



Research article

Effect of roasting on the chemical composition, functional characterisation and antioxidant activities of three varieties of marble vine (*Dioclea reflexa*): An underutilised plantMary A. Ajatta^{a,*}, Stephen A. Akinola^b, Oluwatooyin F. Osundahunsi^c, Olufumilayo S. Omoba^c^a Department of Food Science and Technology, Bamidele Olumilua University of Education, Science and Technology, Ikere-Ekiti, Ekiti State, Nigeria^b Department of Microbiology, North-West University Mafikeng Campus, Mmabatho, 2735, South Africa^c Department of Food Science and Technology, Federal University of Technology, Akure, Ondo State, 340001, Nigeria

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ABSTRACT

Marble vine (*Dioclea reflexa*) seeds were roasted using the conditions in runs generated from Response Surface Methodology with temperature ranging from 110 to 200 °C and time (10–40 min). Proximate composition, antioxidant activities (DPPH, ABTS, FRAP, metal chelation OH and Lipid peroxidation) and Fourier Transform Infrared Spectroscopy (FTIR) were carried out on unroasted and roasted flours. Roasting increased the crude fibre content (2.74–5.08 %) of black variety compared to others. However, a slight denaturation of protein was observed when compared to unroasted samples. A significant increase in all the antioxidant activities compared to the control was also observed compared to unroasted flours. The FTIR showed functional groups such as ketones, aldehydes and carbonyl group upon roasting. Roasting temperature at (110 °C) had more effect than roasting time (10, 25 and 40 min). Hence, roasting at 110 °C could enable the release of food nutrients and improve the functionality of marble vine seed flour.

1. Introduction

The awareness of the nutrients and functional benefits associated with foods such as vegetables, fruits, grains, legumes, nuts and more importantly, the dietary phytochemicals have led to an increase in their consumption [1]. Aside this, the rising global population had led to an increase in the requirements for new plant materials that could serve as food [2]. Most population in the developing nations is a victim of malnutrition due to poor access to high quality protein. In Nigeria, legumes such as bean, cowpea, and groundnut are widely consumed to compliment low and expensive animal sources of protein. Thus, the need to utilise plant source of protein with a high nutrient composition to ameliorate malnutrition and possibly serve as functional foods. Hence, the search for plant-based food with an excellent protein source and antioxidant that is comparable to soybean have led to the discovery of marble vine (*Dioclea reflexa*) seed.

Marble vine (*Dioclea reflexa*) is an underutilised plant seed that belongs to the family Leguminosae and subfamily Papilionoidea [3]. Previous studies on *D. reflexa* showed its remarkable medicinal properties such as antioxidant and anti-inflammatory activities, and prophylaxis

against diseases [4]. The seed of *D. reflexa* is ground into powder and used as soup thickener in the Eastern part of Nigeria. There are three known variants of the seed that thus have the potential to be used in functional food formulations due to its high protein and phytochemical contents [4]. The protein content of marble vine seed ranged from 26% to 33 % [5] with limiting amino acids such as lysine and threonine when compared with pigeon pea, cowpea and soybean [6]. Ajatta et al. [4] reported that three varieties of marble vine seeds are rich in dietary phenolic compounds which can help in the prevention and management of degenerative diseases such as cancer, hypertension, diabetes and cancer.

Roasting have been reported to improve the nutritional quality of leguminous seeds [4]. It is one of the most common processing methods used in Africa and this helps to improve the functional properties of food products [7]. The application of dry heat to food facilitate browning reaction thereby improving the nutritional property of leguminous seeds [8]. Although heat treatment has been reported to boost the antioxidant property of foods [9, 10], it could cause significant changes to product quality when heated at high temperature. Roasting is desirable and significant to improve colour, flavour, crispiness, and crunchy texture in

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products [11, 12]. In recent studies, phenolic compounds in foods have been reported to have antioxidant properties and have gained more attention as valuable food ingredients [13]. Previous studies also showed that temperature and duration of thermal processing strongly results in physical and chemical changes [14]. Katsube et al. [15] reported that polyphenolic compounds are highly thermolabile and can easily undergo degradation at high temperatures.

In Nigeria, processing methods such as roasting and boiling of *D. reflexa* have been used traditionally for food processing, however the effect of roasting variables on nutrient and antioxidant properties is yet to be reported. Several studies have reported the advantage of roasting on nutrient quality of legumes such as cashew nuts [1], hazelnut [12] and sesame [2]. Based on the advantages that roasting has on nutrient and antioxidant properties of food, it is imperative to investigate the effect of roasting conditions on *D. reflexa* seeds to enhance its application in food process and formulation. This study reports for the first time the effect of roasting time and temperature on the nutritional and antioxidant properties of *D. reflexa* seeds.

2. Materials and method

2.1. Chemicals and reagents

Three varieties of marble vine (*Dioclea reflexa*) seeds (black, dark brown and light) were obtained from a local farm in Akure, Ondo state, Nigeria. The seeds were distinguished and authenticated at the Department of Crop, Soil and Pest Management at the Federal University of Technology Akure. All reagents used in the study were of analytical grade.

2.2. Sample preparations

Three variant seeds of marble vine (*Dioclea reflexa*) were, sorted, washed with water containing sodium hypochlorite to remove foreign matters and dried at room temperature. Same seed samples were used in Ajatta et al. [4] on "effect of roasting on the phytochemical properties of three varieties of marble vine (*Dioclea reflexa*) using response surface methodology". The seeds (black, dark brown and light) were roasted in forced hot-air oven at the range of (110–220 °C) for 10–40 min using runs obtained from the design expert software as shown in Table 1. After roasting the samples were cooled in a desiccator and reduced into fine particles (1–3 mm) using attrition mill (model MS-223, Fritsch, Taipei, Taiwan). Samples were sieved in a 250 µm screen and the resulting seed flour was stored in a plastic container and seeds were determined in triplicate for proximate analyses.

Table 1. Generated runs from central composite design for optimisation.

Runs	Roasting temperature (°C)	Roasting time (min)
1	110	25
2	110	10
3	110	40
4	155	25
5	155	40
6	200	10
7	200	40
8	200	25
9	155	25
10	155	25
11	155	25
12	155	25
13	155	10

2.3. Sample extraction

The roasted flour sample was soaked with 80% Methanol at a ratio 1:5 (weight: volume) for 8 h. The mixture was sonicated for 30 min at 40 °C temperature and were filtered through a Whatman filter No. 1. Re-extraction was repeated on the residue to ensure maximum extraction. The resultant solution was concentrated by evaporating to dryness at 50 °C under reduced pressure by vacuum rotary evaporator to yield a crude extract, freeze-dried and the dried extracts were stored at 4 °C for further analysis.

