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Research Article

Effect of Cocoa Bean (*Theobroma cacao*) Powder on the Physicochemical, Antioxidant and Acceptability Properties of Bread Produced from Blends of Selected Cereals Flour

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Abstract

Wheat bread contains most of the important and essential nutrients but deficient in antioxidant rich polyphenol compounds. Cocoa was used as functional ingredient to be incorporated in bread as it contains substantial amount of phenolic compound. This study was designed to establish the potential of cocoa flour addition on wheat-cereal bread in terms of its nutritional compositions, physical, antioxidant and sensory characteristics. The fat and protein contents of the bread samples in this study ranged between 9.45-10.83 % and 11.25-13.87 % while the fiber and ash were 2.66-3.86 % and 1.13-2.89 % respectively. The loaf weight and volume ranged between 112.98-312.55 g and 400.00-470.00 cm^3. The result also showed a significant increase in the DPPH free radical scavenging activities of the samples with increase in the cocoa flour with the value ranged between 55.00-75.00 %. The total phenolic and flavonoids ranged between 4.59-6.98 Mg/g GAE and 6.77-9.91 Mg/g QE. The developed bread samples were rated higher than the control sample as regards the organoleptic properties. The study revealed that $W_{60}M_{20}C_{20}$ (60 % wheat, 20 % maize, 20 % cocoa flour) proved to have higher nutritional composition, free radical scavenging activities potential and total phenolic compounds. Therefore, $W_{60}M_{20}C_{20}$ bread could serve as functional bread and can be used in nutritional intervention for management and prevention of hyperglycemia.

INTRODUCTION

Bread is the most common products among all baked products of wheat. It's extensively consumed and enjoyed by both children and grown-ups from different socio-economic status in Nigeria, therefore leading to its high daily demand and consumption [1,2]. It's having been found to contain a rich source of energy, protein, vitamins especially the B vitamins, minerals and dietary fibre. Recent developments have emphasized on healthy eating by enhancing the application of indigenous crops such as cereals and legumes in baking industries [3]. Bread can act as excellent energy source and through fortification; it can be used as carrier for folate, copper, thiamine, zinc, iron phytic acid, minerals and melanoidins [4]. Bread can also be a plausibly good carrier for delivery of phenolic antioxidants and fibre polysaccharides at reasonable concentrations [5]. The mindfulness of the consumers on the benefit of eating healthy and functional foods is globally increasing and health-conscious consumers prefer food that provides health benefits beyond the basic nutrition [6,7]. Thus, there's a trend to producing functional confectionary products made from compound flours and health promoting diets from non-wheat flour known as functional constituents [8]. The utilization of refined wheat flour alone and other ingredients result in confectionaries deficient in essential components of grains like dietary fibre and phytochemical that has some health benefits [9,10]. Wheat and other cereal flours on their own are good source of calorie and other nutrients but their antioxidant capacity is low as a result refining processing, hence the need to composite it with cocoa powder in other to ameliorate its antioxidant capacity.

Acha (*Digitaria exilis*) is an underutilized crop lately gaining popularity. Like other grains, acha has been reported to be rich in amino acids, particularly in methionine and cystine [11]. Studies have also shown that the methionine level in acha is doubly the set up in egg protein leading to the fact that acha grains could be used to supplement many diets that are low in the essential amino acids. Okeme et al. [12], have reported acha as an excellent nutritional complement to legumes as most legumes are deficient in methionine and cystine. It is also a good source of vitamins, minerals, fibre, carbohydrate, lipids, proteins and ash [13].

Maize (*Zea mays* L.) is one of the most planted cereal grain in the world and represents a pivotal source of human food, livestock feed, fuel and fibre [14]. It's the third most important cereal grain worldwide after wheat and rice [15]. Maize is a staple food grain for large corridor of the world including Africa, America and Asia and is consumed in numerous forms such as infant foods, snacks and main dishes [16]. Maize grain contains 65-84 % starch, 9-10 % protein, 3-5 % fat, 3 % ash and 2-3 % fibre [17]. Maize provides numerous of the B vitamins and essential minerals but lacks some other nutrients such as vitamin B_{12} and vitamin C but poor source of calcium, folate and iron [18].

