



Physicochemical and Antioxidant Properties of Whole-Wheat Biscuits Incorporated with *Moringa oleifera* Leaves and Cocoa Powder

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Authors' contributions

This work was carried out in collaboration between all authors. Author CFA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author VOO identified the species of plant and performed the statistical analysis. Author OSA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the effect of *Moringa oleifera* leaf and cocoa powder on the quality characteristics and antioxidant properties of biscuits.

Study Design: Two-way ANOVA.

Place and Duration of Study: Department of Food Science and Technology, Federal University of Technology, Akure, Ondo State, Nigeria, between March 2014 and December 2014.

Methodology: Biscuits were prepared from different blends of whole-wheat flour, *Moringa oleifera* leaves (MLP) and cocoa powder (CCP) in the ratios of 100:0:0, 95:5:0, 90:10:0, 90:5:5 and 85:10:5 w/w respectively. The choice of these levels was made on the initial acceptability trials of the baked product. The effect of MLP and CCP on the physical, nutritional and antioxidant properties of the

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biscuits were evaluated.

Results: The replacement of whole wheat flour with increasing level of MLP from 0 to 10% decreased the spread ratio of biscuits (from 9.83 to 7.35) while addition of 5% CCP to the moringa-supplemented biscuit increased the spread ratio of the biscuits (from 7.35 to 9.91). The protein content ranged from 10.99 to 14.73% while the fat content varied from 13.19 to 14.46%. The ash, fibre and carbohydrate contents ranged from 2.23 to 2.63%; 2.45 to 4.35% and 64.77 to 69.86% respectively. The iron and calcium contents ranged from 1.57 mg% to 6.23 mg% and 29.17 mg% to 52.47 mg% respectively. The protein, crude fibre, iron, and calcium of the biscuits increased with increasing quantity of MLP and CCP. Sensory evaluation showed that biscuits incorporated with 10% MLP were acceptable and addition of 5% CCP to the moringa-supplemented biscuit produced biscuits with more acceptable colour and taste. Antioxidant properties of biscuits increased with increasing quantity of MLP and CCP.

Conclusion: The results have shown the possibility of incorporating 10% *Moringa oleifera* leaves and 5% cocoa powder in biscuits production in order to improve the nutritional and antioxidant properties of biscuits.

Keywords: Whole-wheat flour; *Moringa oleifera*; cocoa powder; spread ratio; nutritional properties; antioxidant properties; biscuits.

1. INTRODUCTION

Moringa oleifera is the most known and widely cultivated variety of the genus *Moringa*, family Moringaceae [1]. *Moringa oleifera*, also known as drumstick, is a tree native to India but has been planted and domesticated in many other countries including Nigeria and is gaining wide acceptance as medicine and food [2-4]. The leaves are highly nutritious, being a significant source of β -carotene, vitamin A and C, protein, iron, calcium and potassium [5-7]. The growing popularity of the crop is not only attributable to its high nutritional quality but more importantly acclaimed medicinal value of nearly every part of the tree [8]. The medicinal properties of the plant have been attributed to high concentrations of phytochemicals found in different parts of the plant [9]. Phytochemical screening of various solvent extracts of *Moringa oleifera* leaves showed that the leaves contained high polyphenolic content and acts as a good source of natural antioxidants [6,7].

Cocoa (*Theobroma cacao* L) is an important crop in the economics of several countries such as Nigeria, Ghana, Ivory Coast, Indonesia and Malaysia [10]. Cocoa powder and its derivatives have been reported to contain significant amount of dietary flavonoids and exhibit greater antioxidant capacity than many other flavanol-rich foods and food extracts, such as green and black tea, red wine, blueberry, garlic, and strawberry [11,12]. 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, ameliorate endothelial dysfunction and act as antioxidants [13-16]. These health benefits of cocoa have been attributed to their high polyphenol compounds, mainly flavanols. Flavanols are found in the non-fat cocoa solids portion of the cocoa bean, which comprises about 47% of the bean, the remainder being cocoa butter [17]. During processing, cocoa beans are usually separated into two major products; namely, cocoa powder and cocoa butter. Cocoa powder is made from finely milled cocoa beans, which are pressed to remove most of the cocoa butter, resulting in powder that is typically 88% to 90% non-fat solid and 10% to 12% cocoa butter [17]. Hence, the concentration of the non-fat cocoa solids material makes natural cocoa powder one of the richest sources of procyanidins in the diet [18]. Natural cocoa powder has been reported to contain threefold or more flavanol-monomers, procyanidins and antioxidant activity than dark chocolate on equal weight basis [19-21]. Cocoa powder is used in confectionery products and also in non-confectionery products such as flavoured milks, cereals, ice cream, cookies, biscuits, cakes, and other dessert.