2.4. Design of experiment

The central composite design of RSM (Design-Expert version 8.3.0.1, Stat-Ease, Minneapolis, MN, USA) was used to obtain the experimental runs. The lower and upper boundaries for the roasting temperature and time were 110–200 °C and 10–40 min respectively as described by Ajatta et al. [4]. The impact of the independent variables (temperature and time) on responses (proximate and antioxidant properties) were evaluated in three varieties of *D. reflexa* seed flours and determined in triplicate determinations. The second order polynomial Eq. (1) was used to determine the effect of independent variables (process variables) on the responses as reported in our previous study [4].

$$Y = \beta_0 + \sum 2i = 1biXi + \sum 2i = 1biX2 \sum 2i = 1biX \quad (1)$$

2.5. Determination of chemical composition of marble vine (*Dioclea reflexa*) seed

2.5.1. Proximate composition

The crude protein, crude fat, moisture and ash content of the seed flours were determined according to the methods described [16]. The proximate composition of *Dioclea reflexa* seeds were determined in triplicate determinations. The total carbohydrate content was calculated by difference from 100.

2.6. Determination of the antioxidant's properties of marble vine seed varieties

2.6.1. ABTS scavenging ability

The method described by Re et al [17] was used to evaluate the 2, 2-azino-bis -3-ethylbenthiazoline-6-sulphonic acid (ABTS) scavenging ability of the *Dioclea reflexa* seed extract. Seven (7) mM of ABTS aqueous solution with 2.45 mM K₂S₂O₈ was used to prepare ABTS stock solution and was kept in the dark for 16 h. The absorbance of the ABTS stock solution at 734 nm was adjusted to 0.70 ± 0.02 using ethanol. To 0.2 ml of the aliquot seed extracts dilution, the ABTS solution was added and was incubated for 15 min at room temperature. The scavenging ability of the seed extracts was read at 734 nm and the Trolox equivalent of the scavenging capacity.

2.6.2. Determination of ferric reducing antioxidant potential (FRAP)

The FRAP assay measures the reduction of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-azoniacyclopenta-1,4-diene chloride to blue ferrous complex under acidic condition (pH 3.6). The FRAP unit is the reduction of one mole of Fe (III) to Fe (II). The FRAP assay was determined based on the method described by Wong et al. [18]. Briefly, 200 µl of sample extract was added to 3 ml of FRAP reagent that was prepared with a mixture of 300 mM of sodium acetate buffer (pH 3.6), 10 mM of 2,4,6-Tri (2-pyridyl)-s-triazine (TPTZ) solution, and 20 mM of FeCl₆H₂O at the ratio 10:1:1 (v:v). The reaction mixture was incubated for 30 min at 37 °C. The absorbance was measured at 593 nm, and the percentage of inhibition (antioxidant) was calculated as described in Eq. (2).

$$\% \text{ Inhibition} = \frac{A_{593\text{nm}} \text{ of Sample} - A_{593\text{nm}} \text{ of Control}}{A_{593\text{nm}} \text{ of Sample}} \times 100.2.8 \quad (2)$$

where $A_{593\text{nm}}$ refers to the absorbance measured at 593 nm wavelength.

2.6.3. Determination of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging ability

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) scavenging ability of the seed extract was determined according to the method described by Gyamfi et al. [19]. One millilitre of 0.4 mM methanolic solution of DPPH was mixed with 1 ml aliquot of seed extract. The mixture was left in the dark for 30 min at room temperature. The absorbance of the mixture was then measured at a wavelength of 516 nm (Genesys 10 UV/visible scanning UV-Spectrophotometer, Thermo Scientific, Germany).

2.6.4. Fe^{2+} chelation assay

The Fe^{2+} chelating ability of the seed extracts were determined using a modified method of Minotti and Aust [20] as modified by Oboh and Ademosun [21]. Aliquot (200 mL) of freshly prepared 500 μM FeSO_4 was added to a reaction mixture containing 218 μL saline, 168 μL of 0.1 M Tris-HCl (pH 7.4), and 25 μL of the seed extracts. The reaction mixture was incubated for 5 min, and 13 μL of 0.25% 1, 10-phenanthroline solution was added. Absorbance was measured at a wavelength of 510 nm using a UV-spectrophotometer.

2.6.5. Inhibition of the Fenton reaction (Degradation of deoxyribose)

The method of Halliwell and Gutteridge [22] was used to evaluate the ability of the methanolic extracts to prevent $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ induced decomposition of deoxyribose. Amount (0–100 μL) of the seed extract was added to a reaction mixture containing 40 μL of 500 μM FeSO_4 , 120 μL of 20 mM deoxyribose, 400 μL of 0.1 M phosphate buffer, and the volume were made up to 800 μL with distilled water. The reaction mixture was incubated at 37 °C for 30 min after which the reaction process was stopped by the addition of 0.5 mL of 2.8% trichloroacetic acid (TCA) followed by addition of 0.4 mL of 0.6% thiobarbituric acid (TBA) solution. The tubes containing the reaction mixture were incubated in boiling water for 20 min and the absorbance was measured at 532 nm in a UV-spectrophotometer.

2.7. FTIR spectroscopy

The FTIR spectra of raw and roasted seed (110 °C, 25 min) of *Dioclea reflexa* were determined using FTIR spectrometer system (Cary 630 FTIR, Agilent Technologies, USA) at room temperature and spectra were acquired in the range of 4000–650 cm^{-1} . The results were evaluated using Resolution Pro software version 2.5.5 (Agilent Technologies, USA).

2.8. Statistical analysis

Data were analysed statistically using response surface methodology (Design Expert software version 8.3.0.1 version). In order to correlate the response variable to the independent variables, multiple regressions were used to fit the coefficient of the polynomial model of the response. The quality of the fit of the model was evaluated using analysis of variance (ANOVA). Results were analysed using SPSS statistical package (Version 17.0) through the analysis of variance (ANOVA), Duncan New multiple range test was used to determine significant differences in mean of the samples at $p < 0.05$. All values were expressed as mean \pm SD.

