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Mineral Elements Bio-Accessibility and Antioxidant Indices of Blanched *Basella rubra* at Different Phases of *in vitro* Gastrointestinal Digestion

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ABSTRACT: The present investigation was designed to evaluate the mineral element bio-accessibility and antioxidant indices of blanched *Basella rubra* at different phases of simulated *in vitro* digestion (oral, gastric, and intestinal). The phenolic composition of processed vegetable was determined using high-performance liquid chromatography (HPLC)-diode-array detection method. Mineral composition, total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), and total antioxidant activity (TAA) of the *in vitro* digested blanched and raw vegetable were also determined. HPLC analysis revealed the presence of some phenolic compounds, with higher levels (mg/g) of polyphenols in raw *B. rubra* (catechin, 1.12; *p*-coumaric acid, 6.17; caffeic acid, 2.05) compared with the blanched counterpart, with exeption of chlorogenic acid (2.84), that was higher in blanched vegetable. The mineral content (mg/100 g) showed a higher value in enzyme treated raw vegetable compared to their blanched counterparts, with few exceptions. The results revealed a higher level of some of the evaluated minerals at the intestinal phase of digestion (Zn, 6.36/5.31; Mg, 5.29/8.97; Ca, 2,307.69/1,565.38; Na, 5,128/4,128.21) for raw and blanched respectively, with the exception of Fe, K, and P. The results of the antioxidant indices of *in vitro* digested *B. rubra* revealed a higher value at the intestinal phase of *in vitro* digestion, with raw vegetal matter ranking higher (TPC, 553.56 mg/g; TFC, 518.88 mg/g; FRAP, 8.15 mg/g; TAA, 5,043.16 μM Trolox equivalent/g) than the blanched counterpart. The studied vegetable contains important minerals and antioxidant molecules that would be readily available after passing through the gastrointestinal tract and could be harnessed as functional foods.

Keywords: *Basella rubra*, phenolic compounds, bio-accessibility, mineral content, antioxidant indices

INTRODUCTION

Leafy vegetables constitute an indispensable component of human diets in Africa and West Africa in particular. It has been estimated that over 60 species of leafy vegetables are consumed in Nigeria alone (1). Numerous studies have conclusively shown that plant foods are good sources of minerals (2-6). Green plants absorb the minerals they need for diverse metabolic processes from the soil. The complex organic compounds manufactured in plant leaves include antioxidant molecules for protection against oxidative solar radiation (7). Leafy vegetables are, therefore, important dietary sources of minerals, trace elements, and phytochemicals with health-protective and immune-strengthening properties.

The dominant basic elements in plants and vegetables are calcium, potassium, iron, sodium, zinc, magnesium, manganese, and selenium (8). Mineral elements are crucial for many body functions which include transportation of oxygen, normalizing of the nervous systems, stimulation of growth, and maintenance and repair of tissues and bones. Some of these elements are needed in small-amounts (few milligrams) per day, and when absorbed in excess, may be toxic and cause damage to the body (9).

Most vegetables and fruits have been reported to possess antioxidant activities which allow them to scavenge both reactive oxygen species and electrophiles, inhibit nitrosation, chelate metal ions, and modulate certain cellular enzyme activities. It has been established that an appreciable part of the antioxidant activities of vegetables and fruits are related to phenolic compounds (10,11).

B. rubra belongs to the family Basellaceae and of the

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genus *Basella*, commonly called Indian spinach, climbing spinach, Asian spinach, 'Amunututu' (Yoruba tribe in Nigeria). *B. rubra* is a versatile green leafy vegetable and revered in some African and East Asian cultures for its wholesome phyto-nutrient profile (12). It holds an incredibly good amount of vitamins, minerals, and antioxidants (6). *B. rubra* leaves are good sources of minerals like potassium, manganese, sodium, calcium, magnesium, copper, iron, phosphorus, selenium, and zinc (6). However, to understand the potential health benefit of *B. rubra*, it is essential to determine the bio-accessibility or bio-availability of the phyto-nutrient in the gut and to measure the ratio of the ingested vegetal material, which is available for utilization in the human system.

It has been established that not all nutrients are absorbed with equal efficacy after passing through the gastrointestinal tract in the human body during digestion process. This is due to the fact that components available to be absorbed and utilized in the body may vary quantitatively and qualitatively due to the physical properties of the food matrix (13). Moreover, the bioavailability of compounds in plant products may differ because of interactions between their chemical structures and macromolecules within the food matrix, as well as their uptake rates into the body (14).