Biscuit, a popular example of bakery product is a ready-to-eat snack that possesses several attractive features including wide consumption base, relatively cheap, more convenient with long shelf-life and have ability to serve as vehicles for important nutrients [22,23]. They are usually available in different forms, taste and shape. The major ingredients are flour, fat, sugar and water while other ingredients such as milk, salt and

aerating agent can be included. They can also be enriched or fortified with other ingredients in order to meet specific nutritional or therapeutic needs of consumers. In recent years, numerous studies have shown the potential of utilizing natural plant such as green leafy vegetable in cookies and biscuits production; Singh and Kawatra [24] showed the potential of amaranthus leaves as a source of iron and β -carotene in pakora, vada, namakpal, kurmure, biscuit and cake. Extract of amla, *Moringa oleifera* leaves and raisins were used as natural antioxidants in biscuits [25]. Mint leaves [26] and cauliflower leaves [27] have been incorporated in biscuits as sources of natural antioxidant. Dachana et al. [28] showed the potential of *Moringa oleifera* leaves as a source of protein, minerals, fiber and β -carotene in cookies. They reported that incorporation of *Moringa oleifera* leaves in cookies improved its nutritional qualities but as the level increased from 0 to 15% the colour of the cookies became green. The present work is carried out to study the effect of *Moringa oleifera* leaves (MLP) and cocoa powder (CCP) on the nutritional qualities and antioxidant properties of biscuit. Addition of cocoa powder to moringa-substituted biscuit could improve the colour and enhance the nutritional and antioxidant properties of such biscuit.

2. MATERIALS AND METHODS

2.1 Materials

Fresh *Moringa oleifera* leaves were purchased from Oba market in Akure, Nigeria. Authentication of the leaves was carried out in the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. Cocoa powder (CCP) was obtained from COOP Cocoa Limited, Akure-Owo Expressway, Akure, Nigeria. Whole-wheat flour, margarine (fat), sugar powder, skimmed milk powder, baking powder and sodium chloride were purchased from Oba market in Akure, Ondo State. DPPH (2, 2-diphenyl-1-picrylhydrazyl) and other antioxidant reagents were purchased from Sigma (Sigma Chemicals, St. Louis, MO). All chemicals used were of analytical grade.

2.2 Preparation of Moringa Leaf Powder (MLP)

The *Moringa oleifera* leaves powder was prepared by cleaning and washing with distilled water after which it was subjected to shade

drying for 3 weeks. The dried moringa leaves were blended and sieved through a 2 mm sieve. It was then packed in a plastic bag, sealed and stored at room temperature (27°C) prior to use.

2.3 Preparation of Flour Blends

Preliminary investigation was carried out by preparing biscuits with 100:0:0, 95:5:0, 90:10:0, 85:15:0, 90:5:5, 85:10:5 and 80:15:5 of whole-wheat flour, MLP and CCP respectively. These samples were evaluated for sensory attributes in comparison with the control. The following blend were selected and used for further analysis based on the sensory evaluation result; 95:5:0, 90:10:0, 90:5:5 and 85:10:5.

2.4 Production of Biscuits

The biscuits were prepared according to the methods reported by Omoba and Omogbemile [29] with the following formulations- 100g composite flour, 60 g sugar powder, 30 g margarine, 3 g skimmed milk powder, 1.0 g baking powder, and 1.0 g sodium chloride. Briefly, margarine and sugar were creamed at low speed for 3 mins, using a Kenwood Chef Food mixer Model A901. The dried ingredients, which composed of composite flour, skimmed milk powder, baking powder and sodium chloride, were added and mixed with the cream for 2 mins at low speed, to obtain firm dough. The dough was manually rolled out to a height of 5 mm and cut into circular shapes using a 4.3 cm diameter cutter. The dough pieces were transferred onto a baking tray lined with aluminum foil. Biscuit dough were baked in a pre-heated Unox steam convection oven at 160±2°C for 20 mins and cooled for 30 mins at ambient temperature. Biscuits were vacuum-packed in a thick low-density polyethylene bags and stored at 4°C.

