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**COMPARATIVE STUDY OF THE ANTIOXIDANT ACTIVITIES OF METHANOLIC
EXTRACT AND SIMULATED GASTROINTESTINAL ENZYME DIGEST OF
BAMBARA NUT (*Vigna subterranean*)**

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ABSTRACT

The present investigation seeks to compare the antioxidant activities of solvent extracted (methanol) and *in vitro* enzyme digested Bambara nut. Bambara nut was extracted with methanol and another portion was subjected to an *in vitro* human digestion model that stimulated the composition of the gastrointestinal tract. The HPLC-DAD phenolic analysis revealed the presence of some phenolic acids and flavonoids, with reduced levels of most of the identified compounds after cooking process. The result of the antioxidant indices (total phenol, total flavonoid, ferric reducing antioxidant power, ABTS and DPPH radical scavenging activities) of the methanolic extract revealed a higher potency for the raw methanolic extract compared with the cooked methanolic extract. The assessment of the *in vitro* enzyme digested Bambara nut for antioxidant action revealed a high activity for the enzyme digested Bambara nut compared with the methanolic extract. Conversely, the cooking process improved the antioxidant activity after *in vitro* enzyme digestion. The result of this study indicates that the selected legume has some phyto-constituents with some measure of antioxidant action and that the phyto-constituents will be better released after passing through the gastrointestinal tract, and therefore could be harnessed as a functional food with potential health benefits.

Keywords: Antioxidant Activity; Bambara nut; Methanolic Extraction; *In vitro*

INTRODUCTION

Leguminous seeds are important sources of proteins, energy and other nutrients in the diets of large population of people around the world; they form excellent source of lysine, methionine and tryptophan, other water-soluble vitamins (riboflavin, niacin and folacin) and minerals: phosphorus, iron and magnesium (Ekpeyoung and Borchers, 1980; Doughty and Walker, 1982; Ramcharan and Walker, 1985; Food and Agriculture Organisation, 2012). In addition to dietary fiber and the basic nutrients, legumes also contain many health-promoting components, which include phenolic compounds. Phenolic compounds in legumes may be classified in simple terms into phenolic acids (derivatives of benzoic acid or cinnamic acid), flavonoids and tannins (Salawu *et al.*, 2014).

Grain legumes are grouped into two classes; major and minor species. The major species include the industrial legumes such as soybean and groundnut, common beans (*Phaseolus vulgaris*), chicken pea (*Cicer arietinum*), and pea (*Pisum sativum*). Minor species exist in a wide range of diversity either as cultivated or wild species across various regions of the world and are usually cultivated by the traditional farmers; they are also referred to as neglected, underutilized, under-cultivated or lesser-known legumes (Omitogun *et al.*, 2001; Aremu *et al.*, 2006). The underutilized legumes include; Bambara groundnut, Jack bean,

Mung bean, Pigeon pea, Rice bean, African yam bean, Crab's eye pea, locust bean, Velvet bean, Riverhemp, and Sword bean (Aremu *et al.*, 2006; Agbolade *et al.*, 2013).

A number of studies on the nutraceutical value of well-known legumes such as common beans, lentils, peas and soya beans have been conducted. However, a wide gap exists regarding the nutraceutical potential of underutilized legumes such as Bambara groundnut, Jack bean, Mung bean, Pigeon pea, Rice bean, African yam bean, Crab's eye pea, locust bean, Velvet bean, Riverhemp, and Sword bean. Exploring underutilized legume resources is of great importance considering their rich nutraceutical value and the presence of bioactive compounds (Bhat and Karim, 2009). Bambara groundnuts (*Vigna subterranean* L. verdc) have a long history of cultivation and are predominantly grown in drier areas with short inconsistent rainfall in the Sub-Saharan Africa (FAO, 2001). Bambara nut are not popularly consumed legume but in some places mostly in the rural areas they are grown for their seeds which are used for dietary purposes.

Free radicals which are highly reactive chemical substances that travel round the body and cause damage to the body cells have been implicated in various human ailments such as cancer, rheumatoid arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis and AIDS (Lee *et al.*, 2000;

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Pourmorad *et al.*, 2006). Antioxidants possess the ability to scavenge these radicals and destroy them

before they cause damage to the body cells (Oboh and Rocha, 2007). Antioxidants comprise some

vitamins, minerals, carotenoids, polyphenols, which are all present in a variety of foods like grains, fruits, vegetables, legumes, dairy products, spices (Oboh and Rocha, 2007). A number of studies have shown that legumes are rich in antioxidants; peanut, lentils and soybean (Zou *et al.*, 2011), Bambara groundnut (Nyau, 2013), African yam bean and (Oboh *et al.*, 2009).

