



E-ISSN: 2278-4136  
P-ISSN: 2349-8234  
[www.phytojournal.com](http://www.phytojournal.com)  
JPP 2021; 10(3): 50-55  
Received: 04-02-2021  
Accepted: 14-03-2021

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## Effects of *Moringa oleifera* leaf powder on some chemical, phytochemical and antioxidant properties of bread: Potential control for NCDs

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DOI: <https://doi.org/10.22271/phyto.2021.v10.i3a.14069>

**Abstract**

Poor diet has been associated with several chronic diseases including obesity, cardiovascular diseases, and type 2 diabetes. Bread is widely consumed even though they are high in carbohydrates and low in essential nutrients. This study evaluated the nutritional, phytochemical and antioxidant properties of bread supplemented with *Moringa oleifera* leaf powder. Bread samples were prepared by supplementing wheat flour with moringa leaf powder at the levels of 3.0, 5.0, 7.0, 10.0 and 15.0% and their nutritional components and *in vitro* antioxidant properties were determined using standard protocols. Results of nutritional quality showed that M10 (10% moringa: 90% flour) bread had enhanced levels of ash (1.84 to 2.47%), protein (8.05 to 14.66%), fat (10.32 to 13.05%) and fiber (0.65 to 1.31%) with reduced moisture (12.09 to 11.45%) and carbohydrate (67.05 to 57.06%) contents, respectively. Phytochemical and antioxidant properties also showed that M10 bread had the highest total phenol (0.5 to 1.24 mg GAE/g extract), flavonoid (1.55 to 9.01 mg QE/g extract), alkaloid (5.39 to 13.48 mg QE/g extract), reducing power potential (0.29 to 3.18 mg AAE/g extract) and DPPH radical scavenging ability (45.13 to 77.65%). While total tannins (0.29 to 0.56 mg TAE/g extract), total saponins (2.27 to 4.52 mg QE/g extract) and FRAP (1.16 to 1.66 mg AAE/g extract) activities were significantly ( $p < 0.05$ ) high in the M7 (7% moringa: 93% flour) bread. The result of this study indicates that supplementation of bread with *Moringa oleifera* leaf powder improved the nutritional and antioxidant properties of bread and can therefore be incorporated as functional food for the control of NCDs and improvement of health status.

**Keywords:** Bread, moringa, composite flour, antioxidant, nutritional composition

**Introduction**

Poor dietary choices are a leading risk factor for illness, disability, and death worldwide [1]. In a study of health effects of dietary risks conducted for the Global Burden of Disease (GBD), results obtained showed that poor diet was responsible for 11 million [95% uncertainty interval (UI) 10–12] deaths and 255 million (234–274) DALYs [2]. Further results revealed the impact of poor diets on death and disease from non-communicable diseases (NCDs) from consumption of major foods and nutrients across 195 countries. Cardiovascular disease was the leading cause of diet related deaths (10 million deaths) and 207 million DALYs, followed by cancers (913 090 deaths and 20 million DALYs) and type 2 diabetes (338 714 deaths and 24 million DALYs). From which more than 5 million (95% UI 5–5) diet-related deaths and 177 million (163–192) diet-related DALYs occurred among adults aged younger than 70 years [2]. Diets rich in plant foods are increasingly recommended to lower the risk of these cardiometabolic diseases [3]. In Nigeria, NCDs are the second largest cause of deaths and there has been small degree of progress on attaining the diet related NCD targets with 13.1% of women (aged 18 years and over) and 4.6% of men still living with obesity. At the same time, diabetes is estimated to affect 6.0% of adult women and 6.3% of adult men [4]. Therefore, the need for an overhaul in the dietary environment to improve health and reduce chronic diseases cannot be overemphasized.

Vegetable intake has been frequently linked with improved gastrointestinal health and decreased risk of some chronic diseases such as heart attack, some types of cancer and diabetes [5]. However, fruits and vegetable intake still fall short of the recommended daily intake (5 A Day) [6] with just 31% of adults, 32% of 65- to 74-year-olds and 8% of teenagers meeting this target. Adults aged 19 to 64 ate a reported average 4.2 portions of fruit and vegetables per day. Older people aged 65 to 74 ate 4.3 portions and teenagers ate just 2.7 portions per day [6].