2.5 Determination of the Physical Characteristics of Biscuits

The diameter was measured with a calibrated ruler as described by Ayo et al. [30]. The thickness (T, mm) of biscuits was measured by stacking six biscuits on top of one another and taking the average value. The weight of the biscuits was measured by weighing on a Mettler model weighing balance. The spread ratio of biscuits was calculated by dividing values of the diameter by the thickness values [31].

2.6 Determination of Proximate Composition of Biscuits

Biscuit samples were analyzed for moisture content, crude fat, ash, crude protein, crude fibre, carbohydrate as previously described [32]. The iron and calcium contents of biscuits were determined by Atomic Absorption Spectrometry and flame photometry methods described in the AOAC method 965.09 [32].

2.7 Sensory Evaluation of Biscuits

Sensory evaluation of the samples was carried out for consumer acceptance and preference using ten (10) untrained panelists selected at random from the Federal University of Technology, Akure campus, Nigeria. The biscuit samples were evaluated for taste, aroma, crispiness, colour and general acceptability using 9-point hedonic scale where 9 and 1 represent extremely like and extremely dislike respectively.

2.8 Preparation of Aqueous Extract of the Biscuits

Aqueous extracts of the biscuits were prepared by soaking 10 g of the milled biscuits in 100 ml of distilled water for 24 h at 37°C; the mixtures were filtered, centrifuged and supernatants were stored in the refrigerator for subsequent analysis [33].

2.9 Determination of Total Phenol Content

The total phenol content of the extracts was determined using the method reported by Singleton et al. [34]. Appropriate dilutions of extract were oxidized with 2.5 ml of 10% Folin-ciocalteu's reagent and neutralised by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated at 45°C for 40 mins, and the absorbance was measured at 765 nm with the JENWAY Model 6305 UV-Visible spectrophotometer (Barlworld Scientific, Dunmow, United Kingdom). The total phenol content was calculated using Gallic acid as standard.

2.10 Determination of Total Flavonoid

The total flavonoid content of the extracts was determined using a slightly modified method reported by Meda et al. [35]. An aliquot of 0.5 ml of the extract was mixed with 0.5 ml methanol, 50 µl of 10% AlCl₃, 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. Thereafter the absorbance of the mixture was measured at 415 nm with the JENWAY Model 6305 UV-Visible spectrophotometer (Barlworld Scientific, Dunmow, United Kingdom). The total flavonoid content was calculated using quercetin as standard.

2.11 DPPH Free Radical Scavenging Ability

The free radical scavenging ability of the extracts against DPPH (1, 1- diphenyl-2-picrylhydrazyl) free radical was evaluated as described by Aluko and Monu [36]. An appropriate dilution of the extract (1 ml) was mixed with 1 ml of the 0.4 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min before measuring the absorbance at 516 nm. The DPPH free radical scavenging ability was calculated with the respect to the reference (which contains all the reagents without test sample). Free radicals scavenging ability was expressed as percentage (%) inhibition.

2.12 Determination of 2, 2'-azinobis (3-ethylbenzo-thiazoline-6-sulphonic acid) ABTS Scavenging Ability

The ABTS scavenging ability of the extracts was determined according to the method described by Re et al. [37]. ABTS was generated by reacting ABTS aqueous solution (7 mM) with K₂S₂O₈ (2.45 mM, final concentration) in the dark for 16 h and adjusting the absorbance at 734 nm to 0.700 with ethanol. An aliquot of 0.2 ml of the extract was then added to 2.0 ml of ABTS solution and the absorbance was measured at 734 nm after 15 mins. The total antioxidant capacity was calculated and expressed as trolox equivalent.

2.13 Determination of Ferric Reducing Antioxidant Power (FRAP)

The reducing power of the aqueous extract of the biscuit was determined by assessing the ability of the extract to reduce FeCl₃ solution as described by Zhang et al. [38]. Briefly, biscuit extracts (2.5 ml) were mixed with 2.5 ml of 200 mM of sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Thereafter 2.5 ml of 10% Trichloroacetic acid was also added and centrifuged at 2000 rpm for 10 min. An aliquot of

5.0 ml of the supernatant was mixed with 5.0 ml of distilled water and 1.0 ml of 0.1% FeCl₃. The absorbance was measured at 700 nm against a reagent blank. The reducing property was calculated and expressed as Gallic acid equivalent.