3. Result and discussion

3.1. Effect of roasting on the proximate composition of marble vine seed varieties

The effect of roasting temperature and time on the proximate composition of marble vine (*Dioclea reflexa*) seed flour is presented in Table 2. Roasting temperature and time were observed to have a significant effect on the proximate composition of the seed varieties. Protein content ranged from 17.50 ± 0.02 to $22.79 \pm 0.02\%$ in the black variety, dark brown (16.47 ± 0.18 – $29.46 \pm 0.13\%$) and light brown (16.84 ± 0.13 – $24.32 \pm 0.04\%$) variety. For the black and dark brown seed varieties, the optimal roasting condition was at 110 °C for 10 min. There was no significant difference in the roasting time (10, 25 and 40 min) at roasting temperature of 110 °C in the light brown variety. After roasting the highest protein content ($25.54 \pm 0.13\%$) was obtained in the dark brown variety and the lowest ($20.22 \pm 0.20\%$) was in the light brown variety. As the roasting temperature and time increases, a significant decrease in the protein content of the seed flour was observed compared to the control (unroasted seed flour). The observed decrease in protein content at increasing roasting temperature and time could be attributed to denaturation of protein as a result of the heat applied and polymerisation of amino acid during the processing operation. Similar findings were observed by Santos et al. [23] that protein content of roasted walnut flour decreased as roasting conditions increases [2].

The moisture content ranged from 4.25 ± 0.24 to $9.58 \pm 0.07\%$ in the seed of black variety, dark brown (4.40 ± 0.01 – $10.92 \pm 0.01\%$) and light brown (5.19 ± 0.03 – $10.13 \pm 0.01\%$) variety. The best minimum roasting conditions for the three varieties was at 200 °C with variation in roasting time (10, 40 and 25 min) for black, dark brown and light brown seed varieties respectively. There was a reduction in the moisture content from (9.58 ± 0.07 – $4.25 \pm 0.24\%$), (10.92 ± 0.01 – $4.40 \pm 0.01\%$) and (10.13 ± 0.01 – $5.19 \pm 0.03\%$) in the black, dark brown and light brown *D. reflexa* seed flour as roasting temperature and time increased. Similar observation was reported by Lawal et al [2]. that M.C of toasted Sesame seed flour reduced from 3.84 to 1.33% at an increasing toasting time (10–30 min). The low moisture content of the seed flour can be associated with evaporation of moisture from the seed as a result of heat applied which could enhance the keeping quality and shelf of the seed flour. The low moisture will aid the inhibition of microbial proliferation due to lower level of water activity and moisture available for biochemical reactions. Roasting temperature and time favour the moisture content of the seed varieties, the low moisture content obtained was lower than the control.

The crude fibre and ash content of the seed flour were higher compared to the control sample. The crude fibre content of the flour ranged from 2.74 ± 0.02 to $5.08 \pm 0.03\%$, 2.60 ± 0.05 to $4.08 \pm 0.02 \pm 0.05\%$ and 1.52 ± 0.05 to $3.72 \pm 0.14\%$ for black, dark brown and light brown varieties respectively. The best roasting condition for the highest crude fibre content of the seed varieties was at 110 °C for 25 min. However, the black varieties had the highest value ($5.08 \pm 0.03\%$) at 110 °C, 25 min, compared to the values 4.08 ± 0.02 and $3.72 \pm 0.14\%$ for dark brown and light brown varieties respectively. The high fibre content observed could help improve the nutritional profile of the tested seed sample. High fibre content in the diet could help improve digestion of food, control serum lipids and glycemic index, thereby reducing the risk associated with high cholesterol and chronic heart diseases [24]. The result obtained support the observation of Ahmed et al. [25] who reported an increase in the crude fibre content in roasted Sesame seed flour. The result obtained is in agreement with the reports that roasting increases the crude fibre and ash content of food samples [2, 26].

The fat content of the samples ranged from 9.23 ± 0.07 to $12.84 \pm 0.02\%$; 8.28 ± 0.05 to $12.24 \pm 0.02\%$ and 9.12 ± 0.02 to $10.42 \pm 0.11\%$ for black, dark brown and light brown varieties respectively. The black

Table 2. Proximate composition of three varieties of marble vine (*Dioclea reflexa*) seed.