The methods commonly used for the extraction of phytochemical constituents in legumes and other plant foods are usually organic solvents (Oboh *et al.*, 2009; Nyau, 2013). While the products investigated clearly demonstrate a range of antioxidant contents, they do not necessarily relate to the available antioxidants when the legumes are degraded by the enzymes and juices of the gastrointestinal tract which can be released during passage through the gastrointestinal tract.

Therefore, the present investigation seeks to assess the phenolic composition of Bambara nut as legume and analyze its antioxidant potential using the conventional solvent extraction and the simulated *in vitro* enzyme gastrointestinal digestion methods.

MATERIALS AND METHODS

Chemicals

2-Deoxy-D-ribose (Cat No - #D5899), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

diammonium salt (Cat No -#11557), 2,2-Diphenyl-1-picrylhydrazyl (Cat No - #D9132) Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), hydrogen peroxide (H₂O₂), ferrous sulphate, potassium dichromate (K₂Cr₂O₇), Ferric chloride (FeCl₃), Methanol, Folin-Ciocalteu's phenol reagent, sodium bicarbonate, aluminium chloride, potassium acetate, sodium phosphate dibasic, sodium phosphate monobasic disodium phosphate, potassium ferricyanide, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), orthophosphoric acid, quercetin, hydrochloric acid, sulphuric acid, chloroform, calcium chloride, vitamin C, tannic acid, sodium carbonate, aluminium chloride, gallic acid, quercetin, ascorbic acid, glacial acetic acid were obtained from Sigma chemical company, USA. The chemicals used were of analytical grades while the water was glass distilled.

Sample treatment and preparation

A large quantity of Bambara nuts were washed with water to remove dirty particles and stones, after which they were divided into two equal portions. The first portion was milled raw, while the second portion was cooked by boiling at 100⁰C for four hours, cooled, sun-dried and then milled into fine powder. The milled samples were stored in

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plastic containers at room temperature in the Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria until used.

Preparation of methanolic extracts

About five gram (5g) of the milled samples were dissolved in 80 mL of 1% acidified methanol. The extraction was allowed for 24 hrs and the extract was then filtered through whatman filter

paper no 42. The filtrate was put in a standard flask and then made up to 100 mL mark with methanol. Thereafter, the filtrate was put inside amber bottle and stored at -4 °C prior use for antioxidant indices determination.

***In vitro* enzymatic procedure**

The *in vitro* digestion using sequential enzymatic steps is based on a slightly modified method reported by Deigado-Andrade *et al.*, (2010). Two grams of the Bambara nut samples was weighed and dissolved in 40 ml of distilled water. 300µl of alpha amylase (32.5 mg of alpha amylase was dissolved in 25 ml of 1mM calcium chloride at pH 7) was added to the tubes. The tubes were incubated in a shaking water bath set at 37 °C and at 80strokes/min. After 10 minutes, the pH was adjusted to 2 using concentrated HCl. After 30 minutes incubation, 2mg pepsin which was dissolved in 1ml of 0.05 M HCl was added to the tube. The tubes were then incubated again using the same incubation condition. After further 20 minutes of shaking the tubes, the pH was adjusted to 6 using 0.5 M NaOH. Then 10 ml of pancreatin (3 gram of pan creatin was dissolved in 20 ml distilled water) was added and the tubes were incubated in a shaking water bath set at 37 °C for 20 minutes. The pH was adjusted finally to 7.5 using NaOH

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and epicatechin, 325 nm for chlorogenic, caffeic and ellagic acids, and 365 nm for isoquercitrin, quercitrin, quercetin, rutin, kaempferol and luteolin. The samples and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock

(simulating pH conditions in the small intestine). The tubes were incubated for 10 minutes in a shaking water bath set at 37 °C. The digested sample was incubated at 100 °C for 4 minutes to inactivate the enzymes and the digested sample was then centrifuged for 60 minutes at 3200 g and then the soluble fraction was kept in the refrigerator for antioxidant analysis. The insoluble fraction was discarded. An undigested control was also done with the same procedure used for the *in vitro* digestion but without the enzymes.