Bio-accessibility of phyto-nutrient is a measure of the amount of plant nutrients that are soluble in gastrointestinal digestive enzymes which are ultimately available for utilization in humans. It can also be described as any potentially available part of a nutrient after gastrointestinal digestion. Bioavailability of various phyto-nutrients is determined by either *in vivo* administration to similar species to humans, e.g., rats or *in vitro* methods by simulating digestive system conditions in the laboratory. Therefore, the use of *in vitro* methods will address the issue of animal rights that is gaining a global concern. *In vitro* procedures involve the simulation of the gastric and intestinal digestive conditions in the laboratory. The results obtained by *in vitro* methods are based on the formation of digestive products that are soluble. The determined values thus correspond to bio-accessible fractions which represent maximum concentrations of elements soluble in gastrointestinal media (15).

There is, however, limited information on the effect of *in vitro* gastrointestinal digestion on the stability and bio-accessibility of minerals and antioxidant molecules from vegetables. Hence, the objective of this work is to evaluate the actual antioxidant activities and mineral composition by simulating the gastrointestinal digestion *in vitro* on the processed vegetal matter.

MATERIALS AND METHODS

Materials

α-Amylase, pepsin, and pancreatin were bought from Fluka Chemie (Wahlkreis Rheintal, Switzerland) and Merck KGaA (Darmstadt, Germany). Other chemicals used in the experiments were of analytical grade and were used without further purification. Freshly prepared solutions of digestive enzymes were used in the experiments. All the solutions were prepared in distilled water. α-Amylase was prepared by dissolving 32.5 mg of α-amylase in 25 mL of 1 mM calcium chloride at pH 7; pepsin solution was obtained by dissolving 10 mg pepsin in 5 mL of 0.05 M HCl (pH 2). Pancreatin solution was prepared by dissolving 3 g of pancreatin in 20 mL distilled water and the pH adjusted to 7.5 after incubation.

Sample treatment and preparation

Fresh *B. rubra* leaves were carefully detached from its succulent stem. The leaves were carefully selected, washed, sliced, and divided into two portions. One portion was oven dried (hot box oven, size 2) raw at 40°C and milled to powder while the other portion was blanched, oven dried at 40°C and milled to fine powder. Both processed samples were stored in air tight plastic containers at room temperature (27～32°C).

In vitro enzymatic digestion

The *in vitro* digestion was done using sequential enzymatic steps based on a slightly modified method reported by Deigado-Andrade et al. (16). It involved three distinct stages (oral, gastric, and total gastro-intestinal digestion) and each stage was terminated by inactivating the enzyme.

Oral digestion

Ten grams (10 g) of the milled *B. rubra* (blanched and raw) samples were weighed and dissolved separately in 200 mL of distilled water. Three hundred microliters (300 μL) of α-amylase was added to the tubes (simulating pH conditions in the mouth). The tubes were incubated in a shaking water bath set at 37°C for 40 min at 80 strokes/min. After 40 min, the enzyme was inactivated by boiling in water at 100°C for 4 min after which the samples were centrifuged for 60 min at 3,200 *g*. The clear soluble supernatant was separated and stored at −4°C prior to analysis. A non-enzymic digest (control sample digested without enzyme) was also obtained by following the same digestion scheme without enzyme.

Gastric digestion

Ten grams (10 g) of the milled *B. rubra* (blanched and raw) samples were weighed and dissolved separately in 200 mL of distilled water. Three hundred microliters (300

μL) of α-amylase was added to the tubes (simulating pH conditions in the mouth). The tubes were incubated in a shaking water bath set at 37°C for 40 min at 80 strokes/min. After 40 min, the pH was adjusted to 2 using concentrated HCl. After 40 min, 10 mg pepsin was added to the tubes, (simulating pH conditions in the stomach). The tubes were then incubated in a shaking water bath at 37°C for 40 min and at 80 strokes/min. After another 40 min, the enzyme was inactivated by boiling in water at 100°C for 4 min after which the samples were centrifuged for 60 min at 3,200 *g*. The clear soluble supernatant was obtained and stored at −4°C prior analysis. A non-enzymic digest (control sample digested without enzyme) was also obtained by following the same digestion scheme without enzyme.