Cocoa powder is a plant product that has not enjoyed wide consumer acceptance in Nigeria as a result of its bitter aftertaste and astringency. It is lately entered global recognition as a functional food product/ingredient in the food and confectionary industry for numerous applications [19]. Cocoa powder has been reported to confer health-promoting benefits including promotion of cardiovascular health, reduction of low-density lipoprotein (LDL) cholesterol and oxidation of LDL to prevent atherosclerosis or plaque formation. It also elevates high density lipoprotein (HDL) cholesterol owing to its rich content of natural antioxidants and it have been reported to exhibit greater antioxidant capacity than many other flavanol-rich foods and food extracts including red wine, blueberry, garlic, strawberry and green and black tea [20-22]. Nutritionally cocoa powder is a rich source of protein, vitamin A, riboflavin and nicotinic acid, minerals including iron, calcium, copper, magnesium, phosphorus, potassium, sodium and zinc which may serve the dual purpose of enriching food with vital nutrients and health-promoting bioactive compounds [23]. This study therefore, estimated the capabilities of the composite of these flours (wheat, maize acha, and cocoa) in bread production

MATERIALS AND METHODS

Source of Raw Materials

Maize and acha grains used for this research were obtained from a local market in Saki, Nigeria while other bakery ingredients were obtained from a supermarket also located in Saki. The cocoa powder was gotten from COOP Cocoa Limited, Akure-Owo Expressway Akure, Nigeria. All chemical used are analytical grade purchased from Sigma (Sigma Chemicals, St. Louis, MO).

Methods

Processing of Maize and Acha Flours: Cleaning and sorting of the grains was done to remove any unwanted materials. The grains were washed with clean water for about four (4) times and later dried in a cabinet dryer (07AFHPN3371D1ZL, New Delhi, India) at 50°C for 5 h. The samples were then allowed to cool and mill with a hammer mill (Jurgens, Bremmer, Germany) and package in air-tight containers at room temperature until further use.

Bread Dough Formation: The bread dough was produced using Straight dough method. This method involves the addition of all ingredients at the mixing stage, then kneading to form the dough as described by Cauvain [24]. Wheat/Composite flour (100 g), sugar (12.5 g), salt (2.5 g), milk (12.5 g), yeast (0.5 Mc Farland standard), fat (20 g) and water (50 ml).

Production of Bread Using wheat Composite Flour Blends: The modified method of [25], was used for the production of the bread samples. The bread samples were produced in batches by manually mixing and kneading each of the flour blends with other ingredients in a stainless-steel bowl. After thorough kneading, the dough was allowed to ferment and develop for 1h at 35℃ before being knocked back and then molded into cylindrical shape. The molded dough was placed in a well-oiled baking pan and proofed for 30 min at room temperature before baking in an oven which was already pre-heated and set at 210℃. The dough was brought out from the oven and immediately de-panned by knocking out. The knocked-out bread was allowed to cool, weigh and measure before being packed in polyethylene bag and kept at room temperature.

Composite Flour Formulation for Bread Production: Wheat and composite flours preparation were blended in different proportion. Wheat flour was used as control sample as shown in Table 1.

Chemical Analysis

Determination of the Proximate Composition of Composite Flour and Bread: Proximate compositions of the bread samples were determined using (AOAC 2020). Carbohydrate content was determined by difference.

Carbohydrate (%) = 100 - (% Moisture + % Fat + % Ash + % Crude fibre + % Crude protein)

Determination of Functional Properties of Composite Flours

Table 1: Composite flour formulation (%)

Samples code	Wheat	Maize	Acha	Cocoa
$W_{80}M_{10}C_{10}$	80	10	✓	10
$W_{70}M_{15}C_{15}$	70	15	✓	15
$W_{60}M_{20}C_{20}$	60	20	✓	20
$W_{80}A_{10}C_{10}$	80	✓	10	10
$W_{70}A_{15}C_{15}$	70	✓	15	15
$W_{60}A_{20}C_{20}$	60	✓	20	20
WHT	100	✓	✓	✓

WMC: Wheat-maize-cocoa composite flour
WAC: Wheat-acha-cocoa composite flour
WHT: Wheat flour

Determination of Water and Oil Absorption Capacity: Water absorption capacity of flours was analysed suing the modified method of Anyasi et al., Briefly, about 1 g of the flour was weighed into 15 ml centrifuged tubes. 10 ml of distilled water was added to each sample and thoroughly mixed for 2 min and allowed to stand at room temperature for 30 min and centrifuged for 20 min at 3000 centrifugal forces. The oil absorption capacity of the flours was also examined by the method of Anyasi et al., Briefly, about1 g of the flour was carefully mixed with 10 ml of cooking oil in 15 ml centrifuge tubes. The samples were allowed to stand for 30 min and centrifuged for 20 min at centrifugal force. Oil absorption was determined as percent oil bound per gram flour.

Determination of Bulk Density: The modified method of Mariotti et al., was used to determine the bulk density of flours. About 500 ml cylinder was filled with flour samples until the contents were tightly packed. A ratio between the sample weight and the volume of the cylinder was used to analyse the bulk density (g/ml) of flours.