2.14 Statistical Analysis

Data were collected as means of three separate determinations and subjected to two-way analysis of variance using Statistical Package for Social Statistics (SPSS 15.0). The significant differences ($p \leq 0.05$) between the mean values were determined using the Duncan's Multiple Range Test.

3. RESULTS AND DISCUSSION

3.1 Effect of MLP and CCP on the Physical Properties of Biscuits

The results of the effect of MLP and CCP on the physical properties of biscuits are presented in Table 1. The replacement of whole-wheat flour with increasing level of MLP from 0 to 10% decreased the spread ratio from 9.83 to 7.35 due to the decrease and increase in diameter and thickness, respectively. Similar results had been reported for cookies prepared from composite flour [39,28,40]. The observed decrease in spread ratio has been attributed to dilution of gluten and less water available for gluten hydration [39]. McWatters [41] reported that use of composite flour increases dough viscosity and forms aggregates by competing with limited free water available in cookie dough. In contrast, addition of 5% CCP increases the spread ratio of moringa-supplemented biscuits as evident in biscuits containing 5% MLP and 5% CCP (sample D) and those containing 10% MLP and 5% CCP (sample E).

3.2 Nutritional Characteristics of Biscuits

The moisture content of biscuits ranged from 3.05 to 3.76% (Table 2). The protein and fat contents varied from 10.99 to 14.73% and 13.19 to 15.00%, respectively. The ash, fibre and carbohydrate contents ranged from 2.23 to 2.63%, 2.45 to 4.35% and 64.77 to 69.86%, respectively. There was significant increase in protein and fibre content of biscuits with addition of MLP and CCP with the highest protein and fibre contents observed in sample E. The iron content of whole wheat flour biscuit (sample A)

was 1.57 mg%, which increased significantly when incorporated with MLP and CCP. The calcium content of biscuits increased from 29.17 mg% to 52.47 mg% when MLP and CCP were added to the biscuits. The significant increase in the calcium content could be due to the presence of higher calcium content in MLP [6,7]. The results indicate that MLP and CCP-supplemented biscuits possess higher nutritive profile than the control with significant increase in protein, fibre, iron and calcium. Similar improvements in the nutritional characteristics, such as dietary fibre, protein, iron and calcium have been reported for green leafy vegetables-supplemented cookies and biscuits [26,28,40,39,42].

3.3 Effect of MLP and CCP on Sensory Characteristics of Biscuits

The sensory characteristics of biscuits supplemented with MLP and CCP are presented in Table 3. The sensory scores for colour, aroma and taste of all the supplemented biscuits were lower when compared to those of control (sample A). The colour and taste of Sample D and E (biscuits containing MLP and CCP) were more acceptable than those of Sample B and C (biscuits containing MLP). This could be attributed to the addition of 5% CCP which helped to mask the greenish colour of MLP and improved the taste of the biscuit. Past studies had reported the changes in colour of green leafy vegetables-supplemented cookies from golden brown to green [28,40]. The overall acceptability scores were 8.0, 5.7, 5.7, 6.1 and 6.5 for sample A, B, C, D and E, respectively. The results show that acceptable biscuits could be prepared using 5% and 10% MLP. Moreover, addition of 5% CCP to the MLP biscuits produced biscuits with more acceptable colour and taste. Gyedu-Akoto and Laryea [43] reported that addition of cocoa powder to biscuits supplemented with cassava flour enhanced the flavour and colour of the biscuits.

3.4 Phenolic Contents of Aqueous Extracts of Biscuits Supplemented with MLP and CCP

Polyphenols are plant secondary metabolites, which are able to reduce reactive oxygen species (ROS) by donating hydrogen atoms of phenolic hydroxyls and by transferring electrons from such phenolic hydroxyls or phenoxide anions [44]. Among the dietary constituents, polyphenolics have been reported to play a significant role as

antioxidants in the protective effect of plant-derived foods and contribute immensely to the sensory quality such as color, taste and flavor of foods [45,46]. In the present study, total polyphenolic content of aqueous extracts of the biscuits are presented in Table 4. Total phenolic content (TPC) was expressed as milligrams of Gallic acid equivalent (GAE) per gram (mg/g) of dry biscuit samples. Sample A (control) had the lowest total phenol content of 1.01 mg/g GAE while sample E had the highest total phenol content of 5.42 mg/g GAE. The total phenol content in cocoa powder-containing biscuits is similar to the total phenol value of 4.01 mg/g