Intestinal digestion

Ten grams (10 g) of the milled *B. rubra* (blanched and raw) samples were weighed and dissolved separately in 200 mL of distilled water. Three hundred microliters (300 μL) of α-amylase was added to the samples (simulating pH conditions in the mouth). The samples were incubated in a shaking water bath at 37°C for 40 min and at 80 strokes/min. After 40 min, the pH was adjusted to 2 using concentrated HCl and incubated at 37°C for another 40 min in a shaking water bath. Ten milligrams (10 mg) of pepsin was added to the samples, (simulating pH conditions in the stomach and incubated at 37°C for 40 min at 80 strokes/min in a shaking water bath. After another 40 min, the pH was adjusted to 6 using NaOH. After 20 min, 50 mL of pancreatin was added to the samples and incubated in shaking water at 37°C for 40 min. The pH was finally adjusted to 7.5 using NaOH (simulating pH conditions in the small intestine) and incubated for 40 min in a shaking water bath at 37°C. After 40 min, the enzymes were inactivated by boiling in water at 100°C for 4 min after which the samples were centrifuged for 60 min at 3,200 *g*. The clear soluble supernatant was obtained and stored at −4°C prior analysis. A non-enzymic digest (control sample digested without enzyme) was also obtained by following the same digestion scheme without enzyme.

Mineral evaluation

Sixty milliliters (60 mL) of the digested samples and their respective control without enzyme treatments were evaporated to one-third its initial volume (20 mL) by boiling in an autoclave, after which 20 mL 1% HNO_3 was added and heated further to 1/2 its initial volume. It was allowed to cool before pouring in a sample bottle and labeled. Appropriate dilution was made for each element before analysis. The standard for each element using the suitable metal salt was prepared. The standard for each metal was aspirated into the flame along with the digest and control and their respective concentration in mg/L were read for each sample and the absorbance of the standards was noted. Atomic absorption spectrophotometer was used for the analysis of Mg, Mn, Zn, Fe, Cu, and Pb, while UV visible spectrophotometer using vanadomolybdate yellow method was used for the analysis of P, a flame photometer was used for Na, K, and Ca as described in the official methods of the Association of Official Analytical Chemists (17).

Identification of phenolic compounds using high-performance liquid chromatography (HPLC)-diode-array detection (DAD)

B. rubra extracts at a concentration of 15 mg/mL was injected by means of a model SIL-20A Shimadzu Auto sampler (Shimadzu Corporation, Kyoto, Japan). Separations were carried out using Phenomenex C_{18} column (4.6 mm×250 mm×5 μm particle size). The mobile phase was solvent A=water : formic acid (98:2, v/v) and solvent B=acetonitrile. The gradient program was started with 95% of A and 5% of B until 2 min and changed to obtain 25%, 40%, 50%, 60%, 70%, and 80% B at 10, 20, 30, 40, 50, and 80 min, respectively, following the method described by Boligon et al. (18). The sample and mobile phase were filtered through 0.45 μm membrane filter (Millipore, Billerica, MA, USA) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in water : acetonitrile (1:1; v/v) at a concentration range of 0.030～0.500 mg/mL. Quantifications were carried out by integration of the peaks using the external standard method, at 254 nm for gallic acid and ellagic acid, 280 nm for catechin, 326 nm for chlorogenic, *p*-coumaric, and caffeic acids, and 366 nm for quercetin and apigenin. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 600 nm). All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve as defined by Brito et al. (19).

Total phenolic content (TPC)

The TPC of the digested extracts was determined by the Folin-Ciocalteu assay as described by Waterman and Mole (20), with slight modification. One hundred microliters (100 μL) of sample digest was pipetted into a test tube containing 2 mL of distilled water, 500 μL of Folin reagent was added and the mixture was mixed. Subsequently, 1.5 mL of Na_2CO_3 solution (7.5 g/100 mL) was

added to the solution and the volume was made up to 10 mL. It was incubated at 37°C for 2 h (green colored solution obtained), after which the absorbance of the mixture was measured at 760 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer, San Diego, CA, USA). The standard used was tannic acid and the results were expressed as mg tannic acid equivalent per gram of the sample.