Determination of Solubility and Swelling Capacity: The solubility and swelling capacity (SC) of flour samples were determined using the method described by Chatakonda et al., with some slight modifications. About 1 g of flour sample was weighed into a 50 ml centrifuge tube containing 40 ml deionized water and vortexed for 30 min. The tubes were heated in a thermostatically regulated water bath at 85°C for 20 min and cooled to room temperature. The tube and its contents were centrifuged at 2200 rpm for 15 min and the clear supernatant carefully decanted into a pre weighed petri dish. The weight of the residue/sediment was noted. The water in the supernatant was evaporated and the difference in weight of petri dish was recorded as the weight of soluble fraction. Solubility and swelling power (SP) were calculated using these equations:

Solubility (%) = (*weight of soluble fraction*)/ (*weight of sample*) × 100

Swelling Capacity (%) = (*weight of sediment*)/ (*weight of sample* × (100-*solubility*) × 100

Determination of Foaming Capacity: The method of Jitngarmkusol et al., was used for the determination of the foaming capacity of the samples with some slight modifications. About 2 g of each flour sample was mixed with 100 ml of distilled water and the suspension was whipped with a kitchen blender. The whipped suspension was moved into a 250 ml graduated cylinder. Volumes of the whole mixture were recorded before and after whipping. The foaming capacity was calculated using the equation:

Foaming capacity (%) = $(V_1-V_2)/V_3) \times 100$

Where: V_1 is the volume of initial mixture and V_2 is the volume of the mixture after whipping and V_3 is the volume of the foam after 5 h.

Determination of the Physical Properties of the Bread: The loaf volume was measured by rapeseed displacement immediately after baking; loaf weight was determined by weighing the samples on electronic balance scale. The Specific volume was measured by dividing the volume by the weight as described by Maneju et al. [26].

Specific volume = Volume/weight (cm3/g).

Preparation of Bread Extract

Preparation of Powdered Bread: Bread was prepared for analysis according to Michalska *et al*., with slight modification. The bread was sliced to one cm thick and dried in oven at 40 ℃ for 24 h and finally grinded to obtain dried and powdered bread.

Preparation of Bread Crude Extract: About 1 g of the composite flour enriched with cocoa powder was dissolved in 100 ml of distilled water and centrifuged at 3000 rpm for 10 min. The supernatant was collected and kept at 40 ℃ for the analyses [27]. The extract was used for the determination total phenolic content (TPC) and DPPH scavenging activity.

Determination of the Antioxidant Activity of the Bread: The method of De Ancos *et al.*, [28], was used to assess the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of bread. Briefly about 10 µl aliquot of the acidified methanolic extract was mixed with 90 µl distilled water and 3.9 ml of methanolic 0.1 M DPPH solution. The mixture was thoroughly mixed by vortex equipment. The mixture was stored in the dark for 30 min and the absorbance was read at 515 nm. The result was expressed as percentage inhibition of the DPPH radical.

Determination of Total Phenol Content: The total phenol content of the extracts was determined using the method of Singleton et al., [29]. Briefly, an appropriate dilution of extract was oxidized with 2.5 ml of 10 % Folinciocalteau's reagent and neutralized by 2.0 ml of 7.5 % sodium carbonate. The reaction mixture was incubated at 45°C for 40 mins, and its absorbance was read at 765 nm with the UV-Visible spectrophotometer. The total phenol content was calculated using Gallic acid as standard.

Total Phenol (mg/g) = $(Abs_{sam} * Conc._{std}) / (Abs_{std} * Conc._{std})$.

Determination of Total Flavonoid: The total flavonoid content of the extracts was determined using a slightly modified method of Meda et al., [30]. Briefly, an aliquot of 0.5 ml of the extract was mixed with 0.5 ml methanol, 50 µl of 10% $AlCl_3$, 50 µl of 1 mol/l potassium acetate and 1.4 ml of distilled water, and allowed to incubate at room temperature for 30 min. The absorbance of the mixture was measured at 415 nm with UV-Visible spectrophotometer. The total flavonoid content was calculated using quercetin as standard.

Sensory Evaluation

The bread samples were subjected to organoleptic analysis within 24 h of baking. The samples were evaluated by using a 60 untrained panelist using a 9-point hedonic scale of where 9 (like extremely) to 1 (dislike extremely) for appearance, texture, taste, flavor and overall acceptability [31].

Statistical Analysis

The data were analyzed using SPSS version 23.00. The analysis of variance (ANOVA) was used to determine significant differences between the means (P<005) while the means was separated using the Duncan multiple range test (DMRT).