Run	Independent variable	Dependent variables (experimental data)															
		Black variety					Dark brown variety					Light brown variety					
		Temp x ₁ (°C)	Time x ₂ (min)	M.C	Fibre	Ash	Fat	Protein	M.C	Fibre	Ash	Fat	Protein	M.C	Fibre	Ash	Fat
1	110	25	20.70 ^b ± 0.33	5.34 ^c ± 0.26	5.08 ^b ± 0.03	4.02 ^b ± 0.01	11.82 ^b ± 0.03	20.58 ^d ± 0.02	6.42 ^b ± 0.01	4.08 ^a ± 0.02	9.63 ^c ± 0.15	20.23 ^b ± 0.23	8.82 ^d ± 0.04	3.72 ^a ± 0.14	2.82 ^a ± 0.03	9.42 ^c ± 0.02	
2	110	10	20.76 ^b ± 0.04	6.16 ^b ± 0.12	4.99 ^b ± 0.02	3.78 ^c ± 0.01	12.84 ^b ± 0.02	25.54 ^b ± 0.13	7.55 ^c ± 0.02	3.77 ^c ± 0.18	9.60 ^c ± 0.06	20.22 ^b ± 0.20	9.63 ^b ± 0.02	3.52 ^a ± 0.03	1.45 ^a ± 0.02	9.89 ^c ± 0.02	
3	110	40	20.04 ^c ± 0.05	5.85 ^b ± 0.02	5.02 ^c ± 0.02	4.23 ^c ± 0.03	11.73 ^b ± 0.03	21.43 ^c ± 0.11	7.73 ^b ± 0.11	3.79 ^c ± 0.01	2.77 ^c ± 0.00	20.20 ^b ± 0.10	9.37 ^c ± 0.01	3.23 ^a ± 0.03	2.63 ^b ± 0.04	9.90 ^c ± 0.01	
4	155	25	18.69 ^b ± 0.03	5.24 ^f ± 0.01	2.88 ^f ± 0.03	3.43 ^d ± 0.02	10.34 ^c ± 0.11	20.18 ^c ± 0.01	7.24 ^d ± 0.02	3.72 ^{cd} ± 0.14	2.31 ^b ± 0.54	19.24 ^c ± 0.52	6.62 ^b ± 0.17	3.20 ^d ± 0.05	1.63 ^b ± 0.02	9.93 ^b ± 0.19	
5	155	40	18.63 ^c ± 0.34	5.54 ^d ± 0.17	3.45 ^e ± 0.11	3.02 ^f ± 0.07	10.43 ^d ± 0.05	19.20 ^b ± 0.21	4.65 ^e ± 0.10	3.63 ^d ± 0.15	3.41 ^{cd} ± 0.03	19.18 ^c ± 0.22	8.52 ^c ± 0.23	1.52 ^d ± 0.05	1.62 ^d ± 0.18	9.54 ^b ± 0.03	
6	200	10	17.85 ^b ± 1.05	4.25 ⁱ ± 0.24	3.94 ^e ± 0.23	3.03 ^f ± 0.53	10.83 ^c ± 0.06	17.33 ^c ± 0.05	5.72 ^b ± 0.08	2.68 ^f ± 0.05	2.33 ^b ± 0.19	18.38 ^b ± 0.03	5.73 ^b ± 0.20	2.91 ^f ± 0.02	1.83 ^b ± 0.14	9.12 ^b ± 0.02	
7	200	40	17.50 ^b ± 0.02	4.58 ^h ± 0.04	3.63 ^{dv} ± 0.25	2.47 ^g ± 0.05	10.81 ^c ± 0.02	16.47 ^b ± 0.18	4.40 ^m ± 0.01	3.49 ^e ± 0.13	2.82 ^f ± 0.04	16.84 ^b ± 0.13	5.22 ^b ± 0.35	1.84 ^b ± 0.06	1.42 ^b ± 0.03	9.23 ^b ± 0.06	
8	200	25	17.63 ^b ± 0.16	5.53 ^d ± 0.03	3.90 ^c ± 0.43	2.57 ^g ± 0.14	10.43 ^d ± 0.10	16.82 ^b ± 0.04	5.31 ^l ± 0.00	3.43 ^f ± 0.01	3.11 ^e ± 0.11	10.02 ^d ± 0.08	5.19 ^b ± 0.03	3.18 ^c ± 0.03	1.72 ^b ± 0.05	10.23 ^c ± 0.04	
9	155	25	19.48 ^d ± 0.02	5.28 ^f ± 0.02	3.73 ^d ± 0.01	3.27 ^g ± 0.19	09.23 ^b ± 0.07	19.44 ^c ± 0.05	6.53 ^l ± 0.34	3.42 ^f ± 0.04	2.84 ^f ± 0.03	18.48 ^b ± 0.03	7.30 ^b ± 0.01	2.42 ^g ± 0.23	2.05 ^e ± 0.03	10.42 ^b ± 0.11	
10	155	25	18.48 ^f ± 0.02	5.22 ^g ± 0.03	3.50 ^e ± 0.02	3.31 ^g ± 0.02	10.32 ^c ± 0.07	19.22 ^b ± 0.09	5.20 ^k ± 0.22	3.21 ^b ± 0.05	3.52 ^e ± 0.41	19.77 ^c ± 0.07	6.68 ^b ± 0.02	2.44 ^g ± 0.03	2.12 ^f ± 0.11	9.63 ^b ± 0.03	
11	155	25	19.40 ^d ± 0.04	5.38 ^e ± 0.02	3.44 ^e ± 0.02	3.28 ^g ± 0.01	9.73 ^f ± 0.13	20.03 ^c ± 0.03	6.62 ^l ± 0.00	3.93 ^b ± 0.02	2.95 ^f ± 0.00	19.24 ^c ± 0.11	7.05 ^f ± 0.05	2.41 ^g ± 0.03	1.22 ^k ± 0.06	10.12 ^d ± 0.01	
12	155	25	18.80 ^e ± 0.01	5.61 ^c ± 0.03	3.49 ^e ± 0.02	2.53 ^g ± 0.42	9.34 ^g ± 0.13	19.34 ^b ± 0.02	6.63 ^l ± 0.02	3.36 ^g ± 0.02	3.42 ^{cd} ± 0.15	10.21 ^{bc} ± 0.04	19.73 ^c ± 0.304	7.35 ^f ± 0.03	2.44 ^g ± 0.74	1.78 ^h ± 0.03	9.65 ^b ± 0.01
13	155	10	19.04 ^{de} ± 0.12	5.64 ^c ± 0.02	4.08 ^b ± 0.06	3.50 ^d ± 0.05	10.23 ^c ± 0.02	19.30 ^b ± 0.04	5.78 ^h ± 0.14	4.00 ⁿ ± 0.02	2.78 ^g ± 0.05	10.1 ^d ± 0.03	19.40 ^d ± 0.04	7.26 ^f ± 0.11	3.54 ^e ± 0.01	10.33 ^b ± 0.26	
Con	-	-	22.79 ^a ± 0.02	9.58 ^a ± 0.07	2.74 ^h ± 0.02	3.82 ^c ± 0.14	9.68 ^f ± 0.15	29.46 ^f ± 0.13	10.92 ^a ± 0.01	2.60 ^o ± 0.05	2.67 ^g ± 0.03	24.32 ^a ± 0.04	10.13 ^a ± 0.01	3.12 ^e ± 0.02	2.01 ^e ± 0.01	9.21 ^b ± 0.02	

Data were expressed as mean ± SD and analyzed using one-way ANOVA followed by Duncan's multiple range test (DMRT) (n = 3) (p < 0.05). Means with different alphabetical superscripts in the same column are significantly different (p < 0.05). Con: control, M.C: moisture content.

variety have high fat content (12.84 ± 0.02%) at roasting temperature of 110 °C for 10 min compared to the dark (12.24 ± 0.02%) and light brown (10.42 ± 0.11%) varieties at 155 °C for 25 min. The fat content of the seed varieties obtained in this study suggests that *D. reflexa* seed could be a good source of energy and enhance taste and acceptability. Fat is an important nutrient in diet due to its ability to store and promote the absorption of fat-soluble vitamins in the body [27].