Total flavonoid content (TFC)

The TFC of the digested extracts was determined using a slightly modified method reported by Mier et al. (21). Briefly, 0.5 mL of digested sample was mixed with 0.5 mL distilled water pipetted into a test tube and 50 μL of 10% $AlCl_3$, 50 μL of 1 mol/L potassium acetate, and 1.4 mL of distilled water; it was allowed to incubate at 37°C for 30 min. Thereafter, the absorbance was measured at 415 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer). The TFC was calculated using quercetin as standard (0～100 μg/mL), the TFC of the digested samples along with its various control was determined in triplicates and the results were expressed as mg quercetin equivalent per gram of the sample.

Ferric reducing antioxidant power (FRAP)

The reducing power of the digested extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (22). Briefly, 1 mL of the various digested extract was mixed with 1 mL 200 mM sodium phosphate buffer (pH 6.6) in a separate test tubes and 1 mL of 1% potassium ferricyanide added. The mixture was incubated at 50°C for 20 min. After incubation, 1 mL of 10% trichloroacetic acid was added to the mixture. This mixture was centrifuged at 650 rpm for 10 min. Two mL of the supernatant was mixed with an equal volume of distilled water and 0.4 mL of 0.1% $FeCl_3$ added. The absorbance was measured at 700 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer). FRAP was expressed as mg ascorbic acid equivalent per gram of the sample.

Total antioxidant activity (TAA)

TAA of the digested extracts was determined using the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) antiradical assay reported by Awika et al. (23). The $ABTS^{\cdot+}$ (mother solution) was prepared by mixing equal volumes of 8 mM ABTS and 3 mM $K_2S_2O_8$ (both prepared using deionized water) in a volumetric flask, which was wrapped in foil and allowed to react for a minimum of 12 h in the dark. The working solution was prepared by mixing 1 mL of the mother solution with 29 mL phosphate buffer (pH 7.4). A range of Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) standard solutions (100～1,000 μM) were prepared. The working solution (2.9 mL) was added to the digests and the various control extracts (0.1 mL) or Trolox standard (0.1 mL) in a test tube and vortexed. The test tubes were allowed to stand for exactly 30 min at room temperature. The absorbance of the standards and samples were measured at 734 nm with a Lambda EZ150 spectrophotometer (Perkin Elmer). The results were expressed as μM Trolox equivalent per gram of the sample.

Statistical analysis

All analyses were run in triplicates. Means were then computed using Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA) and ANOVA using the Duncan's multiple tests by using SPSS 11.09 for Windows (IBM SPSS, Inc., Armonk, NY, USA).

RESULTS AND DISCUSSION

Mineral content

The mineral contents (Mg, Zn, Fe, Na, K, Ca, and P; mg/100 g) of raw and blanched *B. rubra* subjected to different phases of human digestion (oral digestion, gastric digestion, and intestinal digestion) and their respective controls are shown in Table 1. The results showed high mineral content for raw enzyme treated sample compared to their blanched counterparts, with few exceptions. The reduction in the amount of minerals after blanching may be attributed to loss into the boiled water used for blanching. This is in agreement with previous reports where reductions in the level of some minerals in vegetables were observed after boiling (24,25). Mineral loss to water medium may however be beneficial in cases where the liquid is retained for consumption e.g. in preparation of stews or soups, as is usually practiced in Nigeria. In these scenarios, minerals lost from the food sample are still available for consumption (24,25). Conversely, a higher amount of some minerals were also observed after blanching. This might be associated with the fact that cooking increases the release of some minerals from food matrix by mainly reducing anti-nutrient levels in the food samples that may chelate the minerals (26).

The results also showed varied amount of evaluated minerals at each phase of simulated human digestion *in vitro*. The result revealed a higher level of some of the evaluated minerals in the intestinal phase of *in vitro* digestion (Zn, 6.36/5.31; Mg, 5.29/8.97; Ca, 2,307.69/1,565.38; Na, 5,128.21/4,128.21) for raw and blanched, respectively. Kulkarni et al. (27) also observed that bioaccessibilities of wheat grass minerals were higher after intestinal *in vitro* gastrointestinal digestion than during gastric digestion. Iron, on the other hand, is higher at the oral phase of *in vitro* digestion for raw *B. rubra* (5.17), potassium is higher at gastric phase of *in vitro* digestion

Table 1. Mineral composition (mg/100 g) of *in vitro* digested raw and blanched *Basella rubra*