Reformulation of foods is a strategy that has the potential to introduce macro/micro-nutrients into the diet and promote health. *Moringa Oleifera* (MO) leaves are abundant in healthy antioxidants and bioactive compounds and they have proven to be helpful in numerous chronic conditions, including hypercholesterolemia, high blood pressure, diabetes, insulin resistance, non-alcoholic liver disease, cancer and overall inflammation [7, 8]. Baked foods such as bread, biscuits, buns, donuts, and sausage rolls are basic staple in many homes in Nigeria. Bread is even more staple than rice, cassava products or any of the popular staples perceived as an exclusive preserve of Nigerians' culinary culture. All genders, all ages, all tribes, both the rich and the poor consume these products, and it comes in various sizes, shapes, compositions, and price tags to meet the needs of different categories of consumers. It is a grab and go staple that goes with almost anything, that is why there is virtually no household in Nigeria where not consumed. Bread is produced from simple ingredients and it is one of the most popular cereal-based products in many countries. Due to their simplicity and wide consumption, bread can be enriched and fortified by components that are beneficial to human health. Hence, this study evaluated the effects of moringa oleifera leaves on the chemical, phytochemical, and antioxidant properties of bread.

## Materials and Methods

### Sample collection

The *Moringa oleifera* leaves were collected from Eboakhula area of Ekpoma, Esan-west local government area, Edo state, Nigeria. Identification was done by a plant biologist in the Department of Plant Biology and Biotechnology, Ambrose Alli University, Ekpoma, Nigeria. Commercial wheat flour, sugar, salt, yeast, and bread improver was obtained from a local market.

### Blend formulation

The method described by [9] was adopted in this study. Six different samples of blends were produced and coded as M0, M3, M5, M7, M10 and M15. Sample M0 served as the control and contained 100% wheat. Samples M0, M3, M5, M7, M10 and M15 consisted of wheat/*Moringa oleifera* leaf flours and the other ingredients for bread production.

### Baking process/bread production

The six blends of composite flour were baked into bread using the straight dough method [10]. All dry ingredients were weighed and mixed using machine food processor (Kenwood KM 201, England) for about 10 minutes at low speed to obtain dough. The produced dough was covered with food wrapper to prevent excessive moisture loss and left to rise at room temperature for 60 minutes. Then, the dough was punched and kneads again to release the air inside. The kneaded dough was then transferred into baking pans greased with plasticized fat and covered with greased bread wrapper. The dough was left to ferment for 90 minutes at room temperature in the baking pans. The fermented dough was then allowed to undergo proofing at 40 °C for 90 minutes and then baked at 250 °C for 30 minutes. The bread was cooled to room temperature and used for analysis.

### Sample extractions

Bread samples were sliced (3 cm width and 1 cm thickness) and air-dried for 24 hours. The dried material was blended to obtain powdered bread samples. One gram each of the

powdered samples was weighed and extracted in 100 ml of 80% aqueous methanol for 24 h at 25 °C on an orbital shaker. The extract was further filtered using Whatman filter paper (No. 1) and the filtrate obtained was centrifuged at 3500 rpm for 15 min. Thereafter, the supernatant was collected and used for further studies.

### Determination of proximate composition

The moisture content, ash, crude fibre, crude protein, and fat content of composite bread were determined using the method of AOAC [11]. Total carbohydrate was calculated by the difference.

### Determination of total phenol content

Total phenol content was determined according to the Folin and Ciocalteu's method [12]. Concentrations (0.2 - 1 mg/mL) of gallic acid were prepared in methanol. Then, 0.5 mL of the sample (1 mg/mL) was mixed with 2.5 mL of a ten-fold diluted Folin- Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The mixture was left to stand for 30 min at room temperature then absorbance read at 760 nm. All determinations were performed in triplicates with gallic acid utilized as the reference control [12]. Total phenol content of the extracts was then extrapolated from the standard curve.