RESULTS AND DISCUSSION

Proximate Composition of the Bread Made from the Composite Flour

The proximate composition of bread made from wheat-maize and wheat-acha composite flour enriched with cocoa powder in Table 2 shows that the moisture value of the bread samples ranged from 7.50 to 9.89 % for wheat (WHT) and wheat-maize composite bread ($W_{80}M_{10}C_{10}$). The moisture value of the wheat-acha composite bread were found to be significantly (p> 0.05) higher that the control sample (WHT) with ($W_{80}A_{10}C_{10}$), ($W_{70}A_{15}C_{15}$) and ($W_{60}A_{20}C_{20}$) has the values of 8.90, 8.67 and 8.45 % respectively. The values reported in this study was significantly (p< 0.05) lower than 20.61-33.22 % for bread samples made from wheat-bambara groundnut composite flour Oguntuase *et al.*, [32]. The values however were found to be significantly (p> 0.05) less than the values of 13.40-14.15 % for bread made from wheat-Rosehip composite flour [33]. The moisture values of bread in the present study were similar to values of 7.80 to 9.01 % for wheat-leafy vegetable-based bread reported by Okoye and Ezeugwu [34]. Food products that have high moisture content have a tendency to rapidly generate high amount and rate of microorganism, hence reduced the storage life of baked food products. The bread moisture values in this study showed that it can be kept for a reasonable longer time due to its slightly low moisture. The fat and protein content of the bread ranged from 9.45 to 10.83 % (WHT and $W_{60}M_{20}C_{20}$) and 11.25 to 13 87 % (WHT and $W_{60}M_{20}C_{20}$) respectively. The result showed increase in the fat and protein values as cocoa powder inclusion increases. The values obtained for fat content of the bread was significantly (p< 0.05) less than the values of 1.78-2.38 % obtained for whole wheat-bambara groundnut bread while the protein content was found to be within the range of 8.65-18.41 % observed by Yusufu and Ejeh [35]. The protein value was also similar to the value of 8.00-15.57 % for wheat-leafy vegetable-based bread [36]. The increase in protein value of the bread samples could be as a result of inclusion effect of the high protein found in wheat and cocoa flours as study have shown that cocoa protein ranged between 11-15% [37], while wheat contain about 11-13 % protein [38]. The increased protein in the bread samples could be a significant step to improving the nutrient intake of the consumers especially, the amino acid concentration. The fiber value of the bread ranged from 2.66 to 3.86 % ($W_{80}A_{10}C_{10}$) and ($W_{60}M_{20}C_{20}$) while the ash value ranged from 1.13 to 2.89 % (WHT) and ($W_{60}M_{20}C_{10}$) respectively. The values of fiber and ash obtained in this study is found to be significantly (p> 0.05) greater than the values of 1.52 and 1.91 % for wheat bread enriched with hazelnuts and walnuts [39]. The values were however close to 2.77 and 2.44 % for whole wheat-bambara groundnut bread [35]. Enrichment of the cocoa powder with the composite flour greatly increased the fiber and ash content of the bread. Inclusion of flours obtained from by-products rich in phenolic compounds such as cocoa powder has been found to increase ash and fiber content of baked food products [40]. The carbohydrate values of the bread samples ranged from 59.40 to 67.77 % for (WHT) and ($W_{60}M_{20}C_{20}$). This value was similar to 53.05 to 69.85 % for whole wheat-bambara groundnut bread [35]. However, the values were significantly (p< 0.05) lowered to 71.04-73.66 % for wheat-Rosehip bread reported by [33]. The observed decrease in carbohydrate of the bread made from the composite flour enriched with cocoa powder compared to the wheat bread have been reported for bread enriched with moringa seed flour and soy-flour [41,42].

