without cocoa powder); 100% CF (100% improved variety cassava bread without cocoa powder); 100% CF bread + FL (100% improved variety cassava bread + cocoa powder); 50% CF + 50% WF bread + FL (50% improved variety cassava + 50% wheat flour bread + cocoa powder); 20% CF + 80% WF bread + FL (20% improved variety cassava + 80% wheat flour bread + cocoa powder); 10% CF + 90% WF bread + FL (10% improved variety cassava + 90% wheat flour bread + cocoa powder); 100% WF bread + FL (100% wheat flour bread + cocoa powder).

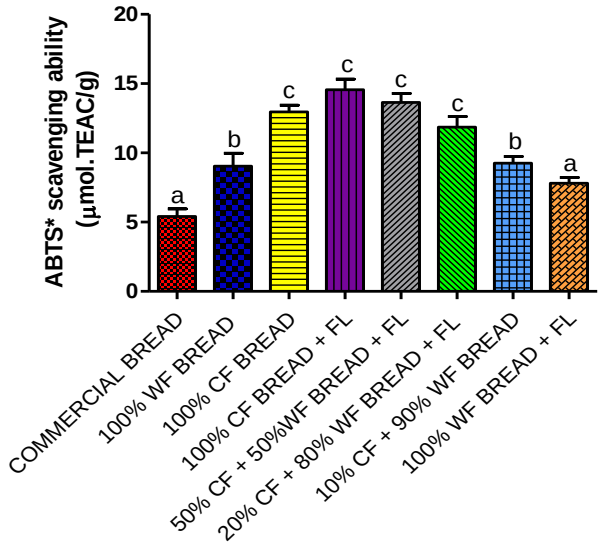

Figure 3: ABTS^{+} scavenging ability of free polyphenol extracts of the bread samples.
Values represent mean ± deviation of the replicate reading.
KEY: Commercial bread; 100% WF (100% Wheat flour bread without cocoa powder); 100% CF (100% improved variety cassava bread without cocoa powder); 100% CF bread + FL (100% improved variety cassava bread + cocoa powder); 50% CF + 50% WF bread + FL (50% improved variety cassava + 50% wheat flour bread + cocoa powder); 20% CF + 80% WF bread + FL (20% improved variety cassava + 80% wheat flour bread + cocoa powder); 10% CF + 90% WF bread + FL (10% improved variety cassava + 90% wheat flour bread + cocoa powder); 100% WF bread + FL (100% wheat flour bread + cocoa powder).

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

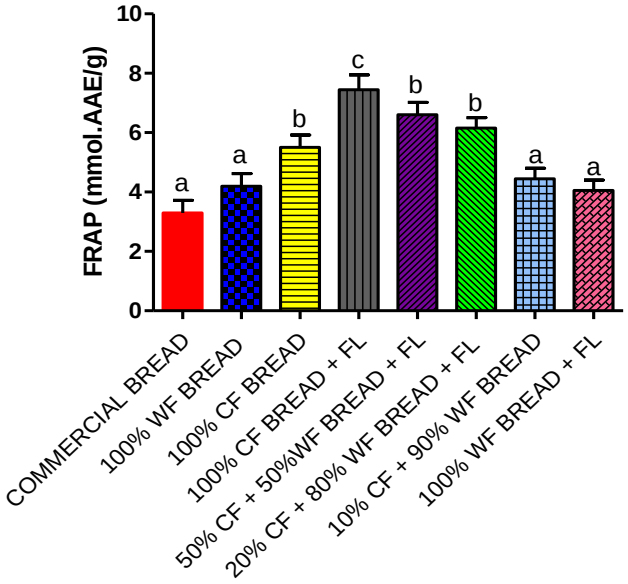

Figure 4: Ferric reducing property of free polyphenol extracts of the bread samples.
Values represent mean ± deviation of the replicate reading.
KEY: Commercial bread; 100% WF (100% Wheat flour bread without cocoa powder); 100% CF (100% improved variety cassava bread without cocoa powder); 100% CF bread + FL (100% improved variety cassava bread + cocoa powder); 50% CF + 50% WF bread + FL (50% improved variety cassava + 50% wheat flour bread + cocoa powder); 20% CF + 80% WF bread + FL (20% improved variety cassava + 80% wheat flour bread + cocoa powder); 10% CF + 90% WF bread + FL (10% improved variety cassava + 90% wheat flour bread + cocoa powder); 100% WF bread + FL (100% wheat flour bread + cocoa powder).