3.2. Antioxidant capacity of marble vine (*Dioclea reflexa*) seed variety

The effect of varied roasting temperatures on the DPPH radical scavenging activity of marble vine seed varieties is presented in Table 3. The 1, 1-diphenyl-2-picrylhydrazyl scavenging ability of the seed extract is based on its ability to reduce DPPH radical and it is an effective method to determine the antioxidant activity of plant food due to its speed, ease, and reliability. The DPPH scavenging ability of the roasted seed ranged from 8.06 ± 0.03 to 29.40 ± 0.24% for black seed, dark brown (7.88 ± 0.04 to 29.28 ± 0.31%) and light brown variety (7.88 ± 0.02 to 79.25 ± 0.02%). The roasting condition for optimal DPPH scavenging activity was obtained at 110 °C for 10 min in the black and light variety while it was 110 °C for 25 min in the dark brown variety. Increasing roasting time had significant (p ≤ 0.05) effects on the antioxidant activities of seed extract at 110 °C compared to the control (unroasted sample). There was a decreased in DPPH radical scavenging activity at a more prolonged roasting temperature in all seed varieties studied which we suppose could be due to phenolic degradation in the samples.

The ABTS (2, 2-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) radical scavenging activity increased at roasting temperature of 110 °C. The ABTS scavenging ability of the *D. reflexa* seed ranged from 32.77 ± 0.14 to 78.02 ± 0.44%, 15.87 ± 0.27 to 64.30 ± 0.41% and 16.73 ± 0.05 to 68.47 ± 0.03% in black, dark brown and light brown varieties respectively. The black variety had the highest value (78.02%) compared with the Dark brown (64.30%) and light brown (68.47%) respectively. Roasting at 110 °C for 10 min was observed for black variety, 110 °C for 40 min and 110 °C for 25 min were observed for dark brown and light brown varieties respectively for ABTS scavenging activities. A similar trend was observed in DPPH scavenging activities of the three varieties as obtained in ABTS radical scavenging abilities of the tested extracts. The DPPH radical scavenging activity showed a positive correlation with ABTS at roasting temperature of 110 °C. The result obtained for the scavenging activity of the seed varieties is an indication that the major compounds contributing to the scavenging activity of the seeds can be activated using heat treatment at a similar temperature. The molecular structure of ABTS and DPPH is presented in Figure 1. The increase in scavenging abilities (DPPH and ABTS) after roasting as observed in marble vine seeds can be attributed to the release of phenolic from the bound fractions and/or the formation of Maillard reaction products which possess antioxidant activity. This is in agreement with Carciochi, et al. [28] on effect of roasting conditions on the antioxidant compounds of quinoa seeds.

Ferric reducing power of a food extract indicates its anti-oxidative activity [1]. The ability of food extract to convert Fe³⁺ to the Fe²⁺ at 593 nm indicates its reducing potential as absorbance increases resulting in high reducing power as shown in Figure 2. The ferric reducing antioxidant potential (FRAP) of the *Dioclea reflexa* seed extract ranged from 1.73 ± 0.02 to 1.88 ± 0.01% in black variety, dark brown (1.75 ± 0.44–1.85 ± 0.24%) and light brown (1.80 ± 0.04–1.92 ± 0.03%) variety of *Dioclea reflexa*. There were slight significant differences in the reducing power activities of the seed extracts as both the roasting temperature and time increases, however, significant differences were observed between the roasted samples and the control. At all roasting temperature and time considered, the light *Dioclea reflexa* seed had the highest ferric reducing antioxidant property than the black and dark brown seed varieties. A similar increase in the reducing power after heat treatment of camelina seed was reported by Terpin et al. [29] as well as Jan et al. [30] for kalonji seed flour. The results obtained suggests that heat treatment

Table 3. Effect of roasting temperature and time on the antioxidant activities of three varieties of marble vine (*Dioclea reflexa*) seed.