Table 2: Proximate composition (%) of the composite flours and bread

Samples	Moisture	Fat	Protein	Fibre	Ash	Carbohydrate
FLOUR						
$W_{80}M_{10}C_{10}$	$4.02^c±0.01$	$10.18^c±0.12$	$11.39^c±0.11$	$2.35^c±0.02$	$1.45^c±0.01$	70.61e±0.02
$W_{70}M_{15}C_{15}$	$4.12^b±0.03$	$10.33^b±0.13$	$12.66^b±0.12$	$2.55^b±0.01$	$1.54^b±0.02$	68.80f±0.03
$W_{60}M_{20}C_{20}$	$4.31^a±0.02$	$10.57^a±0.14$	$12.73^a±0.11$	$2.75^a±0.03$	$1.69^a±0.03$	67.95g±0.03
$W_{80}A_{10}C_{10}$	$3.62^f±0.01$	$8.15^f±0.12$	$10.11^f±0.12$	$2.07^f±0.01$	$1.35^f±0.02$	74.70b±0.02
$W_{70}A_{15}C_{15}$	$3.67^e±0.01$	$8.55^e±0.01$	$10.19^e±0.01$	$2.67^d±0.03$	$1.45^e±0.03$	73.47c±0.03
$W_{60}A_{20}C_{20}$	$3.87^d±0.01$	$8.85^d±0.01$	$10.34^d±0.03$	$2.61^e±0.01$	$1.53^d±0.03$	72.80d±0.02
WHT	$3.10^g±0.03$	$7.89^g±0.01$	$10.01^g±0.03$	$2.01^g±0.03$	$1.25^g±0.01$	75.74a±0.02
BREAD						
$\mathbf{W_{80}M_{10}C_{10}}$	$9.89^a±0.03$	$10.45^c±0.13$	$13.45^c±0.13$	$3.35^c±0.03$	$2.69^c±0.01$	60.17d±0.03
$\mathbf{W_{70}M_{15}C_{15}}$	$9.25^b±0.03$	$10.71^b±0.13$	$13.65^b±0.13$	$3.47^b±0.03$	$2.78^b±0.01$	60.14d±0.02
$\mathbf{W_{60}M_{20}C_{20}}$	$9.15^c±0.03$	$10.83^a±0.13$	$13.87^a±0.12$	$3.86^a±0.03$	$2.89^a±0.01$	59.40e±0.02
$\mathbf{W_{80}A_{10}C_{10}}$	$8.90^d±0.01$	$10.25^f±0.12$	$12.55^f±0.12$	$2.66^f±0.03$	$1.64^f±0.01$	64.00b±0.03
$\mathbf{W_{70}A_{15}C_{15}}$	$8.67^e±0.01$	$10.33^e±0.13$	$12.65^e±0.12$	$2.78^e±0.03$	$1.69^e±0.01$	63.88c±0.02
$\mathbf{W_{60}A_{20}C_{20}}$	$8.45^f±0.01$	$10.45^d±0.13$	$12.78^d±0.12$	$2.99^d±0.03$	$1.77^d±0.01$	63.56c±0.03
WHT	$7.50^g±0.01$	$9.45^g±0.13$	$11.25^g±0.12$	$2.90^g±0.03$	$1.13^g±0.01$	67.77a±0.03

Mean(±) Values with different alphabetical superscripts in a column differ (P > 0.05) significantly

WMC: Wheat-maize-cocoa composite flour

WAC: Wheat-acha-cocoa composite flour

WHT: Wheat flour

Functional Properties of the Composite Flours

Table 3 shows the result of the functional properties of the composite flours. The bulk density values ranged from 0.56 to 0.88 g/ml for (WHT) and ($W_{60}M_{20}C_{20}$). The results obtained from this present study were similar to 0.45 to 0.0.86 g/ml for wheat-finger millet composite flour as reported by Mudau *et al*., Omak and Okafor, reported same results of bulk density for wheat-millet-pigeon pea flour which varied from 0.64 to 0.84 g/ml. Flour with bulk density less than 1 g/ml can be used in producing low bulk weaning foods and high-energy foods and it also facilitate easy storage, transport and marketing due to less volume of packaging materials requirement for any flour products. Water absorption capacity of the composite flour was 2.15 to 2.75 g/ml for (WHT) and ($W_{60}M_{20}C_{20}$). Water absorption capacity values were significant ($p< 0.05$) different between ($W_{80}M_{10}C_{10}$) and ($W_{80}A_{10}C_{10}$), however, there were no significant different between ($W_{70}M_{15}C_{15}$) and ($W_{60}M_{20}C_{20}$). It is worth noting that as the cocoa flour concentration increases, the water absorption capacity of the composite flour increases. Similar results were observed for cereal-pulse-fruit seeds composite where an increased in water absorption capacity was recorded in baked food products as reported by Chandra *et al*., and Menou *et al*. Increased in water absorption capacity has been linked with increase in amylose leaching, solubility and loss of starch crystalline structure. It has been reported that dough from composite flours absorbs more water than the one from wheat flour and this may be utilised in the development of different food products such as sausages, dough and other bakery foods.