reported for chocolate cookies [17]. The flavonoid content of the biscuits ranged from 0.22 to 1.39 mg/g QE. The control sample (A) also had the least flavonoid content when compared to the supplemented biscuits. Moringa leaves and cocoa powder have been known as rich sources of polyphenols [6,7,12,18]. Hence, the observed increase in phenolic compounds of the supplemented biscuits could be attributed to MLP and CCP that were added to them. Similar increase in polyphenolic compounds was observed in biscuits supplemented with bambara groundnut and orange peel when compared to 100% whole wheat biscuits [47].

Table 1. Effect of MLP and CP on the physical characteristic of biscuit

Samples	Diameter (cm)	Weight (g)	Thickness (cm)	Spread Ratio
A	5.53±0.06 ^a	12.22±1.30 ^a	0.57±0.06 ^{ab}	9.83±1.01 ^a
B	5.10±0.17 ^{bc}	11.63±1.98 ^a	0.67±0.12 ^a	7.81±1.37 ^b
C	4.87±0.07 ^c	11.03±1.94 ^a	0.66±0.02 ^a	7.35±0.27 ^b
D	5.20±0.10 ^b	12.06±1.61 ^a	0.53±0.06 ^b	9.83±1.16 ^a
E	5.30±0.17 ^b	10.41±0.00 ^a	0.54±0.03 ^b	9.91±0.95 ^a

Values in the same column with different superscript are significantly different ($P \leq 0.05$). Means \pm standard deviations of triplicate samples. Key: A= 100% whole-wheat flour biscuit; B = 95% whole-wheat flour + 5% MLP; C = 90% whole-wheat flour + 10% MLP; D = 90% whole-wheat flour + 5% MLP + 5% CCP; E = 85% whole-wheat flour + 10% MLP + 5% CCP

Table 2. Effect of MLP and CCP on nutritional characteristics of biscuits

Parameters/ Samples	A	B	C	D	E
Moisture (g %)	3.65 ^{ab} ±0.05	3.05 ^c ±0.01	3.19 ^c ±0.23	3.76 ^a ±0.12	3.37 ^{bc} ±0.24
Protein (g %)	10.99 ^d ±0.07	12.70 ^c ±0.44	13.37 ^b ±0.23	13.99 ^b ±0.09	14.73 ^a ±0.41
Fat (g %)	14.39 ^b ±0.11	14.46 ^b ±0.09	13.19 ^d ±0.33	15.00 ^a ±0.14	13.83 ^c ±0.43
Ash (g %)	2.31 ^a ±0.25	2.45 ^a ±0.08	2.23 ^a ±0.61	2.63 ^a ±0.15	2.31 ^a ±0.07
Fibre (g %)	2.45 ^d ±0.20	3.22 ^c ±0.27	4.06 ^{ab} ±0.32	3.47 ^{bc} ±0.35	4.35 ^a ±0.26
Carbohydrate (g%)	69.86 ^a ±0.45	67.17 ^b ±0.67	67.15 ^b ±1.13	64.91 ^c ±0.44	64.77 ^c ±0.59
Iron (mg %)	1.57 ^d ±0.14	1.77 ^d ±0.13	4.83 ^b ±0.09	2.80 ^c ±0.08	6.23 ^a ±0.13
Calcium (mg %)	29.17 ^e ±0.25	36.63 ^d ±0.42	43.73 ^b ±1.07	41.17 ^c ±0.53	52.47 ^a ±0.58

Values in the same row with different superscript are significantly different ($P \leq 0.05$). Means \pm standard deviations of triplicate samples. Key: A= 100% whole-wheat flour biscuit; B = 95% whole-wheat flour + 5% MLP; C = 90% whole-wheat flour + 10% MLP; D = 90% whole-wheat flour + 5% MLP + 5% CCP; E = 85% whole-wheat flour + 10% MLP + 5% CCP