Estimation of Phenolic Compounds in Bambara Nut using HPLC-DAD

Reverse phase chromatographic analyses were carried out under gradient conditions using C₁₈ column (4.6 mm x 150 mm) packed with 5µm diameter particles; the mobile phase was water containing 2% acetic acid (A) and methanol (B), and the composition gradient was: 5% of B until 2 min and changed to obtain 25%, 40%, 50%, 60%, 70% and 100% B at 10, 20, 30, 40, 50 and 80 min, respectively, following the method described by Amaral *et al.* (2013) with slight modifications. The Bambara nut (raw and cooked) was analyzed at a concentration of 15 mg/mL. The flow rate was 0.7 mL/min, injection volume 50µl and the wavelength were 254 nm for gallic acid, 280 nm for catechin

solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.025 – 0.300 mg/mL for isoquercitrin, quercitrin, quercetin, rutin, luteolin and kaempferol; and 0.040 – 0.250 mg/mL for gallic acid, chlorogenic acid, caffeic acid, ellagic acid, catechin and epicatechin.

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, as defined by Sabir *et al.* (2012). LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

ANTIOXIDANT INDICES

Total phenolic content (TPC)

The total phenolic content of the extract and the digest was determined by the Folin-Ciocalteu assay as described by Waterman and Mole (1994). 500 μ L of Folin reagent was added and mixed with a solution containing 100 μ L of the extract and 2ml of distilled water. 1.5 mL of 7.5% sodium carbonate was then added to the solution and the volume was made up to 10mL with distilled water. The mixture was left to stand for 2 h after addition of the sodium carbonate and the absorbance of the mixture was

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by Oyaizu (1986). Briefly, appropriate dilution of each extract (1ml) was mixed with 1ml of 200 mM sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. Each mixture was incubated at 50°C for 20 min and then 1 ml of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 2ml of the supernatant was mixed with 2ml of distilled water

measured at 760 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer, USA). The standard used was tannic acid and the result was expressed as mg tannic acid equivalents per gram of the sample.

Total flavonoid content (TFC)

The total flavonoid content of the extract and the digest determined using a slightly modified method reported by Mea *et al* (2005). Briefly, 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 μ L of 10% $AlCl_3$, 50 μ L of 1 mol L^{-1} potassium acetate and 1.4 mL water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of each reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using quercetin as standard by making use of a seven point standard curve (0-40 μ g/ml or 0-100 μ g/ml), the total flavonoids content of samples 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 extract and the digest was determined by assessing the ability of each extracts to reduce $FeCl_3$ solution as described

and 0.4 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant power was determined in triplicate and expressed as mg ascorbic acid equivalent/g of the sample.

ABTS antiradical assay

Antioxidant activity of the extract and the digest was determined using the 2, 2'-azinobis-3-

ethylbenzothiazoline-6-sulfonic acid (ABTS) antiradical assay (Awika et al., 2003). The ABTS⁺ mother solution was prepared by mixing equal volumes of 8 mM ABTS and 3 mM potassium persulphate (K₂S₂O₈) in a volumetric flask, which was wrapped with foil and allowed to react for a minimum of 12 h in dark. The working solution was prepared by adding 2.5 ml of the mother solution with 7.5 ml phosphate buffer (pH 7.4). A range of trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-carboxylic acid) standard solutions (100–1000 µM) were prepared in acidified methanol. The working solution (2.9 ml) was added to the methanol extracts (0.1 ml) or Trolox standard (0.1 ml) in a test tube

and mixed with a vortex. The test tubes were allowed to stand for exactly 30 min. The absorbance of the standards and samples were measured at 734 nm with a Lambda EZ150 spectrophotometer. The results of the triplicate experiments were expressed as µM Trolox equivalents/g sample, on dry weight basis

DPPH antiradical assay

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RESULTS AND DISCUSSION

Experimental studies have shown that legumes exhibit significant antioxidant activity (Beninger and Hosfield 2003; Lee *et al.*, 2004; Heimler *et al.*, 2005). It is widely accepted that significant antioxidant activity of food is related to high phenolic composition.

The DPPH assay was done according to the method of Brand-Williams *et al.*, 1995, with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100mL methanol and then stored at -20°C until needed. The working solution was obtained by mixing 10mL stock solution with 45mL methanol to obtain an absorbance of 1.1 units at 515 nm using the spectrophotometer. Phenol extract and the digest (300µl) were allowed to react separately with 2700 µl of the DPPH solution for 6 h in the dark. Then the absorbance was taken at 515 nm. Results which were determined in triplicates were expressed in µM Trolox Equivalent/g sample.