The oil absorption capacity of the composite flours increased with increasing level of cocoa flour and the value ranged from 2.18 to 2.76 g/ml. The wheat flour recorded the lowest oil absorption capacity while (W60A20C20) have the highest value. The values observed in this study are significantly higher than 0.69-0.92 g/ml for whole wheat-bambara groundnut composite flour reported by Yusufu and Ejei [35]. It is however lower than the values of 120.55 g/g for wheat-finger millet composite flour reported by Mudau et al. The increase in oil absorption capacity may be as result of the presence of more hydrophobic proteins which shows dominance in binding lipids. The oil absorption capacity solely relies on some intrinsic factors such as protein conformation, amino acid and surface polarity or hydrophobicity. The non-polar amino acids chains of protein can form hydrophobic interactions with hydrocarbon chains of lipids. The composite flours in this study hence, has the ability of being utilized in food structural interactions like retention of flavors, improved palatability and shelf life extension in bakery foods where the absorption of fat is important.

The foam capacity of the composite flours ranged from 3.55 to 6.85 % for (WHT) and (W60M20C20). This value was less than 19.89-26.60 % for whole wheat-bambara composite flour [35]. There were significant difference between the control samples and other developed composite samples. Foam capacity improves the textural consistency of food systems because of its high percentage of porosity intended for the production of baked food products and also acts as functional agent in other food formulations. The water solubility index and swelling power ranged from 60.00 to 88.00 % and 30.00 to 39.00 % for (WHT) and (W60A20C20) respectively. The water solubility index and swelling power are indicators of starch hydration as they demonstrate the degree of interaction between the starch chains within both the amorphous and crystalline areas. Water solubility index and swelling power values determine the textural, pasting and thickening properties of starch-based food products. It can be concluded that the composite flours in the study may be suitable to develop consistent dough which may help to produce foods with good eating quality.

Physical Properties of the Bread

The physical properties of the bread samples are presented in Table 4. The loaf weight of the bread samples ranged from 112.98 to 312.55 g for ($W_{80}M_{10}C_{10}$) and (WHT). The present study showed a significant increase in weight in all bread samples than the control sample (WHT). The results obtained in the study were similar to 245.60 g for whole wheat-bambara groundnut bread [35]. The values however were significantly ($p> 0.05$) greater than 134.40 g for bread fortified with with green leafy vegetables [36]. The increase in loaf weight could be attributed to increase moisture absorption and less retention of carbon dioxide gas in the blended dough resulted in heavy dough and thus heavy loaves [43]. The loaf volume and specific volume of the bread ranged from 400.00 to 470.00 cm^3 for (WHT) and ($W_{70}M_{15}C_{15}$) and 1.28 to 3.79 cm^3/g for (WHT) and ($W_{80}M_{10}C_{10}$) respectively. The result showed that the higher the loaf volume and loaf weight, the lower the specific volume of the bread and this assertion

Table 3: Functional properties of the composite flour

Samples	BD (g/ml)	FC (%)	WAC (g/ml)	OAC (g/ml)	SI (%)	SP (%)
$W_{80}M_{10}C_{10}$	0.63^f±0.01	6.77^b±0.02	2.50^b±0.11	2.35^e±0.11	65.00^f±0.12	33.00^d±0.14
$W_{70}M_{15}C_{15}$	0.79^b±0.01	6.43^c±0.03	2.75^a±0.12	2.65^c±0.12	70.00^e±0.13	35.00^c±0.15
$W_{60}M_{20}C_{20}$	0.88^a±0.03	6.85^a±0.02	2.75^a±0.11	3.10^a±0.12	75.00^d±0.14	35.00^c±0.13
$W_{80}A_{10}C_{10}$	0.67^e±0.01	5.84^e±0.01	2.20^e±0.13	2.55^d±0.12	81.00^c±0.13	37.00^b±0.14
$W_{70}A_{15}C_{15}$	0.70^d±0.01	5.95^d±0.03	2.35^d±0.13	2.66^c±0.13	84.00^b±0.13	37.00^b±0.13
$W_{60}A_{20}C_{20}$	0.77^c±0.01	5.95^d±0.03	2.55^c±0.13	2.76^b±0.13	88.00^a±0.13	39.00^a±0.13
WHT	0.56^g±0.01	3.55^f±0.03	2.15^f±0.13	2.18^f±0.13	60.00^g±0.13	30.00^d±0.13

Mean (±) Values with different alphabetical superscripts in a column differ (P > 0.05) significantly