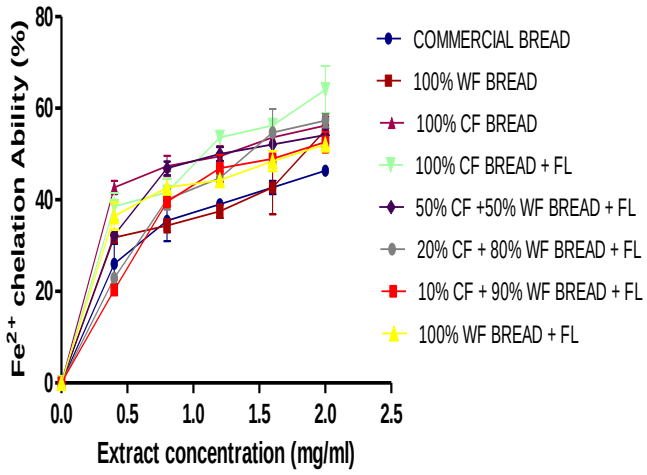

Figure 5: Fe^{2+}chelating ability of free polyphenol extracts of the bread samples.
Values represent mean ± deviation of the replicate reading
KEY: Commercial bread; 100% WF (100% Wheat flour bread without cocoa powder); 100% CF (100% improved variety cassava bread without cocoa powder); 100% CF bread + FL (100% improved variety cassava bread + cocoa powder); 50% CF + 50% WF bread + FL (50% improved variety cassava + 50% wheat flour bread + cocoa powder); 20% CF + 80% WF bread + FL (20% improved variety cassava + 80% wheat flour bread + cocoa powder); 10% CF + 90% WF bread + FL (10% improved variety cassava + 90% wheat flour bread + cocoa powder); 100% WF bread + FL (100% wheat flour bread + cocoa powder).

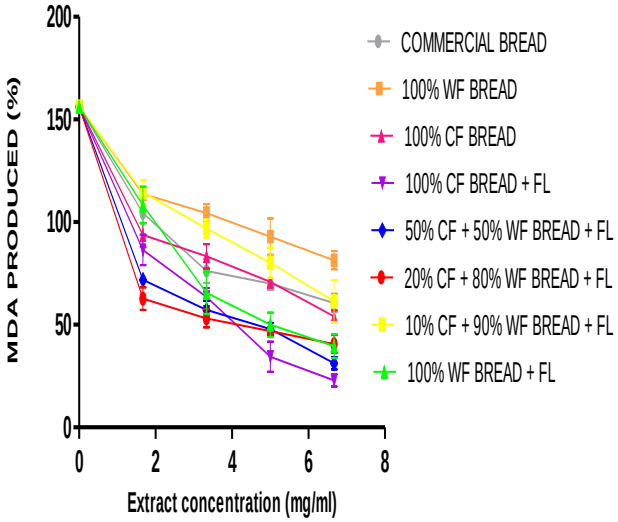

Figure 6: Fe^{2+} induced Lipid peroxidation of the free polyphenol extract of the bread samples.
Values represent mean ± deviation of the replicate reading.
KEY: Commercial bread; 100% WF (100% Wheat flour bread without cocoa powder); 100% CF (100% improved variety cassava bread without cocoa powder); 100% CF bread + FL (100% improved variety cassava bread + cocoa powder); 50% CF + 50% WF bread + FL (50% improved variety cassava + 50% wheat flour bread + cocoa powder); 20% CF + 80% WF bread + FL (20% improved variety cassava + 80% wheat flour bread + cocoa powder); 10% CF + 90% WF bread + FL (10% improved variety cassava + 90% wheat flour bread + cocoa powder); 100% WF bread + FL (100% wheat flour bread + cocoa powder).

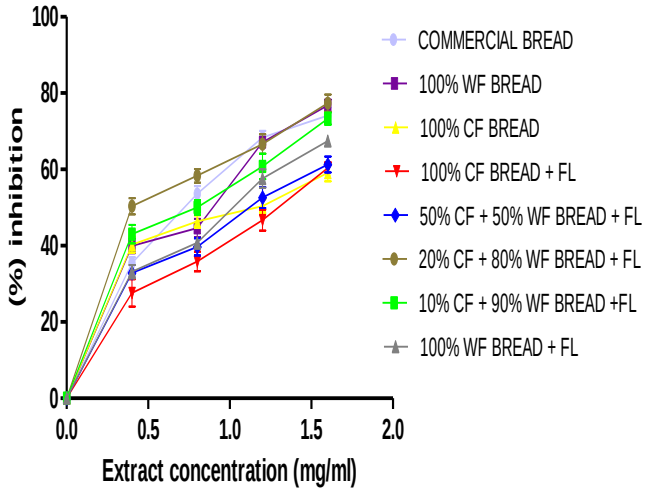

Figure 7: α-amylase inhibition activity of the free polyphenol extract of the bread samples.
Values represent mean ± deviation of the replicate reading.
KEY: Commercial bread; 100% WF (100% Wheat flour bread without cocoa powder); 100% CF (100% improved variety cassava bread without cocoa powder); 100% CF bread + FL (100% improved variety cassava bread + cocoa powder); 50% CF + 50% WF bread + FL (50% improved variety cassava + 50% wheat flour bread + cocoa powder); 20% CF + 80% WF bread + FL (20% improved variety cassava + 80% wheat flour bread + cocoa powder); 10% CF + 90% WF bread + FL (10% improved variety cassava + 90% wheat flour bread + cocoa powder); 100% WF bread + FL (100% wheat flour bread + cocoa powder).

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Inhibition assays for α- amylase and α- glucosidase.