Table 3. Effect of MLP and CCP on the sensory characteristics of biscuits

Parameters/ Samples	A	B	C	D	E
Colour	8.1 ^a	5.7 ^c	5.9 ^c	6.6 ^b	6.9 ^b
Aroma	7.5 ^a	5.4 ^b	5.6 ^b	5.6 ^b	5.9 ^b
Taste	7.8 ^a	4.8 ^c	5.7 ^c	6.5 ^b	6.6 ^b
Crispiness	6.7 ^a	6.9 ^a	5.6 ^b	5.6 ^b	6.3 ^{ab}
Overall acceptability	8.00 ^a	5.7 ^{bc}	5.7 ^{bc}	6.1 ^b	6.5 ^b

Values in the same row with different superscript are significantly different ($P \leq 0.05$). Key: A= 100% whole-wheat flour biscuit; B = 95% whole-wheat flour + 5% MLP; C = 90% whole-wheat flour + 10% MLP; D = 90% whole-wheat flour + 5% MLP + 5% CCP; E = 85% whole-wheat flour + 10% MLP + 5% CCP

3.5 Antioxidant Properties of Aqueous Extracts of Biscuits Incorporated with MLP and CCP

Since the response of antioxidants depends on many factors in a given test, the antioxidant activity of a system can be better characterized by using different assays based on different mechanisms [48]. Hence, several methods are used to evaluate antioxidant activities of natural compounds in foods or biological systems. Among these methods are DPPH and ABTS procedures, which uses 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and 2, 2-Azinobis (3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) as free radical generators, respectively and ferric reducing antioxidant power.

3.5.1 DPPH radical scavenging activities of aqueous extracts of biscuits incorporated with mlp and ccp

DPPH radical is an oil-soluble free radical that becomes a stable product after accepting an electron or hydrogen from an antioxidant. DPPH

radical is stable in methanol and show maximum absorbance at 517 nm. When DPPH encounters a proton-donating substance such as an antioxidant, the radical would be scavenged and the absorbance is reduced. Hence the antioxidant activity of the substance can be expressed as its ability in scavenging the DPPH radical [49]. The DPPH radical scavenging activities of aqueous extracts of biscuits are shown in Fig 1. The supplemented biscuits exhibited better DPPH radical scavenging activity than the control (sample A) while the highest DPPH radical scavenging activity was observed in sample E. This trend is consistent with the total phenolic content results presented for both non-supplemented and supplemented samples (Table 4). For instance, sample E with highest polyphenol content exhibited the highest DPPH radical scavenging activity whereas; lowest DPPH radical scavenging activity was recorded for sample A with lowest polyphenol content. The results show that there is positive relationship between the polyphenolic content and DPPH radical scavenging activity of the biscuit extracts.

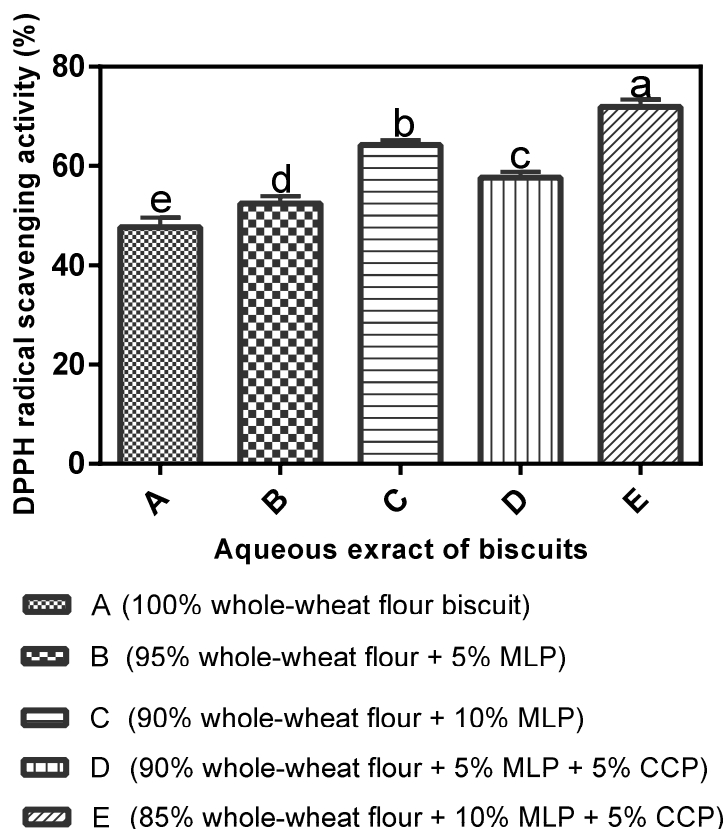


Fig. 1. DPPH radical scavenging activity of aqueous extracts of biscuits incorporated with *Moringa oleifera* leaf and cocoa powder

