

Research Article

Determination of Chemical Composition, Nutritional Quality and Anti-Diabetic Potential of Raw, Blanched and Fermented Wonderful Kola (*Bucholzia coriacea*) Seed Flour

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- Chemical composition
- Anti-diabetic potentials

Abstract

Background: Plants contain nutrient and phytochemicals, which serve as food and medicine to promote good nutritional and health wellbeing in human. *Buchholzia coriacea* is a perennial plant, and its seeds contain nutrient and medicinal properties. The present study, therefore, aimed at determining influence of blanching and fermentation processing techniques on nutrient composition, phytochemicals and anti-diabetic potentials of the seed.

Methods: The *Buchholzia coriacea* seeds were processed as raw, blanched and fermented flour samples. The flour samples were analysed for nutrient composition, phytochemicals and anti-diabetic activity using standard methods after processed as raw, blanched and fermented flour samples.

Results: Proximate composition of the flour samples ranged as follows: moisture contents 7.99 ± 0.44 - 10.12 ± 0.95 g/100g, crude protein 12.92 ± 3.53 - 15.41 ± 1.95 g/100g, crude fibre 2.51 ± 1.29 - 3.54 ± 1.64 . For mineral contents, RWC flour sample had the highest values for Mg, Fe, Na, K, Mn and Co than FWC and BWC flour samples. The Na/K, Ca/P, Ca/Mg, Ca/K and Na/Mg molar ratios ranged as follow: 0.39-0.44, 7.54-8.49, 0.74-1.28, 0.34-0.63 and 0.89-0.98 respectively. Total amino acid profiles of RWC, BWC and FWC were 73.66, 73.31 and 68.96 mg/100g of protein respectively. The predominant polyunsaturated and monounsaturated fatty acids were linoleic acid (29.59 ± 0.00 - 33.27 ± 0.0 mg/100g) and oleic acid (14.29 ± 0.00 - 12.54 ± 0.00 mg/100g), while linolenic acid (8.92 - 9.71 mg/100g) and palmitoleic acid (2.32 ± 0.0 - 2.72 ± 0.00 mg/100g) were the lowest respectively. Essential amino acid index (EAAI) of BWC (51.68%) had the highest value compared with FWC (47.70%) and BWC (45.14%). For predicted-protein efficiency ratio (P-PER), BWC had the highest value (2.12), while FWC had the lowest value (1.77). Predicted-biological values of RWC, BWC and FWC were 43.69%, 44.63% and 40.29% respectively, while nutritional index of the samples ranged from 6.36 in RWC to 8.58% in BWC. Polyunsaturated/saturated fatty acid ratio (P:S), atherogenic index (AI) and lipid preventive score (LPS) of the seed flour samples ranged as follows: 1.16 - 1.50, 0.504 - 0.679 and 141.52 - 158.43 respectively. The oxalate, trypsin, saponin, alkaloid and flavonoid were lower in blanched and fermented samples than in RWC. The molar ratios of phytate/mineral of the flour samples ranged as follows: Phytate: zinc (49.833 - 92.412), phytate/calcium (0.062 - 0.092) and (Ca) (Phytate): zinc (2.056 - 6.353) and phytate: Iron (0.35 - 0.483). The percentage reduction of blood glucose in diabetic-induced rats fed with FWC samples had the highest value, while those rats fed with RWC sample had the lowest value.

Conclusion: The study established that the nutritional efficacy and anti-diabetic potentials of blanched *Bucholzia coriacea* flour sample were higher than raw and fermented flour samples.

INTRODUCTION

Plants are important for human existence and they are the major sources of foods and medicine [1]. Plant-based foods are important components of traditional diets in many parts of developing countries [2]. For instance, plant-based foods are very good sources of protein, fibers, minerals and vitamins [3], and consumption of these vegetables/fruit can help to prevent diabetes, cancers [4,5] and heart disease like stroke, high blood pressure and accumulation of cholesterol [6]. The use of plants

in traditional medicine has a long history in the life of a man, and it remains the mainstay of primary health care in most of the developing countries. Plant-based medicines are used by over 60% of the world population; in both developing and developed countries where modern medicines are predominantly used [7]. In African countries large proportions of the population depends solely on herbal medicines for its primary health care needs.