Figures 7 and 8 show the dose-dependent inhibition of α-amylase and α- glucosidase activities by extracts from composite bread made from an improved variety cassava/wheat flour. Inhibition of enzymes involved in starch hydrolysis (α- amylase) and glucose absorption (α-glucosidase) is used to treat type-2 diabetes.[45,32] Many foods and plant extracts have been shown to have hypoglycemic properties and may provide health advantages without the negative side effects.[52] The extracts of 100% CF + FL bread (IC50 = 0.43 ± 0.09 mg/ml) showed the highest inhibition of α- amylase activity, followed by 100% WF bread (IC_{50} = 0.68 ± 0.07 mg/ml), while extracts of commercial bread had the least inhibition (IC_{50} =1.23 ± 0.36 mg/ml). Consequently, the extracts from an improved variety of cassava/wheat flour composite bread inhibited α- glucosidase activity in a dose-dependent way (Figure 8). Interestingly, composite bread containing 10% CF and 90% WF inhibited α- glucosidase activity the most, with an IC50 value of 0.21 ± 0.00 mg/ml, while 100% WF control bread had the least inhibition (IC50 = 0.74 ± 0.07 mg/ml). The findings of Shodehinde and Oboh,[53] on the inhibitory effect of aqueous extracts of Amala made from unripe plantain flour on salivary α- amylase enzyme activity was consistent with the results of α- amylase and α-glucosidase inhibitions of the extract of an improved variety cassava/wheat flour composite bread.

Different flours such as cereals (maize, rice, sorghum, millet) and tubers rich in starch (cassava, cocoyam, sweet potato, yam) and protein-rich flours (cowpea, soybean) have been used in bread making to partially substitute wheat flour in bread.[54,55,56,57,58,59,60,61] Shittu et al.,[62] assessed the effect of baking time and temperature on some physical properties of bread produced from composite cassava –wheat flour. The authors were able to optimize the baking process based on some storage and consumption qualities of the composite cassava – wheat flour. Previous study from our research group revealed the sensory attributes, nutritional values and glycemic indices of bread blend produced from cocoa powdered flavoured yellow fleshed cassava/wheat composite flour *in-vitro* (Ajani et al.,[63]). The result obtained show that the bread was generally accepted by respondents, had good nutritional properties, high potassium level, low glycemic indices, and inhibited key enzymes (α-amylase and α-glucosidase) linked to type-2-diabetes (Ajani et al.,[63]).

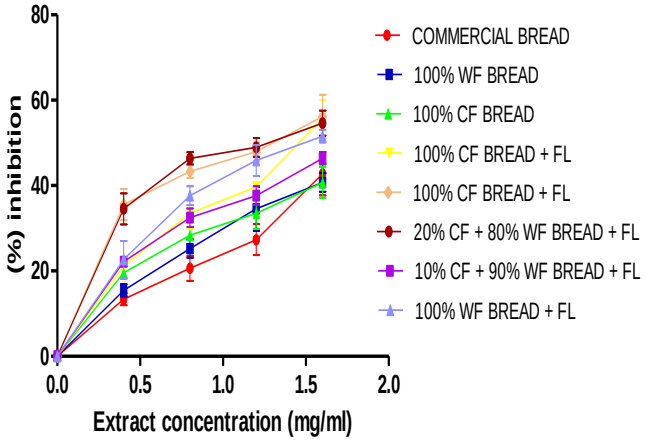

Figure 8: α-glucosidase inhibition activity of the free polyphenol extract of the bread samples.

Values represent mean ± deviation of the replicate reading.

KEY: Commercial bread; 100% WF (100% Wheat flour bread without cocoa powder); 100% CF (100% improved variety cassava bread without cocoa powder); 100% CF bread + FL (100% improved variety cassava bread + cocoa powder); 50% CF + 50% WF bread + FL (50% improved variety cassava + 50% wheat flour bread + cocoa powder); 20% CF + 80% WF bread + FL (20% improved variety cassava + 80% wheat flour bread + cocoa powder); 10% CF + 90% WF bread + FL (10% improved variety cassava + 90% wheat flour bread + cocoa powder); 100% WF bread + FL (100% wheat flour bread + cocoa powder).

Conclusion

This present study revealed that the extracts of an improved variety cassava/wheat flour composite bread have strong antioxidant potential; however, 100% CF bread + FL proved to have contained the highest phenolic constituent with greatest antioxidant abilities and highest inhibition of enzymes linked with Type-2-diabetes (α-amylase and α-glucosidase) when compared with other composite bread blends produced. Hence, substitution of wheat flour with an improved variety cassava flour in bread production may serve to have addressed the difficulties of patient with nutritional complication, and management/ prevention of type-2-diabetes mellitus and as well as showing promising functional food recipe. Therefore, its consumption will enhance a high beneficial effect by promoting good health.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors wish to express their gratitude to members of Functional Food and Nutraceuticals Laboratory Unit of the Biochemistry department, Federal University of Technology, Akure for the opportunity given to carry out the research work in their Laboratory.

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