3.5.2 ABTS radical scavenging activities of aqueous extracts of biscuits incorporated with MLP and CCP

ABTS is soluble in both aqueous and organic solvents, and it reacts relatively rapidly compared to DPPH, which normally takes several hours for the reaction to be completed. Although the mechanisms of DPPH and ABTS methods are similar, ABTS model was developed to assay for free radical scavenging ability because of some limitation of DPPH associated with color interference and sample solubility [47]. The results of ABTS radical scavenging activities of biscuits' extracts, expressed as Trolox equivalent antioxidant capacity (TEAC) are shown in Fig. 2.

The ABTS radical scavenging activities of the supplemented biscuits are significantly ($p \leq 0.05$) stronger than the control biscuit. This trend is similar to the trend observed in DPPH radical scavenging activities of the biscuit's extracts (Fig. 1), although there is no significant difference between the ABTS scavenging activity of Sample C and D.

3.5.3 Ferric reducing antioxidant power of aqueous extracts of biscuits incorporated with MLP and CCP

The reducing capacity of a given compound may serve as a significant indicator of its potential antioxidant activity [49].

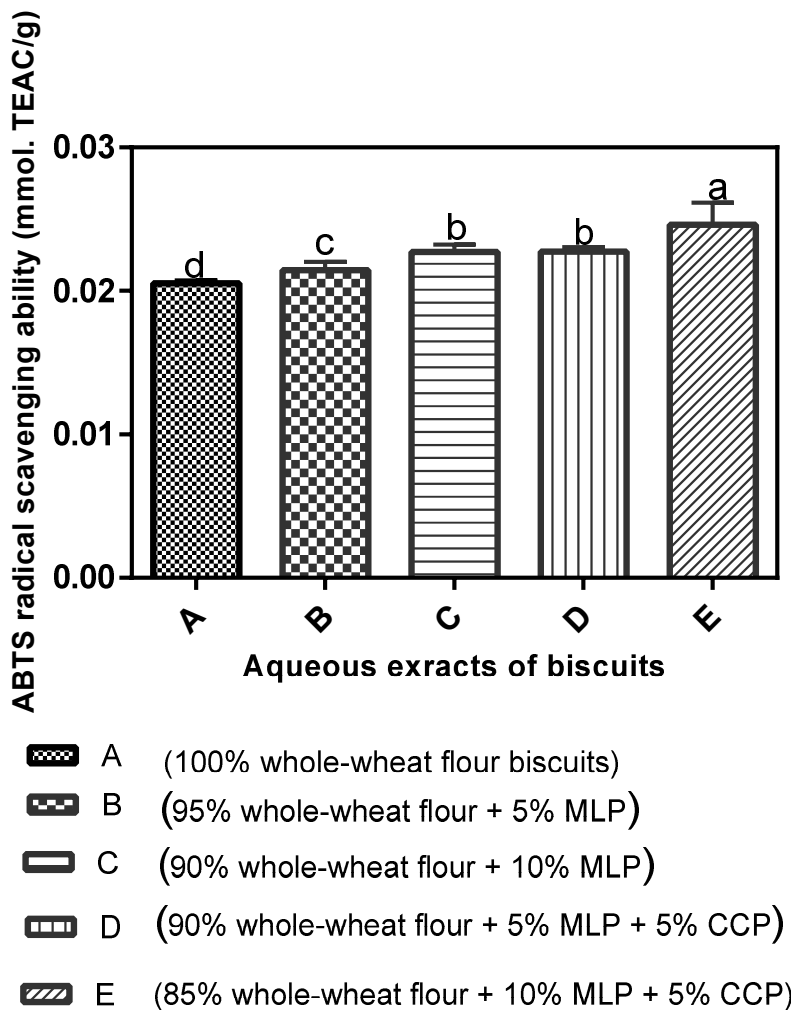


Fig. 2. ABTS radical scavenging activity of aqueous extracts of biscuits incorporated with *Moringa oleifera* leaf and cocoa powder