Sample	Mg	Zn	Fe	Na	K	Ca	P
ROC	6.98±0.06^{e}	4.81±0.35^{d}	3.68±0.47^{de}	181.45±20.16^{ab}	5,036.29±4.03^{f}	1,514.11±2.02^{cd}	4.28±0.25^{g}
ROD	5.33±0.07^{bc}	3.66±0.33^{b}	5.17±0.01^{g}	203.63±2.02^{ab}	4,036.29±4.03^{d}	1,209.68±9.80^{b}	3.18±0.05^{c}
BOC	6.47±0.46^{d}	3.13±0.06^{a}	3.59±0.18^{d}	886.09±1.01^{c}	5,515.12±7.06^{h}	1,915.32±9.10^{f}	3.58±0.05^{d}
BOD	4.82±0.28^{ab}	2.92±0.05^{a}	4.13±0.07^{f}	128.02±5.04^{a}	3,215.73±30.24^{a}	1,814.52±2.78^{e}	3.78±0.05^{e}
RGC	7.56±0.35^{f}	4.59±0.04^{d}	2.98±0.20^{c}	203.51±3.10^{ab}	5,061.98±103.31^{f}	1,033.06±7.70^{a}	2.01±0.05^{a}
RGD	5.51±0.11^{c}	4.61±0.04^{d}	3.40±0.23^{d}	231.40±4.13^{b}	5,309.92±61.98^{g}	1,446.28±8.40^{c}	1.96±0.10^{a}
BGC	5.62±0.10^{c}	5.47±0.18^{e}	1.96±0.02^{ab}	123.97±6.70^{a}	4,130.17±4.13^{e}	2,027.89±4.10^{g}	9.30±0.10^{j}
BGD	4.37±0.05^{a}	4.73±0.02^{d}	1.78±0.14^{a}	125.00±1.03^{a}	6,243.81±2.07^{i}	1,756.20±103.31^{e}	5.89±0.10^{h}
RIC	9.21±0.74^{g}	3.99±0.09^{c}	4.32±0.27^{f}	5,615.38±179.50^{g}	6,333.33±128.21^{j}	1,794.87±9.30^{e}	4.04±0.06^{f}
RID	5.29±0.04^{bc}	6.36±0.15^{f}	4.23±0.07^{f}	5,128.21±2.56^{f}	3,726.92±3.85^{c}	2,307.69±7.50^{h}	2.44±0.13^{b}
BIC	8.86±0.04^{g}	3.63±0.17^{b}	2.21±0.15^{b}	3,623.08±2.56^{d}	3,410.26±5.13^{b}	2,365.38±7.40^{h}	7.95±0.13^{i}
BID	8.97±0.05^{g}	5.31±0.25^{e}	4.02±0.14^{ef}	4,128.21±25.64^{e}	3,769.23±25.64^{c}	1,565.38±8.30^{d}	2.05±0.13^{a}

Values in the same column followed by different letters (a-j) are significantly different (P<0.05).
ROC, raw oral control; ROD, raw oral digest; BOC, blanched oral control; BOD, blanched oral digest; RGC, raw oral control; RGD, raw gastric digest; BGC, blanched gastric digest; BGD, blanched gastric digest; RIC, raw intestinal control; RID, raw intestinal digest; BIC, blanched intestinal control; BID, blanched intestinal digest.

(5,309.92/6,243.81) for raw and blanched *B. rubra* and phosphorous ranked higher at gastric phase of *in vitro* digestion (5.89) in blanched *B. rubra*. The varied level of the evaluated minerals at the different phases of the simulated human digestion may be associated with the fact that the bioavailability of compounds in plant products differ by virtue of their interactions between the chemical structures and macromolecules within the food matrix, as well as their uptake rates into the body during gastrointestinal digestion (28). The results also showed that the blanched digested *B. rubra* have higher mineral composition at total *in vitro* digestion phases than their respective controls (27,29). This, by implication, suggests that the action of the digestive enzymes makes a number of minerals available from the processed vegetable to the body. Overall, the concentration of the evaluated minerals after *in vitro* digestion, were able to meet the Recommended Dietary Allowance as reported by the World Health Organization (30).