Statistical Analysis

All the analyses were run in triplicates. Results were then computed using Microsoft Excel software (Microsoft Corporation, Redmond, WA) and followed by one –way Anova Duncan’s Multiple Range Test (DMRT) to compare the means that showed significant variation by using SPSS 11.09 for windows. The significance level was set at $p < 0.05$.

The HPLC-DAD qualitative estimation of phenolic compounds in raw and cooked Bambara nut (Figure 1a-b) revealed the presence of gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, epicatechin, rutin, isoquercitrin, quercetin, kaempferol and luteolin.

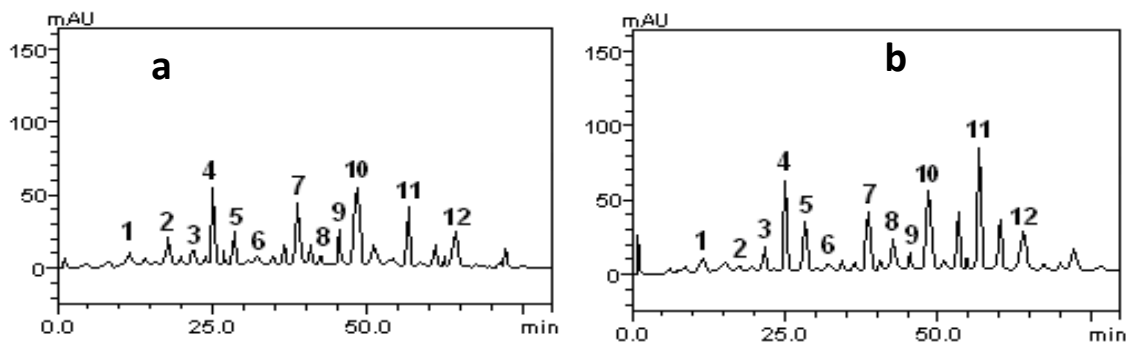


Figure 1 (a-b)– Representative of high performance liquid chromatographic profile of phenolic compounds in (a) Raw Bambara nut and (b) Cooked Bambara nut:

Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), epicatechin (peak 6), rutin (peak 7), isoquercitrin (peak 8), quercitrin (peak 9), quercetin (peak 10), kaempferol (peak 11) and luteolin (peak 12)

The quantitative estimate of the identified phenolic compounds in Bambara nut is as shown in Table 1. The result revealed a substantial reduction in the level of the identified phenolic compounds after cooking (gallic acid: 60.19%; catechin: 58.12%; chlorogenic acid: 78.90%; epicatechin: 66.09%; isoquercitrin: 30.95%; quercitrin: 22.93%; quercetin: 38.94%) with the exception of caffeic acid, ellagic acid, rutin, kaempferol and luteolin where increased values were observed after cooking.

This observation is in agreement with the report of Segev *et al.* (2011), where they reported a reduced

level of phenolic content for chicken pea after cooking. Similarly, the same observation was made by the report of Ukom *et al.*, (2014) and Didier *et al.*, 2014 which showed that cooking decrease the polyphenol content in *Xanthoma maffa* and *Dioscorea alata* tubers respectively after cooking. Phenolic compounds have attracted much interest recently because *in vitro* studies suggest that they have a variety of beneficial biological properties like anti-inflammatory, anti-tumor and anti-microbial activities (Hudson *et al.*, 2000; Ling *et al.*, 2001; Itani *et al.*, 2002; Morimitsu *et al.*, 2002).

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TABLE 1: QUANTITATIVE ESTIMATES OF PHENOLIC COMPOUNDS IN BAMBARA NUT

| Compounds | Raw (mg/g) | Cooked (mg/g) | % Reduction | LOD (µg/mL) | LOQ (µg/mL) |
|-------------|--------------------------|--------------------------|-------------|----------------|----------------|
| Gallic acid | 1.03 ± 0.01 ^b | 0.41 ± 0.02 ^a | 60.19 | 0.021 | 0.069 |