The reducing properties of antioxidants are generally associated with the presence of reductones, which has antioxidant activity by breaking the free radical chain through electron transfer reactions [50]. In this study, the ability of biscuits' extracts to act as a reducing agent by transforming the Fe⁺³/ferricyanide complex to the ferrous state (Fe⁺²) was measured at 700 nm. An increase in absorbance indicates better reducing power of the biscuits' extracts as shown in Fig. 3. The results show that the reducing power activity of extracts from sample A (control) was

significantly lower (p≤ 0.05) than the supplemented biscuit extracts. The observed trend in reducing power activity of biscuit extracts is consistent with those observed in DPPH (Fig. 1) and ABTS (Fig. 2) radicals scavenging activity, which show a strong (p≤0.05) relationship between the phenolic content (Table 4) and overall antioxidant activities of the biscuit extracts. The correlations between the phenolic contents and overall antioxidant activities of various food samples have been reported [47,51,52].

Table 4. Phenolic distribution of aqueous extracts of biscuits incorporated with *Moringa oleifera* leaves and cocoa powder

Extracts/ Parameters	Total phenols (mg/g GAE)	Total flavonoids (mg/g QE)
A	1.01 ^e ±0.03	0.22 ^e ±0.02
B	2.72 ^d ±0.04	0.52 ^d ±0.02
C	4.99 ^b ±0.02	1.27 ^b ±0.05
D	3.66 ^c ±0.04	1.01 ^c ±0.04
E	5.42 ^a ±0.01	1.39 ^a ±0.02

Values in the same column with different superscript are significantly different (P ≤ 0.05). Means ± standard deviations of triplicate samples. Key: A= 100% whole-wheat flour biscuit; B = 95% whole-wheat flour + 5% MLP; C = 90% whole-wheat flour + 10% MLP; D = 90% whole-wheat flour + 5% MLP + 5% CCP; E = 85% whole-wheat flour + 10% MLP + 5% CCP

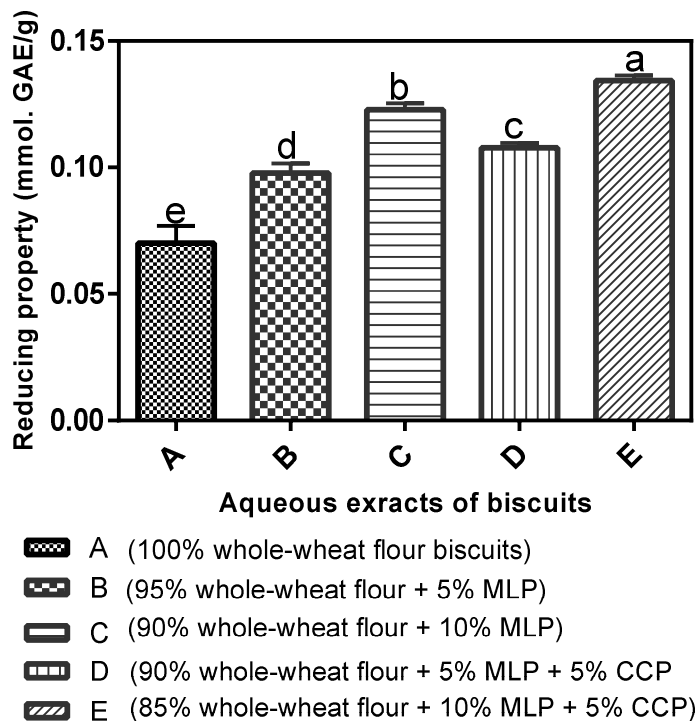


Fig. 3. Ferric reducing antioxidant power of aqueous extracts of biscuits incorporated with *Moringa oleifera* leaf and cocoa powder

4. CONCLUSION

The replacement of whole wheat flour with increasing level of MLP from 0 to 10% decreased the spread ratio of biscuits while addition of 5% CCP to the moringa-supplemented biscuits increased its spread ratio. Sensory evaluation showed that biscuits incorporated with 10% MLP were acceptable and addition of 5% CCP to the MLP biscuit produced biscuits with more acceptable colour and taste. Protein, crude fibre, iron, calcium and antioxidant activity of biscuits increased with increasing amount of MLP and CCP. The results have shown the possibility of utilizing dried *Moringa oleifera* leaves and cocoa powder to improve the nutritional and antioxidant properties of biscuits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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