### Determination of total flavonoid content

The total flavonoid content was determined using the method of Miliuskas *et al.* [13]. Two (2 ml) of 2% AlCl<sub>3</sub> in ethanol was mixed with 2 ml of varying concentration of the extracts (0.1 – 1.0 mg/ml) in methanol. The absorbance was measured at 420 nm after one hour incubation at room temperature. Similar concentrations of quercetin, the positive control was measured. The total flavonoid content was calculated as mg quercetin equivalent/g of extract. Total flavonoids content of the extracts was then extrapolated from the standard curve.

### Determination of total tannins

Total tannins content was determined by Folin-Denis method [14]. The method is based on the measurement of a blue colour formed by the reduction of phosphotungsto-molybdic acid by tannin-like compounds in alkaline medium. One milliliter of extract (1mg/mL) and standard solution of Tannic acid (10-150 µg/mL) was made up to 7.5mL with distilled water. Then 0.5 mL Folin-Denis reagent and 1mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution were added. The volume was made up to 10 mL with distilled water and absorbance was measured at 700nm. Total tannins content of the extracts was then extrapolated from the standard curve and expressed as mg of Tannic acid equivalent /g of extract.

### Determination of total alkaloids content

Total alkaloid level of the extracts was determined following the method reported by Singh *et al.* [15]. The extract (1 mL) was mixed with 1 mL of FeCl<sub>3</sub> solution (0.025 M FeCl<sub>3</sub> in 0.5 M HCL) and 1 mL of 0.05 M of 1, 10- phenanthroline in ethanol. The reaction mixture was incubated in hot water bath at 70 ± 2 °C for 30 minutes. The absorbance of red coloured complex formed was measured at 510 nm against reagent blank. Alkaloid content of sample was extrapolated from the standard curve and expressed as quinine equivalent in mg/g of sample dry weight.

### Determination of saponins content

Estimation of total saponin content was determined by Makkar *et al.* [16] based on vanillin-sulphuric acid colorimetric

reaction with some modification. About 50µl of bread extract was added with 250µl of distilled water. To this, about 250µl of vanillin reagent, 800mg of vanillin in (10mL of 99.5% ethanol) was added and it was mixed well. This solution was kept in a water bath at 60°C for 10min, it was cooled in ice cold water and the absorbance was read at 544nm. The values were expressed as quinine equivalents (mg QE/g extract) derived from a standard curve.

#### Determination of reducing power potential

The reducing power was determined according to the method described by Lai *et al.* [17]. One milliliter of different concentrations of extracts (0.1-1.0mg/mL) in water was mixed with 2.5mL of 0.2M phosphate buffer, pH 6.6 and 2.5mL of potassium ferricyanide. The mixture was incubated at 50°C for 20mins. Thereafter, 2.5mL of trichloroacetic acid (10%) was added to the mixture to stop the reaction. Then 2.5mL of distilled water and 0.5mL of 0.1% FeCl<sub>3</sub> were added, and the absorbance measured at 700nm. Higher absorbance values indicated higher reducing power. Ascorbic acid served as a positive control.

#### Ferric reducing antioxidant power (FRAP)

The FRAP assay was carried out using a modified method of Benzie and Strain [18]. To 1.5 mL of freshly prepared FRAP solution (25 mL of 300 mM acetate buffer pH 3.6, 2.5 mL of 10mM 2,4,6-tripyridyls-triazine (TPTZ) in 40mM HCl, and 2.5 mL of 20 mM ferric chloride (FeCl<sub>3</sub> · 6H<sub>2</sub>O) solution was added to 1mL of the extracts at concentrations of 100 - 600µM. The reaction mixtures were incubated at 37°C for 30 min and the increase in absorbance at 593nm was measured. FeSO<sub>4</sub> was used for the calibration curve and ascorbic acid served as the positive control. FRAP values (expressed as mg Fe (II)/g of the extract) for the extracts were then extrapolated from the standard curve.

#### Determination of 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity

DPPH is a stable free radical with red color (absorbed at 517nm). If free radicals have been scavenged, DPPH will change its colour to yellow. This assay uses this character to show free radical scavenging activity. Radical scavenging activity was done by a slightly modified method of Brand-Williams *et al.* [19].