| | | | | | |
|-------------------|--------------------------|--------------------------|---------|-------|--------|
| Catechin | 2.34 ± 0.03 ^b | 0.98 ± 0.01 ^a | 58.12 | 0.009 | 0.034 |
| Cholorogenic acid | 2.37 ± 0.02 ^b | 0.50 ± 0.01 ^a | 78.90 | 0.034 | 0.113 |
| Caffeic acid | 3.65 ± 0.01 ^a | 3.75 ± 0.03 ^a | -2.74 | 0.015 | 0.045 |
| Ellagic acid | 1.09 ± 0.02 ^a | 1.42 ± 0.01 ^b | -30.27 | 0.007 | 0.0023 |
| Epicatechin | 1.15 ± 0.02 ^b | 0.39 ± 0.01 ^a | 66.09 | 0.028 | 0.092 |
| Rutin | 1.12 ± 0.01 ^a | 3.16 ± 0.03 ^b | -182.14 | 0.035 | 0.117 |
| Isoquercitrin | 0.42 ± 0.01 ^b | 0.29 ± 0.01 ^a | 30.95 | 0.019 | 0.061 |
| Quercitrin | 2.05 ± 0.03 ^b | 1.58 ± 0.02 ^a | 22.93 | 0.008 | 0.026 |
| Quercetin | 6.39 ± 0.01 ^b | 3.94 ± 0.01 ^a | 38.34 | 0.024 | 0.073 |
| Kaempferol | 2.18 ± 0.02 ^a | 3.15 ± 0.01 ^b | -44.49 | 0.007 | 0.023 |
| Luteolin | 1.09 ± 0.01 ^a | 1.67 ± 0.03 ^b | -53.21 | 0.027 | 0.089 |

Results are expressed as mean ± standard deviations (n = 3). Averages followed by different letters differ by Tukey test at p < 0.05. LOD= Limit of detection, LOQ= Limit of qualification. Minus sign indicates percentage increase after processing

It has been established that organic extraction methods do not represent the natural conditions occurring *in vivo* in which antioxidant molecules are subjected to a number of physical and chemical changes during their gastro-intestinal tract (GIT) transit (Gil-Izquierdo *et al.*, 2001). Therefore, the antioxidant parameters were assessed on the methanolic extracts and *in vitro* enzyme digest of processed Bambara nut. This approach will be able to compare the antioxidant potential of the two methods and at the same time provide a base line

data on the actual antioxidant potential of Bambara nut as it passes the gastrointestinal tract.

The result of the total phenolic content (mg TAE/g) of methanolic extract of Bambara nut showed a reduced level after cooking (raw: 81.30 ± 1.91; cooked: 26.02 ± 1.91). The reduction in the phenolic content during cooking might be due to the effect of heat on some sensitive phenolic compounds (Adedayo, 2012; Minaxi *et al.*, 2013; Ukom *et al.*, 201). The result of the total flavonoid (mgQE/g) and ferric reducing property (mg

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AAE/g) of the methanolic extract of Bambara nut (Table 1) also showed a reduced level after cooking; total flavonoid content (raw: 14.47 ± 0.31; cooked: 12.53 ± 0.084); ferric reducing property (raw: 17.47 ± 0.10; cooked: 12.63 ± 0.11). The reduced total flavonoid content is in agreement with the report of Ezeocha and Ojmelukwe (2012).

Flavonoids are potent water-soluble antioxidants which prevent cell damage and have strong anti-haemorrhoidal activity (Okwu and Orji, 2007). Flavonoids have been found to possess antioxidant and anti-inflammatory activities and were also useful for sexual stimulation (Yao, 2004). Flavonoids had the record of being a powerful water

soluble free radical scavengers and powerful antioxidants which could prevent oxidative cell damage, have a potent anticancer activity and inhibit tumour growth (Xiao, 2011). It also contains hydroxyl functional group, which are responsible for antioxidant effect in some medicinal plants. The result of ABTS radical scavenging activity of the

methanolic extract expressed in $\mu\text{M TE/g}$ of the sample (raw: 828.40 ± 6.46 ; cooked 149.18 ± 3.29) and DPPH radical scavenging activity of the methanolic extract expressed in $\mu\text{M TE/g}$ of the sample as shown in Table 2 (raw: 72.24 ± 1.47 ; cooked: 67.44 ± 2.13), equally showed a reduced level after cooking.