WMC: Wheat-maize-cocoa composite flour

WAC: Wheat-acha-cocoa composite flour

WHT: Wheat flour

Table 4: Physical properties of bread made from the composite flour

Samples	Loaf weight (g)	Loaf volume (cm)	Specific volume (cm^3/g)	Loaf width (cm)
$W_{80}M_{10}C_{10}$	112.98^h±0.15	428.01^c±1.24	3.79^a±0.01	6.00^d±0.01
$W_{70}M_{15}C_{15}$	188.89^g±0.17	470.00^a±1.14	2.49^b±0.01	7.00^c±0.01
$W_{60}M_{20}C_{20}$	188.98^f±0.17	462.00^b±1.17	2.44^c±0.01	9.00^a±0.01
$W_{80}A_{10}C_{10}$	218.67^d±0.16	420.00^d±1.29	1.92^d±0.01	8.00^b±0.01
$W_{70}A_{15}C_{15}$	223.67^b±0.16	402.00^e±1.17	1.79^e±0.01	9.00^a±0.01
$W_{60}A_{20}C_{20}$	223.55^c±0.16	402.00^e±1.17	1.79^e±0.01	9.00^a±0.01
WHT	312.55^a±0.16	400.00^f±1.14	1.28^f±0.01	5.00^e±0.01

Mean (±) Values with different alphabetical superscripts in a column differ (P > 0.05) significantly
WMC: Wheat-maize-cocoa composite flour
WAC: Wheat-acha-cocoa composite flour
WHT: Wheat flour

was in accordance with the report of Abdelghafar et al., [44], and Juliana et al., [45], who observed higher loaf weight and volume of bread made from composite flour as a result of less carbon dioxide gas in the blended dough, thus producing dense bread texture. Loaf volume is considered as the most important bread characteristic since it provides a quantitative measurement of baking performance [46]. Loaf volume is said to have positive economic effect on bread and this is because consumers always attracted to higher volume and weight of bread as they belief those bread is more attractive but at the same price with other bread. Lack of weight and volume in bread is an indicator of weak flour or flour low in enzyme activity [47].

DPPH Radical Scavenging Activities, Total Phenolic and Total Flavonoids of the Aqueous Extracts of Bread made from Composite Flour Enriched with Cocoa Powder

The DPPH radical scavenging activities of the aqueous extracts of bread are shown in Figure 1. The antioxidant activity of the sample ranged from 55.00 to 75.00 % for (WHT) and ($W_{60}M_{20}C_{20}$). Antioxidant are chemical substance that acts as oxidation inhibitors and as such inhibit the production of free radicals, hence play a key role in preventing oxidation stress which results to generation of chronic diseases like diabetes, obesity, hypertension and cancers [48,49]. The antioxidant activity generated from this present study indicates that regular and adequate consumption of the formulated bread could inhibit free radicals' formation, thereby prevent occurrence of oxidative stress (Figure 1).

Total Phenols and Total Flavonoids Content of Bread from the Composite Flour

The total phenolic and flavonoids of the bread samples from the composite flour are shown in Table 5. The total phenolic content of the bread expressed in gallic acid equivalent ranged from 4.59 to 6.98 mg/GAE for (WHT) and ($W_{60}M_{20}C_{20}$) while the total flavonoids content expressed in quecetin equivalent ranged from 6.77 to 9.91 mg/QE for (WHT) and ($W_{60}M_{20}C_{20}$). The result of the present study showed that ($W_{60}M_{20}C_{20}$) has the highest total phenols and flavonoid content. It is worth noting that when the percentage of wheat flour was reduced and maize and cocoa powder increased, total phenolic and total flavonoid contents increased. The same trend of result was noted for DPPH radical scavenging activity of the (WHT) and ($W_{60}M_{20}C_{20}$). The total phenolic and flavonoid content of samples in this study is significantly ($p< 0.05$) higher than 3.61 and 1.28 mg/GAE for bread produced from cassava-wheat-cocoa composite flour reported by Richard et al., [50]. However, the total phenolic content of the bread was lower to 26.09 and 39.45 mg/GAE for wheat bread enriched with hazelnuts and walnuts [39]. PhenolicT have long been thought to play a major role in the food sensory qualities such as taste, color and flavor [27]. The global growing interest for diets high in phenolic content has prompted new approaches to enrich diets with plants products possessing high phenolic and antioxidant components [51].

Sensory Characteristic of Bread Made From the Composite Flour

The sensory characteristic of bread made from the composite flour is shown in Table 5 and 6. The mean value for taste, flavor

Table 5: Total phenols and Total flavonoids content of bread from the composite flour

Extract concentration	Total phenols content (mg/g GAE)	Total Flavonoids content (mg/g QE)
$W_{80}M_{10}C_{10}$	6.67±0.03^c	9.63±0.02^c
$W_{70}M_{15}C_{15}$	6.89±0.01^b	9.88±0.03^b
$W_{60}M_{20}C_{20}$	6.98±0.02^a	9.91±0.03^a
$\mathbf{W_{80}A_{10}C_{10}}$	4.66±0.03^f	8.52±0.02^f
$\mathbf{W_{70}A_{15}C_{15}}$	5.45±0.04^e	8.59±0.03^e
$\mathbf{W_{60}A_{20}C_{20}}$	5.78±0.03^d	8.77±0.02^d
WHT	4.59±0.02^g	6.77±0.03^f