The following concentrations of extract were prepared: 0.002, 0.005, 0.01, 0.025, 0.05, 0.075, 0.1, 0.15, and 1mg/mL. Ascorbic acid was used as standard, and the same concentrations were prepared as the test solution. All the solutions were prepared with methanol. 2mL each of the prepared concentrations were placed into test tubes, and 0.5mL of 1mM DPPH solution in methanol was added. The experiments were carried out in triplicates. The test tubes were incubated for 15 minutes at room temperature, and the absorbance read at 517 nm. A blank solution containing the same amount of methanol and DPPH was prepared and the absorbance read. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

#### The radical scavenging activity was calculated using the following formula

DPPH radical scavenging activity (%) =  $[(A_0 - A_1) / (A_0)] \times 100$

Where A<sub>0</sub> was the absorbance of DPPH radical + methanol; A<sub>1</sub> was the absorbance of DPPH radical + sample extract or

standard. The 50% inhibitory concentration value (IC<sub>50</sub>) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radical.

## Results and Discussion

### Proximate analysis of composite bread

The nutritional characteristics of moringa bread are presented in Table (1). The data obtained showed that M10 (10% moringa: 90% flour) composite bread could be considered as good sources of ash, protein, fat, and fiber contents. *Moringa oleifera* leaf addition significantly increased ash from 1.84 in control (100% wheat flour) to 2.47% of M10. Ash content refers to the inorganic residue remaining after either ignition or total oxidation of organic matter in a food sample. The inorganic residue consists primarily of the micronutrients present in the food sample [20]. This significant ( $p < 0.05$ ) increase in ash content could be attributed to the high mineral content of the *Moringa oleifera* leaves. These micronutrients play key roles in glucose metabolism and regulation of insulin resistance. Conversely, there was also an increase in protein content from 8.05 in control to 14.66% of M10. Higher protein content causes flours to have more water absorption capacity and affects other farinograph parameters. Flours with higher protein qualities produce stronger doughs [21]. This significant increase in protein content in composite bread could be attributed to the combined protein content in wheat flours and *Moringa oleifera* leaves. A higher protein in doughs can make it stand rougher handling, improve proof time and temperature, and provide a natural way to make bread last longer. Crude fat content also increased from 10.32 in control to 13.05% of M10. This increase is important as it will help reduce the amount of shortening agent used in baking. There was equally an increase in crude fiber from 0.65 in control to 1.31% in M10 bread, respectively. Dietary fibers can be defined as plant carbohydrates and lignins which cannot be digested and absorbed in small intestine by the conventional digestive enzymes. Dietary fibers aid in maintaining good health and combat various diseases like obesity, diabetes, dyslipidemia, hypertension, colon cancer [22]. Therefore, an increase in fiber will improve metabolism and promote a healthy digestive system. Dachana *et al.* [23] studied the effect of dried *Moringa (Moringa oleifera)* lam) leaves at 0, 5, 10 and 15% on rheological, microstructural, nutritional, textural, and organoleptic characteristics of cookies. They observed that the addition of *Moringa* leaf powder increased ash (0.9 to 1.3, 1.5 and 1.8), fat (14.0 to 14.2, 14.4 and 14.6), protein (8.8 to 10.0, 11.6 and 12.8) and fiber (2.4 to 3.4, 4.3 and 5.3) contents with increasing levels of supplementation. However, moisture (12.09 to 11.45%) and carbohydrate (67.05 to 57.06%) contents decreased significantly ( $p < 0.05$ ) in M10. High moisture content increases microbial activity which makes a product go bad during storage. The decrease in moisture content may be due to the high phenolics content and this will significantly delay deterioration in bread and make it last longer. Similarly, the decrease observed in the carbohydrate content in composite bread may be because of the increase in other parameters examined and combined effect of *Moringa oleifera* leaves. These results agree with that of Summaya *et al.* [24]. In their study, significant improvement in nutritional composition was recorded in wheat – oat composite bread when supplemented with *moringa oleifera* leaf powder. El-Gammal *et al.* [25] also reported similar results in their study of the effect of *Moringa* leaves powder (*Moringa oleifera*) on some chemical and physical properties of pan bread. Addition of *Moringa* leaves

powder to pan bread raised the protein content up to 21.85%, ash (5.21%) and carbohydrates content decreased and reached 59.34%. Treated pan bread with 10% Moringa leaves powder had higher amount of Magnesium (Mg), Calcium (Ca) and Iron (Fe) compared with control sample (from 25.50 to 102.56 mg/100g, 12.85 to 205.60 mg/100g and 4.54 to 12.56 mg/100g, respectively). Sengev *et al.* [26] also studied effect of *Moringa oleifera* leaf powder supplementation at 0%, 1%,