Runs	Temp (°C)	Time (Min)	Black seed variety						Dark brown seed variety						Light brown seed variety						
			DPPH (%)	ABTS (%)	FRAP (%)	MCA (%)	HRS (%)	LPI (%)	DPPH (%)	ABTS (%)	FRAP (%)	MCA (%)	HRS (%)	LPI (%)	DPPH (%)	ABTS (%)	FRAP (%)	MCA (%)	HRS (%)	LPI (%)	
			1	110	25	29.40 ^a ±0.24	78.02 ^a ±0.44	1.88 ^a ±0.02	53.22 ^b ±	79.02 ^a ±0.16	64.98 ^a ±0.23	29.28 ^a ±0.31	59.54 ^b ±	1.85 ^a ±	57.01 ^a ±0.03	66.11 ^a ±0.12	55.07 ^a ±0.01	54.95 ^b ±	66.27 ^b ±	1.91ab ±	55.42 ^a ±0.07
2	110	10	29.34 ^a ±0.02	64.56 ^b ±	1.87 ^b ±	53.13 ^b ±	64.56 ^c ±0.24	53.53 ^d ±	29.05 ^a ±0.25	57.09 ^c ±1.32	1.85 ^a ±0.24	51.22 ^b ±	60.06 ^c ±0.05	49.02 ^b ±	79.25 ^a ±0.02	68.47 ^a ±0.03	1.91ab ±	50.62cd ±	68.84 ^b ±	51.80 ^c ±0.02	0.31
3	110	40	24.48 ^c ±0.02	48.59 ^g ±	1.88 ^a ±	54.96 ^a ±0.12	73.59 ^b ±	62.55 ^b ±	15.83 ^g ±	64.30 ^a ±0.41	1.84 ^b ±	50.71 ^c ±0.31	61.39 ^b ±	40.36 ^d ±	33.43 ^g ±	58.15 ^c ±0.02	1.90 ^{bc} ±0.32	56.78 ^b ±	53.29 ^h ±	42.26 ^h ±	0.05
4	155	25	21.57 ^f ±0.23	49.37 ^e ±0.04	1.87 ^b ±	40.99 ^f ±0.07	49.37 ^g ±	38.33 ^g ±	15.58 ^{gh} ±	42.17 ^g ±	1.84 ^b ±	38.56 ^g ±	46.82 ^e ±0.05	35.79 ^f ±0.06	35.58 ^e ±0.05	44.47 ^h ±	1.90 ^{bc} ±0.03	49.85 ^e ±0.13	1358.64 ^f ±0.11	47.60 ^e ±0.04	0.04
5	155	40	26.23 ^b ±	50.64 ^f ±0.06	1.87 ^b ±	37.62 ⁱ ±0.63	50.32 ^f ±0.45	38.34 ^g ±	15.35 ^h ±	19.08 ⁱ ±0.01	1.84 ^b ±	14.03 ^j ±0.02	21.19 ^j ±0.42	10.15 ^j ±0.25	25.13 ^j ±0.02	42.81 ⁱ ±0.25	1.90 ^{bc} ±0.02	50.99cd ±	71.69 ^a ±0.02	60.65 ^a ±0.01	0.02
6	200	10	17.13 ⁱ ±0.03	62.64 ^c ±	1.84 ^d ±	24.98 ^k ±0.02	12.64 ⁱ ±0.42	21.60 ^j ±0.09	15.41 ^h ±	18.78 ^{ij} ±0.01	1.84 ^b ±	13.97 ^j ±0.03	19.60 ^k ±0.08	17.57 ⁱ ±0.34	29.28 ⁱ ±0.06	47.03 ^f ±0.03	1.89 ^c ±0.02	47.98 ^f ±0.06	62.61 ^d ±	51.57 ^d ±	0.02
7	200	40	21.52 ^f ±0.12	51.43 ^e ±0.20	1.87 ^b ±	27.52 ^j ±0.09	51.34 ^e ±0.21	38.54 ^g ±	7.88 ⁱ ±0.04	15.87 ^k ±0.27	1.83 ^c ±0.64	10.62 ^j ±	17.63 ^m ±	6.59 ^j ±0.04	14.40 ⁱ ±	16.73 ^j ±	1.89 ^c ±0.02	9.59 ^k ±0.05	16.56 ^k ±0.02	5.52 ^j ± 0.01	0.02
8	200	25	20.34 ^g ±	50.35 ^f ±0.12	1.87 ^b ±	41.40 ^e ±	38.52 ⁱ ±0.05	45.26 ^e ±0.04	18.73 ^d ±	17.53 ^j ±0.01	1.83 ^c ±0.02	12.38 ^k ±0.02	19.47 ⁱ ±	8.43 ^k ±0.03	7.88 ^m ±	19.39 ^k ±0.05	1.89 ^c ±0.05	16.01 ^j ±0.35	23.26 ^j ±0.12	12.23 ^k ±0.08	0.02
9	155	25	21.45 ^f ±2.04	42.22 ⁱ ±0.05	1.87 ^b ±	15.33 ⁱ ±	32.22 ^j ±0.03	41.87 ^f ±0.08 ^g	15.56 ^{gh} ±	41.27 ^g ±	1.84 ^b ±	40.06 ^e ±0.02	43.62 ^g ±	34.77 ^h ±	32.50 ^h ±	45.46 ^g ±	1.90 ^{bc} ±0.02	47.78 ^g ±	58.62 ^f ±0.04	46.52 ^f ±0.02	0.02
10	155	25	23.31 ^d ±	48.46 ^g ±	1.87 ^b ±	52.45 ^c ±0.22	50.34 ^f ±0.12	35.56 ⁱ ±0.09	16.59 ^f ±0.05	32.16 ^h ±	1.83 ^c ±0.04	34.63 ⁱ ±0.02	47.70 ^d ±	35.34 ^g ±	35.77 ^d ±	34.35 ^j ±0.01	1.91ab ±	46.85 ^h ±	58.03 ^g ±	46.59 ^f ±0.05	0.14
11	155	25	22.41 ^e ±0.13	56.62 ^d ±	1.86 ^c ±0.05	40.62 ^g ±	47.64 ^h ±	36.43 ^h ±	24.46 ^c ±	32.17 ^h ±	1.83 ^c ±0.02	38.24 ^h ±	36.91 ⁱ ±0.04	41.78 ^c ±0.02	37.52 ^c ±0.14	44.64 ^h ±	1.90 ^{bc} ±0.08	49.78 ^e ±0.04	58.55 ^f ±0.03	33.56 ^j ±0.08	0.02
12	155	25	8.06 ⁱ ±0.03	38.57 ^j ±0.02	1.87 ^b ±	11.52 ^m ±	18.57 ^k ±0.51	7.53 ^k ±0.35	15.58 ^{gh} ±	44.27 ^f ±0.05	1.84 ^b ±	39.60 ^f ±1.04	46.32 ^f ±0.35	37.09 ^e ±0.03	35.72 ^d ±	45.43 ^g ±	1.92 ^a ±	46.55 ^h ±	59.62 ^e ±0.02	41.33 ^h ±	0.06
13	155	10	21.81 ^f ±0.01	47.53 ^h ±	1.87 ^b ±	39.23 ^h ±	47.53 ^h ±	36.49 ^h ±	17.63 ^e ±0.43	50.62 ^e ±0.04	1.83 ^c ±0.02	47.14 ^d ±	42.72 ^h ±	40.62 ^d ±	34.61 ^f ±0.02	47.46 ^f ±0.03	1.80 ^d ±	42.52 ⁱ ±0.07	52.51 ⁱ ±0.10	40.41 ⁱ ±0.05	0.02
Con	-	-	18.73 ^b ±	32.77 ^k ±	1.73 ^e ±	50.74 ^d ±	62.07 ^d ±	56.73 ^c ±	25.47 ^b ±	53.28 ^d ±	1.75 ^d ±	47.81 ^d ±	66.94 ^a ±	41.91 ^c ±	23.78 ^k ±	52.05 ^d ±	1.81 ^d ±	50.14 ^d ±	59.30 ^e ±	45.26 ^g ±	0.18

Data were expressed as mean ± SD analyzed using one-way ANOVA followed by Duncan's multiple range test (DMRT) ($n = 3$) ($p < 0.05$). Means with different alphabetical superscripts in the same column are significantly different ($p < 0.05$). Con: control, FRAP: ferric reducing power activity, MCA: metal chelating activity, LPI: lipid peroxidation inhibition, HRS: Hydroxyl Radical Scavenging Activity.