Mean (±) Values with different alphabetical superscripts in a column differ (P > 0.05) significantly
WMC: Wheat-maize-cocoa composite flour
WAC: Wheat-acha-cocoa composite flour
WHT: Wheat flour

Table 6: Sensory characteristic of bread made from the composite flour

Samples	Taste	Flavor	Appearance	Texture	Overall acceptability
$W_{80}M_{10}C_{10}$	6.15^{c}	7.11^{c}	6.57^{c}	6.59^{c}	6.35^{c}
$W_{70}M_{15}C_{15}$	6.67^{b}	7.15^{b}	6.77^{b}	6.91^{b}	6.55^{b}
$W_{60}M_{20}C_{20}$	6.89^{a}	7.37^{a}	6.87^{a}	7.00^{a}	6.65^{a}
$W_{80}A_{10}C_{10}$	5.00^{f}	5.73^{d}	5.91^{e}	5.45^{d}	5.64^{f}
$W_{70}A_{15}C_{15}$	5.67^{e}	5.73^{d}	5.96^{d}	5.12^{f}	5.76^{e}
$W_{60}A_{20}C_{20}$	5.98^{d}	5.73^{d}	5.11^{f}	5.26^{e}	5.89^{d}
WHT	4.78^{g}	5.12^{e}	5.00^{g}	5.00^{g}	5.02^{g}

Mean (±) Values with different alphabetical superscripts in a column differ (P > 0.05) significantly
WMC: Wheat-maize-cocoa composite flour
WAC: Wheat-acha-cocoa composite flour
WHT: Wheat flour

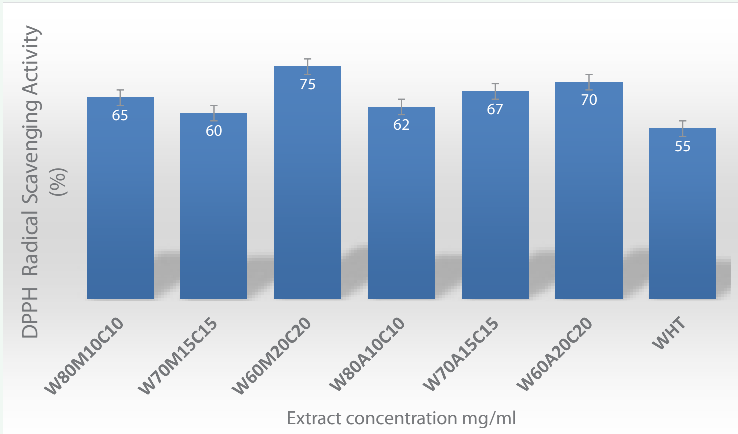

Figure 1 DPPH radical scavenging activity of aqueous extracts of bread made from composite flour.
WMC: Wheat-maize-cocoa composite flour
WAC: Wheat-acha-cocoa composite flour
WHT: Wheat flour

and appearance of the bread ranged from 4.78 to 6.89, 5.12 to 7.37 and 5.00 to 6.87 for (WHT) and ($W_{60}M_{20}C_{20}$) while the texture and overall acceptability were 5.00 to 7.00 and 5.02 to 6.65 for (WHT) and ($W_{60}M_{20}C_{20}$). It was noted that as the level of cocoa powder increased, the sensory properties of the bread increased. This result was in agreement with the result obtained by Bolarinwa et al., [41], whereby fortification of moringa seeds powder in bread significantly improved the sensory characteristics of the bread. It can be concluded that the cocoa powder improved the formulated bread, more importantly, the ($W_{60}M_{20}C_{20}$) bread sample.

CONCLUSION

This present study showed that the developed bread, more importantly $W_{60}M_{20}C_{20}$ (60 %wheat, 20 % Maize, 20 % cocoa) proved to have contain high nutritional content, improve physical quality and enormous amount of antioxidant activities when compare with the other composite bread produced. Hence, this bread samples may be suitable to prevent hyperglycemia and oxidative stress.

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AUTHOR'S CONTRIBUTIONS

AOA conceptualized the project, developed the experimental design and wrote the manuscript. AAO developed the experimental design, conducted the statistical analysis and wrote the manuscript. OOP conducted the experimental designed, developed the manuscript. EOO designed the experiment and wrote the manuscript. AIB. Designed the experiment and wrote the manuscript.

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