2%, 3%, 4% and 5% on some quality characteristics of wheat bread. *Moringa* leaf powder addition significantly ( $p < 0.05$ ) increased the fiber (2.10% to 3.28%), ash (1.10% to 1.65%), protein (9.07% to 13.97%), and ether extract (1.51% to 2.59%), while decreasing moisture content (35.20% to 27.65%). Whereas carbohydrate content of bread with 5% moringa leaf powder decreased to 50.08%.

**Table 1:** Effect of *Moringa oleifera* leaves supplementation on proximate composition of composite bread.

Samples	Moisture	Ash	% Composition			
			Protein	Fat	Fiber	Carbohydrate
M 0	12.09± 0.11 <sup>c</sup>	1.84±0.02 <sup>b</sup>	8.05±0.01 <sup>a</sup>	10.32±0.06 <sup>a</sup>	0.65±0.02 <sup>a</sup>	67.05±0.16 <sup>c</sup>
M 3	11.75±0.15 <sup>b</sup>	0.57±0.02 <sup>a</sup>	10.84±0.14 <sup>b</sup>	16.85±0.11 <sup>d</sup>	0.74±0.03 <sup>b</sup>	59.25±0.29 <sup>d</sup>
M 5	13.01±0.06 <sup>d</sup>	1.79±0.01 <sup>b</sup>	11.49±0.46 <sup>c</sup>	16.64±0.07 <sup>d</sup>	0.77±0.01 <sup>b</sup>	56.30±0.36 <sup>b</sup>
M 7	8.92±0.10 <sup>a</sup>	1.83±0.05 <sup>b</sup>	15.66±0.02 <sup>d</sup>	14.18±0.05 <sup>c</sup>	1.32±0.05 <sup>c</sup>	58.09±0.06 <sup>d</sup>
M 10	11.45±0.04 <sup>b</sup>	2.47±0.02 <sup>c</sup>	14.66±0.12 <sup>c</sup>	13.05±0.03 <sup>b</sup>	1.31±0.02 <sup>c</sup>	57.06±0.13 <sup>c</sup>
M 15	13.63±0.03 <sup>d</sup>	2.43±0.05 <sup>c</sup>	16.97±0.08 <sup>f</sup>	14.24±0.08 <sup>c</sup>	1.36±0.02 <sup>c</sup>	51.37±0.08 <sup>a</sup>

Data are presented as Mean ± Standard error of mean. Values in the same column with different alphabetical superscripts are considered statistically significant ( $p < 0.05$ ). M0=100% wheat flour (control); M3= 3% Moringa oleifera leaf flour: 97% wheat flour; M5= 5% Moringa oleifera leaf flour: 95% wheat flour; M7 = 7% Moringa oleifera leaf flour: 93% wheat flour; M10 = 10% Moringa oleifera leaf flour: 90% wheat flour; and M15 =15% Moringa oleifera leaf flour: 85% wheat flour.

### Effect of *Moringa oleifera* leaves supplementation on phytochemical properties of bread

The quantitative phytochemical constituents of composite bread are shown in Table 2. The results showed a significant ( $p < 0.05$ ) steady increase in value as the concentration of *Moringa* leaves to flour increased with M10 (10% moringa: 90% flour) composite bread having high values in phenol (0.5 to 1.24 mg GAE /g extract), flavonoid (1.55 to 9.01 mg QE/g extract) and alkaloid (5.39 to 13.48 mg QE/g extract) contents, respectively. Phenolics and flavonoids are natural compounds found in many cereal grains. A review published in 2012 found growing consensus for the hypothesis that the specific intake of food and drink containing relatively high concentrations of flavonoids may play a meaningful role in reducing the risk of cardiovascular disease (CVD). Phenolics possess several health properties that confer benefits like antioxidative, anti-inflammatory, antimutagenic and anticarcinogenic properties. It also retains flavor, taste, color and prevents them from oxidative deterioration [27]. Nouman, *et al.* [28] also reported that analysis of hydro-methanolic extracts of *Moringa* leaves revealed a wide range of phenolic compounds. Many authors also reported that the leaves of *M. oleifera* fresh or dried are renowned to be incredible source of antioxidants and they have significantly higher antioxidant content comparing to fruits such as strawberries known for high antioxidant contents [29, 30, 31] This result is consistent