Phenolic composition

Phenolic compounds are commonly found in plants and have been reported to have several biological activities (31-33). Studies have focused on the biological activities of phenolic compounds, which have potential antioxidants and free radical scavenging abilities (34). The HPLC-DAD quantification of phenolic compounds in *B. rubra* is presented in Fig. 1. The qualitative evaluation of phenolic compounds in *B. rubra* revealed the presence of gallic acid, chlorogenic acid, caffeic acid, ellagic acid, *p*-coumaric acid, catechin, quercetin, and apigenin. The presences of phenolic compounds have been reported in leafy vegetables by numerous researchers (32,35-38). Estimate of the phenolic compounds concentrations in the vegetable studied is shown in Table 2. The results revealed a reduction in the level of some of the evaluated phenolic compounds after blanching, with exception of chlorogenic acid, with an elevated value after blanching, while apigenin, ellagic, and gallic acids were not significantly different after blanching. The observed reduction

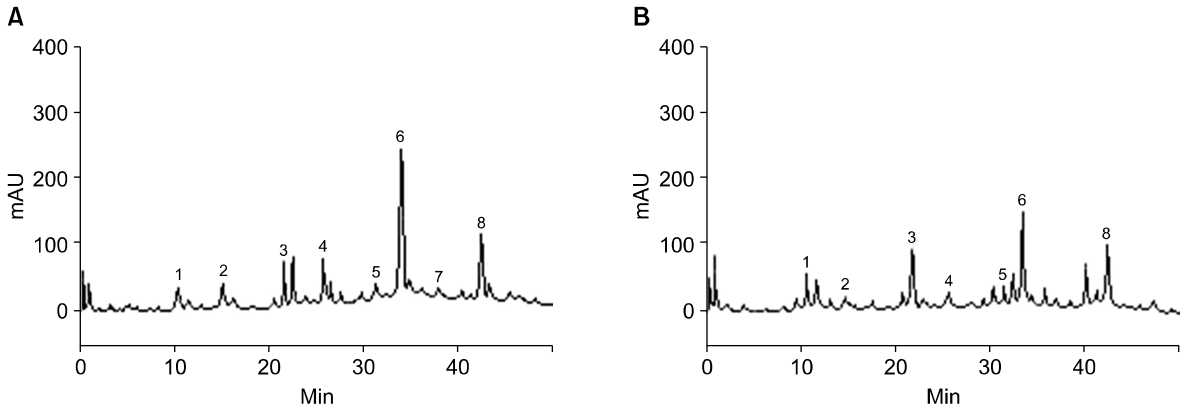

Fig. 1. Representative high performance liquid chromatography (HPLC) profile of *B. rubra* raw (A) and blanched (B) extracts. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), *p*-coumaric acid (peak 6), quercetin (peak 7), and apigenin (peak 8).

Table 2. Quantitative estimates of phenolic compounds in raw and blanched *Basella rubra* digests

Compounds	*B. rubra* raw (mg/g)	*B. rubra* blanched (mg/g)	LOD (μg/mL)	LOQ (μg/mL)
Gallic acid	1.09±0.01[b]	1.13±0.02[b]	0.011	0.036
Catechin	1.12±0.03[b]	0.52±0.01[a]	0.019	0.063
Chlorogenic acid	1.97±0.02[b]	2.84±0.03[c]	0.024	0.080
Caffeic acid	2.05±0.01[b]	0.57±0.04[a]	0.028	0.094
Ellagic acid	0.48±0.04[a]	0.51±0.01[a]	0.007	0.023
p-Coumaric acid	6.17±0.03[d]	4.16±0.01[d]	0.015	0.046
Quercetin	0.43±0.01[a]	–	0.026	0.087
Apigenin	3.01±0.01[c]	2.87±0.03[c]	0.013	0.043

Results are expressed as mean±SD of three determinations. Values in the same column followed by different letters (a-d) are significantly different (P<0.05).
LOD, limit of detection; LOQ, limit of quantification.