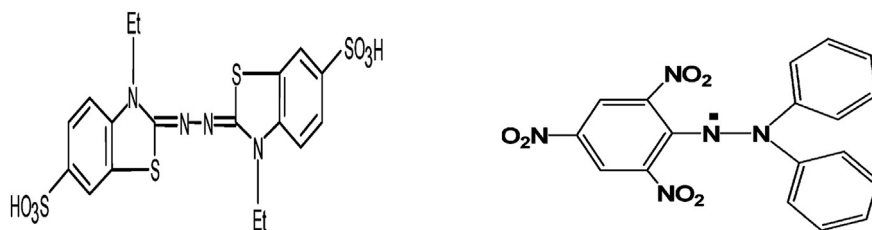


Figure 1. Structure of Structure of (ABTS^{•+}) 2, 2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and (DPPH[•]) 2, 2-diphenyl-1-picrylhydrazyl.

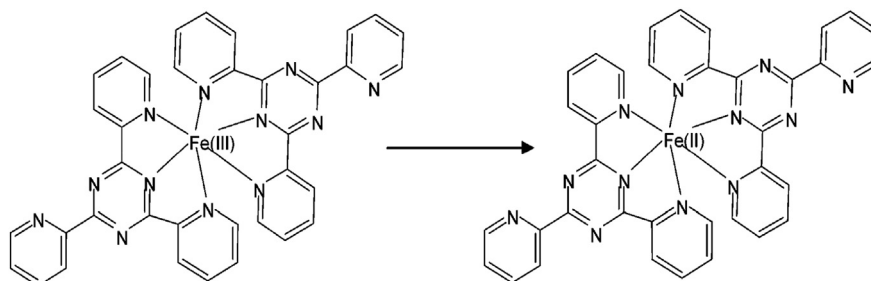


Figure 2. Conversion of Fe³⁺-TPTZ + reducing antioxidant to Fe²⁺-TPTZ (intense blue at 593 nm).

increases the ability of the seeds to donate electron from phenolic compounds regardless of the duration and roasting temperature [28, 31]. However, previous studies have shown that roasting at 110 °C resulted in

a negligible change in total phenolic content and antioxidant activity of cocoa beans, while an increase in temperature at (120 and 150 °C) was reported to reduced the antioxidant potential of cocoa bean (Oracz and

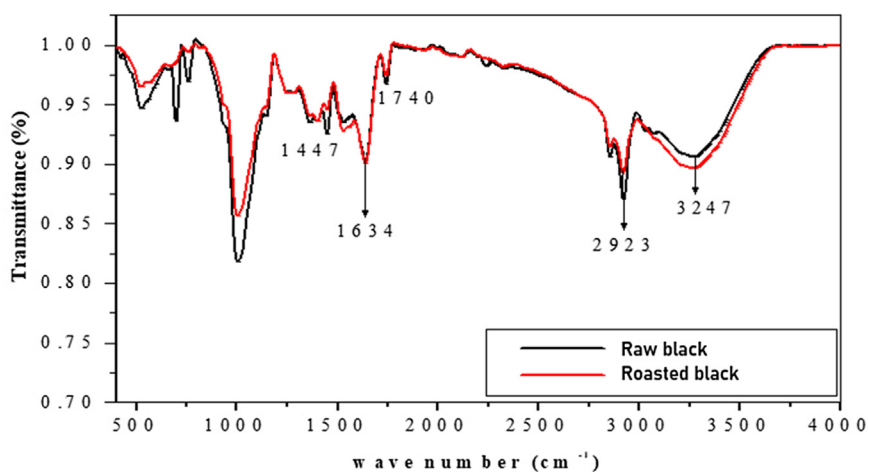


Figure 3. Spectral analysis obtained from FTIR for black *Dioclea reflexa* seed.

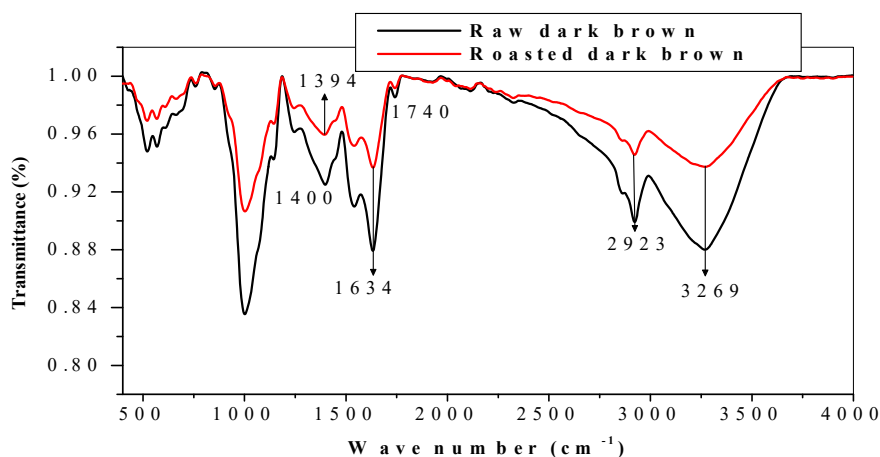


Figure 4. Spectral analysis obtained from FTIR for dark brown *Dioclea reflexa* seed.

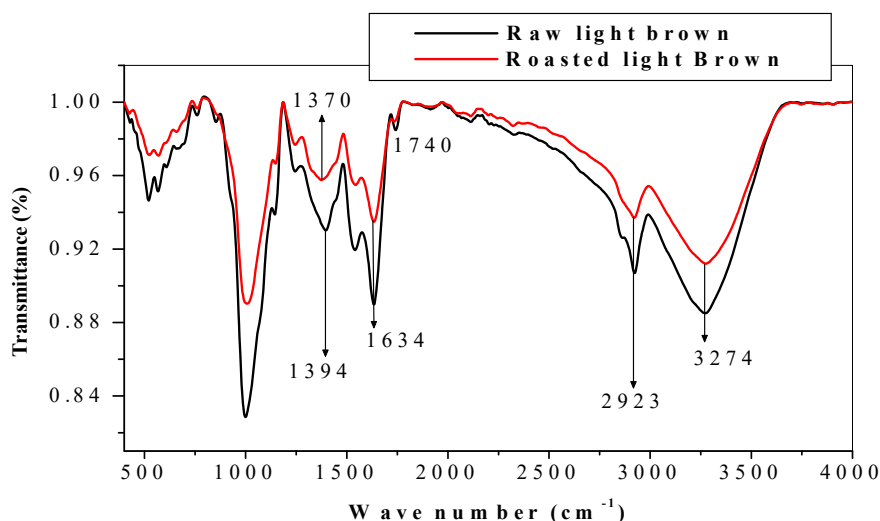


Figure 5. Spectral analysis obtained from FTIR for light brown *Dioclea reflexa* seed.