with that of Bourekoua *et al.* [32] in their study of the evaluation of physical, sensorial, and antioxidant properties of gluten-free bread enriched with *Moringa oleifera* leaf powder (MOLP), the addition of MOLP significantly enhanced the total phenol content of gluten-free products from 0.88 mg GAE/g dw for control bread to 2.39 mg GAE/g dw for bread with 10% MOLP.

However, the bread made with 7% *Moringa* leaf powder was highest in total tannins (0.29 to 0.56 mg TAE /g extract) and saponins (2.27 to 4.52 mg QE/g extract) contents, respectively. The presence in moderate number of tannins is important for its pharmaceuticals and therapeutic significance. Saponins are a class of chemical compound found in many plant species especially in legume plants [33]. It poses a wide range of bioactive activities. In general, all composite bread supplementation recorded enhanced phytochemical constituents when compared to control bread (made with 100% flour). These results agree with that reported by Mushtaq *et al.* [34] in their study of characterization of *Moringa oleifera* leaves and its utilization at 5, 10, 15 and 20% as value added ingredient in unleavened flat bread (chapatti). Total phenol (0.75 to 2.30, 4.82, 6.41 and 8.38) and total flavonoid (0.38 to 1.36, 2.14, 2.98 and 3.66) contents significantly increased with increasing levels of *Moringa* leaf powder.

**Table 2:** Effect of *Moringa oleifera* leaves supplementation on total phenols, total flavonoids, total tannins, total alkaloids and total saponins content of composite bread

Samples	Phenols (mg GAE/g extract)	Flavonoids (mg QE/g extract)	Tannins (mg TAE/g extract)	Alkaloids (mg QE/g extract)	Saponins (mg QE/g extract)
M 0	0.50 <sup>a</sup> ± 0.008	1.55 <sup>a</sup> ± 0.004	0.29 <sup>a</sup> ± 0.026	5.39 <sup>a</sup> ± 0.189	2.27 <sup>a</sup> ± 0.032
M 3	0.73 <sup>b</sup> ± 0.012	4.21 <sup>c</sup> ± 0.023	0.45 <sup>b</sup> ± 0.006	10.62 <sup>b</sup> ± 0.06	3.76 <sup>bc</sup> ± 0.2
M 5	0.86 <sup>bc</sup> ± 0.013	7.43 <sup>d</sup> ± 0.041	0.51 <sup>bc</sup> ± 0.016	11.36 <sup>c</sup> ± 0.16	4.19 <sup>cd</sup> ± 0.007
M 7	1.04 <sup>d</sup> ± 0.038	7.91 <sup>d</sup> ± 0.069	0.56 <sup>c</sup> ± 0.015	12.82 <sup>d</sup> ± 0.16	4.52 <sup>d</sup> ± 0.034
M 10	1.24 <sup>e</sup> ± 0.037	9.01 <sup>e</sup> ± 0.247	0.49 <sup>bc</sup> ± 0.005	13.48 <sup>d</sup> ± 0.06	3.48 <sup>b</sup> ± 0.189
M 15	0.82 <sup>b</sup> ± 0.008	3.36 <sup>b</sup> ± 0.072	0.55 <sup>c</sup> ± 0.002	12.28 <sup>e</sup> ± 0.06	4.10 <sup>cd</sup> ± 0.003

Data are presented as Mean ± Standard error of mean. Values in the same column with different alphabetical superscripts are considered statistically significant ( $p < 0.05$ ). M0=100% wheat flour (control); M3= 3% Moringa oleifera leaf flour: 97% wheat flour; M5= 5% Moringa oleifera leaf flour: 95% wheat flour; M7 = 7% Moringa oleifera leaf flour: 93% wheat flour; M10 = 10% Moringa oleifera leaf flour: 90% wheat flour; and M15 =15% Moringa oleifera leaf flour: 85% wheat flour.