TABLE 2: ANTIOXIDANT INDICES OF BAMBARA NUT USING METHANOLIC EXTRACTION AND SIMULATED *IN VITRO* ENZYME DIGESTION METHODS

| Sample | Total phenol (mg TAE/g) | Total flavonoid (mg QE/g) | Reducing power (mg AAE/g) | ABTS ^{•+} ($\mu\text{M TE/g}$) | DPPH [•] ($\mu\text{M TE/g}$) |
|---------|----------------------------|------------------------------|------------------------------|--|---|
| R-BN-ME | 81.30 ± 1.91 | 14.47 ± 0.31 | 17.47 ± 0.10 | 828.40 ± 6.46 | 74.24 ± 1.47 |
| C-BN-ME | 26.02 ± 1.91 | 12.53 ± 0.084 | 12.63 ± 0.11 | 149.18 ± 3.29 | 67.44 ± 2.13 |
| R-BN-D | 88.41 ± 3.64 | 17.30 ± 0.054 | 27.59 ± 0.12 | 4341.49 ± 10.27 | 24.47 ± 3.27 |
| R-BN-NG | 38.39 ± 5.74 | 13.69 ± 0.55 | 16.27 ± 0.21 | 1983.59 ± 6.72 | 7.54 ± 0.78 |
| C-BN-D | 92.23 ± 3.64 | 20.99 ± 0.25 | 38.44 ± 0.10 | 4375.11 ± 11.81 | 19.67 ± 2.45 |
| C-BN-NG | 38.39 ± 3.31 | 9.95 ± 0.24 | 14.05 ± 0.13 | 1163.26 ± 6.72 | 13.67 ± 3.21 |

Values represent mean \pm standard deviation of triplicate experiments. TAE= Tannic Acid Equivalent; QE= Quercetin Equivalent; AAE= Ascorbic Acid Equivalent; TE= Trolox Equivalent. R-BN-D = Raw Digested Bambara Nut; R-BN-NG = Raw Non-Digested Bambara Nut; C-BN-D = Cooked Digested Bambara Nut; C-BN-NG = Cooked Non-Digested Bambara Nut; R-BN-ME= Raw Bambara Nut Methanolic Extract; C-BN-ME= Cooked Bambara Nut Methanolic Extract

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However, the result of the antioxidant indices of the *in vitro* digested Bambara nuts ranked higher in the raw and cooked Bambara nut respectively (total phenolic content: 88.41 ± 3.64 , 99.23 ± 3.64 ; total flavonoid: 17.30 ± 0.054 , 20.99 ± 0.25 ; reducing power: 27.59 ± 0.12 , 38.44 ± 0.10 and ABTS radical scavenging activity: 4341.149 ± 10.27 , 4375.11 ± 11.81), compared with the non-enzymatic digest (control) and methanolic extract, with the exception

of the DPPH radical scavenging activity where a higher value was recorded for the methanolic extract. Thus, maximum amount of antioxidant compounds were released during *in vitro digestion* process as a result of the activity of the enzymes (α -amylase, pepsin and pancreatin) of the gastrointestinal tract. This observation is in agreement with previous report (Tagliazucchi *et al.*, 2010) that concluded that digestion may be a

determinant in the release of nutritionally relevant compounds from the food matrix. In addition, the result is also in agreement with the report of Gil-Izquierdo *et al.* (2001), who concluded that solvent extraction methods do not represent the natural conditions occurring *in vivo*, in which phenolic compounds are subjected to a number of physical and chemical changes during their gastro intestinal tract (GIT) transit and are released from the food matrix. Conversely, higher antioxidant indices were recorded for the cooked Bambara nut subjected to *in vitro* enzyme digestion when compared with raw digested Bambara nut, with the exception of DPPH radical scavenging activity where a higher value was recorded for the raw *in vitro* enzyme digested Bambara nut. The increase in the antioxidant indices of the *in vitro* digested cooked Bambara nut compared with the raw digest may be due to alterations in chemical structure and composition as a result of heat during boiling (Cohen *et al.*, 2001). Thus, cooking might have enhanced the breakdown of insoluble fiber matrix of Bambara nut thereby

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making its polyphenols more accessible for further breakdown by the enzymes of the gastro-intestinal tract (Tagliazucchi *et al.*, 2010).

CONCLUSION

This present study reaffirmed that the antioxidant values obtained through the conventional solvent extraction method is not exactly the same with what actually happens in the gastrointestinal tract. The result of the study therefore is an indication that the selected underutilized legume possesses antioxidant molecules which would be better released after the gastrointestinal enzyme digestion compared with the solvent extract. Therefore, the consumption of Bambara nut could be harnessed as natural product in the management free radical mediated diseases.

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