Nebesny) [32]. Hence the observation in this study could be attributed to thermal degradation activities in the seed.

Metal chelating ability of a food sample is based on its ability to inhibit the generation of reactive oxygen species (ROS) which are catalyzed by transition metal such as Fe^{2+} and Cu^{2+} in the body, thus, preventing cellular injury. A high amount of iron in food promotes peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals that can themselves attract hydrogen and perpetuate the chain reaction of lipid peroxidation [33]. The chelating of transition metals by food samples may help reduce reactive oxygen species and retard lipid peroxidation. The chelating ability of the seed variety ranges from 11.52 ± 0.02 to $54.96 \pm 0.12\%$ in black seed, 10.62 ± 0.35 to $57.01 \pm 0.03\%$ in dark brown seed and 9.59 ± 0.05 to $56.78 \pm 0.23\%$ in the light variety of *Dioclea reflexa* seed. Among the treatments, dark brown variety had significantly ($p \leq 0.05$) higher chelating activity than the black and light brown seeds varieties. The optimum roasting condition for high metal chelating properties of the seed variety was obtained in the dark brown seed at 110°C for 10 min while in the black and light brown variety at 110°C for 40 min. The ability of the seed extracts to chelate metal activities could be as a result of the activation of o-dihydroxy polyphenols by heat treatment which enhanced its chelating ability [34].

The hydroxyl radical scavenging ability is attributed to the inhibition of the hydroxyl radicals generated through Fenton reaction in the presence of Fe^{2+} and H_2O_2 . Result for the hydroxyl scavenging ability ranged from 12.64 ± 0.42 to $79.02 \pm 0.16\%$ in black variety, dark brown (17.63 ± 0.02 – $66.94 \pm 0.09\%$), and light brown seed variety (16.56 ± 0.02 – $71.69 \pm 0.02\%$). The black seed had the highest hydroxyl scavenging ability while the dark brown had the lowest and was significantly ($p \leq 0.05$) higher than the control except the dark brown variety. The optimum roasting conditions was obtained at 110°C for 25 min in black and dark brown seed varieties and dark brown seed variety (155°C for 40 min), this could be as result of thermal degradation or inactivation of the antioxidants components at high roasting temperature thereby leading to a reduction in the antioxidant activities. The result obtained in the study suggests that heat treatment at 110°C activated the polyphenolic compounds such as flavonoids which may have a pro-oxidative effect in the presence of transition metals [35].

Lipid peroxidation involves the formation and propagation of lipid radicals with many deleterious effects, including the destruction of membrane lipids, metabolic disorders and inflammation, and the production of malondialdehyde (MDA) which is a hallmark of the process. The ability of food samples to inhibit lipid peroxidation is highly desired. The lipid peroxidation abilities of *D. reflexa* ranged from 7.53 ± 0.35 to

$64.98 \pm 0.23\%$ in black variety, dark brown (6.59 ± 0.04 – $55.07 \pm 0.01\%$) and light seed (5.52 ± 0.01 – $60.65 \pm 0.01\%$) variety of *D. reflexa* seeds. The optimum condition for the inhibition of lipid peroxidation was obtained at 110°C for 25 min in black and dark brown varieties, while in light brown variety roasting conditions (155°C for 40 min) was the optimum. The high lipid peroxidation ability of the seed extract can be attributed to the phenolic and flavonoid content in the marble vine seed varieties which inhibits radicals and peroxides.

3.3. Effect of roasting on the functional group of marble vine (*Dioclea reflexa*) seed varieties

The changes occurring in the functional groups of marble vine seed variety was studied by FTIR spectroscopy and the spectral obtained is presented in Figures 3, 4, and 5. The broad and strong absorption bands observed at 3247 cm^{-1} for the three seed variant of roasted *Dioclea reflexa* seed flours indicates alcohol-phenol ($-\text{OH}$) groups [36]. The O–H group reduces the activities of free radicals and could help reduce rancidity in foods. The roasted and unroasted variety of *Dioclea reflexa* seed flours showed an identical pattern of absorption bands which indicates the similarity of compounds present in each treatment. The characteristics band of 2923 cm^{-1} in the seed variety confirmed the carboxylic acid (C–H) bond stretching and bending vibrations both symmetric and asymmetric [37] while bands observed at 1634 cm^{-1} in all the seed varieties are due to peptide group of proteins (C=O) [30]. A vibrational stretch in the carbonyl (C=O) group was observed at 1394 cm^{-1} compared to the control at 1400 cm^{-1} . This disparity could be due to the effect of roasting in the release of aldehydes, ketones that presents characteristics flavour and aroma in the seed.

The alcohol-phenol functional group was affected by the roasting operation while carboxylic acid was not affected by roasting of the black *D. reflexa* seed flour. This supports the assertion that roasting improves the release of phenolic compounds and anti-oxidative properties of food materials. A slight decrease was observed in the absorption band of carbonyls and a decrease in the peptide group of protein with the heat treatment, changes observed may be responsible for the development of aroma and flavour attributable to Maillard reaction. The decrease in the peptide absorption band in the roasted seed variety might be attributed to denaturation of protein in the seed and/or development of the desirable compounds that increase aroma and flavor of the roasted flour which may be associated to the Maillard reaction [30].

3.4. Conclusion

In conclusion, this study showed that the marble vine seeds were significantly affected by roasting and thus improved the nutritional and bioactive properties as against the previous reports of reducing the food quality. Roasting temperature at 110 °C with different roasting time (10, 25 and 40 min) improved the proximate and antioxidant property of the three varieties than those roasted at a higher temperature 150–200 °C. Therefore, the processing conditions used in this study could be applied to *Dioclea reflexa* seed for optimum nutritive properties and could help produce food with good aroma, high nutrients and bioactive quality. This could aid the utilisation of *D. reflexa*, thereby boosting its potential to contribute to nutritional and health status of developing countries, especially among the low-income families. The changes in the functional groups of the three varieties of *D. reflexa* seed by FTIR spectroscopy also showed that it could help in the reduction of rancidity in foods.

Declarations

Author contribution statement

Mary A. Ajatta: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Stephen A. Akinola: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Oluwatooyin F. Osundahunsi, Olufumilayo S. Omoba: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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