### Effect of *Moringa oleifera* leaf supplementation on antioxidant properties of composite bread

The results for the reducing power potential, FRAP and DPPH scavenging abilities is presented in Table 3. There was steady increase in reducing power potential as the concentration of *Moringa* leaves to flour increased except for 15% composite bread, where a slight decrease was observed. M10 bread was significantly ( $p < 0.05$ ) high in reducing power potential ( $3.18 \pm 0.065$  mg AAE / g extract) when compared to other groups and the control (100% flour bread;  $0.29 \pm 0.034$  mg AAE / g extract). Bourekoua *et al.* [32] reported enhanced DPPH scavenging activities for 7.5% and 10% of *Moringa* leaf powder bread. There was also significant ( $p < 0.05$ ) increase in FRAP activities in all supplemented bread compared to the control bread. However, this increase in FRAP activities among the groups is not significantly different from each other. The highest FRAP

(1.16 to 1.66 mg AAE / g extract) activity was observed in 7% composite bread. Equally, the result for DPPH scavenging ability showed that composite bread exhibited enhanced antioxidant and free radical inhibiting activity. Just like most of the phytochemicals, highest activity was found in the 10% composite bread (45.13 to 77.65%). Total antioxidant is measured in terms of radical scavenging activity of free radicals like DDPH radical and by FRAP assay. The enhanced levels recorded in composite bread is due to naturally occurring antioxidants present in the *Moringa* leaves. These results as regards FRAP and DPPH are consistent with that reported by Mushtaq *et al.* [34] where they recorded wide variation in DPPH scavenging activity of Chapattis sample. Addition of *Moringa oleifera* leaves powder increased antioxidant percentage with maximum value observed in 20% (64.32% to 80.52%).

**Table 3:** Antioxidant activities of composite bread supplemented with *Moringa oleifera* leaf powder

Samples	Reducing power (mg AAE/g extract)	FRAP (mg AAE / g extract)	DPPH (% Inhibition)
M 0	$0.29^a \pm 0.034$	$1.16^a \pm 0.027$	$45.13^a \pm 0.59$
M 3	$1.41^b \pm 0.007$	$1.57^b \pm 0.025$	$61.36^b \pm 0.06$
M 5	$1.75^c \pm 0.028$	$1.62^c \pm 0.013$	$63.42^b \pm 0.11$
M 7	$2.44^d \pm 0.023$	$1.66^c \pm 0.002$	$69.55^c \pm 0.09$
M 10	$3.18^e \pm 0.065$	$1.63^c \pm 0.007$	$77.65^d \pm 0.03$
M 15	$2.78^e \pm 0.009$	$1.60^c \pm 0.005$	$73.15^c \pm 0.34$
Trolox	-	-	$93.03^f \pm 1.03$

Data are presented as Mean  $\pm$  Standard error of mean. Values in the same column with different alphabetical superscripts are considered statistically significant ( $p < 0.05$ ). M0=100% wheat flour (control); M3= 3% *Moringa oleifera* leave flour: 97% wheat flour; M5= 5% *Moringa oleifera* leave flour: 95% wheat flour; M7 = 7% *Moringa oleifera* leave flour: 93% wheat flour; M10 = 10% *Moringa oleifera* leave flour: 90% wheat flour; and M15 =15% *Moringa oleifera* leave flour: 85% wheat flour.

### Conclusion

Bread supplemented with 7% and 10% moringa oleifera leaf powder significantly improved on the nutritional composition (fibre, protein, crude fat and minerals) and decreased carbohydrate and moisture content which may likely confer an extended shelf life to the bread. Supplementation also increased phytochemical and antioxidant activities of obtained bread. It can be concluded that from this present study the use of the formulated composite flour can be considered in the preparation of bread enriched with physiological-functional and nutritive properties and can therefore be suggested for incorporation as functional foods for the control and prevention of some diet related non-communicable diseases.

### Acknowledgement

We want to appreciate the laboratory technicians Mr Nasiru Shuaibu and Mr Isaac Samuel for their time and availability during this study.

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