Table 3. Antioxidant indices of raw and blanched *Basella rubra* at different phases of *in vitro* digestion

Sample	*B. rubra* raw	*B. rubra* blanched
Total phenolic content (mg tannic acid equivalent/g dry weight)		
Oral	79.48±0.82[a]	108.71±3.85[b]
Gastric	386.71±4.50[b]	80.02±0.47[a]
Intestinal	553.56±5.83[c]	553.56±5.83[c]
Total flavonoid content (mg quercetin equivalent/g dry weight)		
Oral	302.15±0.00[b]	264.78±0.49[b]
Gastric	260.29±0.15[a]	250.80±0.74[a]
Intestinal	518.88±1.21[c]	488.33±0.68[c]
Ferric reducing antioxidant power (mg ascorbic acid equivalent/g dry weight)		
Oral	5.27±0.02[a]	4.95±0.02[a]
Gastric	5.70±0.01[b]	5.19±0.00[b]
Intestinal	8.15±0.01[c]	8.14±0.01[c]
Total antioxidant activity (mg Trolox equivalent/g dry weight)		
Oral	3,087.84±2.00[a]	3,106.34±2.00[a]
Gastric	3,274.47±0.00[ab]	3,253.83±2.10[ab]
Intestinal	5,043.16±0.00[c]	4,815.03±6.48[c]

Values represent mean±SD of triplicate experiment.
Values in the same column followed by different letters (a-c) are significantly different (P<0.05).

in the levels of some of the identified phenolic compounds is in agreement with previous reports (39,40). However, the increasing amount of chlorogenic acid after blanching might be due to alterations in chemical structure and composition as a result of heat during blanching (41). Thus, blanching might have enhanced the breakdown of insoluble fiber matrix of the vegetable, making some of its polyphenols more accessible.

Antioxidant indices

A number of studies have demonstrated the use dietary components in the control of free radical mediated diseases, such as cancer and cardiovascular problems (42). The result of the antioxidant indices of *B. rubra* at various stages of *in vitro* gastrointestinal digestion (oral, gastric, and intestinal digestion) are shown in Table 3. The results revealed some levels of antioxidant activities at different stages of simulated human gastrointestinal digestion, with a much higher antioxidant activity for the raw digested vegetal matter compared to blanched digested vegetal matter. It has been established that an appreciable part of the antioxidant activities of vegetables and fruits are related to phenolic compounds (10,11,43, 44). Therefore, the reduced antioxidant activity in the blanched digested vegetal matter might be associated with quantitative loss of polyphenols encounterd during the heat treatment (39,40).

In vitro digestion method measures the potential bioavailability of the nutrient, which is the amount of the nutrient liberated from the food material during gastrointestinal digestion, which is, in turn, available for absorption in the body (45). The result of the antioxidant activity of the *in vitro* digested vegetal matter showed higher antioxidant indices (TPC, TFC, FRAP, and TAA) at the intestinal phase of *in vitro* digestion. It has been documented that antioxidant activity from extracts of food materials is not usually the actual antioxidant capacity in the digestive tract (46). It has also been established that the antioxidant activities of plant food is related to their phenolic composition (10,11,43,44). Therefore, the observed higher antioxidant activities at the intestinal phase of gastrointestinal digestion may likely be due to the release of the bound polyphenols after the action of the digestive enzymes.

This implies that the maximum amount of phenolics is likely to be released during *in vitro* digestion process as a result of the activity of the digestive enzymes (α-amylase, pepsin, and pancreatin) of the gastrointestinal tract. The higher antioxidant activity at the intestinal phase of gastrointestinal digestion is in agreement with previous reports (47,48), which reported high polyphenolic contents and antioxidant activities of commercially available juices after gastric and intestinal phase of gastrointestinal digestion. Additionally, it has been reported that higher antioxidant activity obtained during digestion might be due the biotransformation of polyphenolic compounds to other phenolics in mild alkaline conditions (49). Olthof et al. (50) also reported that hydrolyzation of cyanidin and quercetin glycosides by digestive enzymes (pepsin and pancreatin) to cyanidin and quercetin respectively, which have very high antioxidant potentials. This is an indication that the action of the digestive enzymes on the studied vegetal matter during digestion and most especially at the total intestinal digestion phase could make the constituent antioxidant molecules readily available to the human body and thus make it a good candidate in ameliorating free radical related diseases.

The result of this study revealed that the studied vegetable has a number of nutritional important mineral ele-

ments and health promoting phenolic compounds with antioxidant potential. The results also established the availability of the essential minerals after simulated *in vitro* digestion. Furthermore, the results revealed higher antioxidant indices after the intestinal phase of the simulated human digestion. Therefore, the consumption of the studied vegetable could be harnessed as functional food; as a source of nutritionally important mineral element and the prevention of free radical mediated diseases.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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