Buchholzia coriacea or drumstick is found in many parts of African countries like Nigeria, Ghana and Liberia. The tree with

large, glossy leaves and conspicuous cream white flowers in racemes at the end of the branches [8], is a perennial plant which grows as a forest tree and it belongs to the family capparacea. The local name includes "uwuro" (Yoruba), "esson bossi" (Central Africa), "uke" (Ibo), "ovu" (Bini) and Aponmu (Akure). The plant parts commonly eaten are the seed, which are either cooked or eaten raw [9]. *Buchholzia coriacea* is a brain food, which promotes memory, useful in the treatment of hypertension and prevents premature aging [10]. It is evident in some parts of Africa that wonderful kola has the ability to stop migraine headache [10]. The *Buchholzia coriacea* plants serves as food and medicine in many parts of African countries, hence, gave the plant a common name wonderful kolanut.

Buchholzia coriacea plants contain phytonutrients (protein, fiber, fat/oil, carbohydrate, minerals and vitamins) and phytochemicals, which promote good nutritional and health wellbeing in man [10]. Phytochemicals or bioactive plant components are naturally occurring chemical components that give plants color, flavor and smell, and are part of a plant naturally defense system for the plant (disease resistance) [11]. Phytochemical has been considered of crucial nutritional and health important in terms of preventing chronic disease such as cancer, cardiovascular disease and diabetics [5,12]. Plant-based foods contain biologically active components or phytochemicals, and that regular consumption of these phytochemicals has always been associated with health benefits [4]. The therapeutic efficacy of many indigenous plants for various diseases lies in some chemical substances that produce define physiological action in the human body [5]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds [13].

Despite tremendous progress in western medicines, diseases like diabetes, hypertension and cancer are still a major threat in developing countries, due to relatively high cost of synthetic drugs and emergence of drug resistance. Recently, evident has shown that there is a gradual revival of interest in the use of medicinal plants, which has formerly been neglected in developing countries, because of its safety and less side effect especially when compared with synthetic drugs [14]. Therefore, the present study aims at evaluating the effects of processing methods on chemical compositions, glyceamic index and antidiabetic potentials of *Buchholzia coriacea* seeds, which would further enhances its utilization as food or medicine.

MATERIALS AND METHODS

Collection and Identification of Plant

Freshly harvested *Buchholzia coriacea* seeds were brought from Ojee market in Ibadan North-East local Government of Oyo State. The plant seeds were identified and authenticated at Herbarium Unit of Department of Crop Production and Pest, Federal University of Technology, Akure, Nigeria.

Processing of *Buchholzia coriacea* seed into flour

Wonderful kola seeds were processed into raw, blanched and fermented flour as described below:

Raw wonderful kola seed flour

Raw *wonderful kola* seeds were sorted pretreated for 5 min with 200 ppm of bleach containing 5.25% sodium hypochlorite, mixed in deionized water to control microbial growth. The seeds were rinsed, soaked in deionized water (1:3, w/v) for 9 hr at ambient temperature (23–25°C), dehulled, sliced manually and oven dried at 60°C (Plus11 Sanyo Gallenkamp PLC, UK) for 20 hours, milled using Philips laboratory blender (HR2811 model) and sieved using a 60 mm mesh sieve (British Standard) to obtain raw wonderful kola seed flour. The flour was packed in a plastic container seal with an aluminum foil and stored at room temperature (~27°C) until required for use (Figure 1).

Blanched wonderful kola seeds flour

Wonderful kola seeds were sorted pretreated for 5 min with 200 ppm of bleach containing 5.25% sodium hypochlorite, mixed in deionized water to control microbial growth. The seeds were rinsed, sliced into smaller pieces, blanched for 30 minutes, drained, oven dried at 60°C (Plus11 Sanyo Gallenkamp PLC, UK) for 20 hours, milled using Philips laboratory blender (HR2811 model) and sieved using a 60 mm mesh sieve (British Standard) to obtain blanched wonderful kola seed flour. The flour was packed in a plastic container sealed with an aluminum foil and stored at room temperature (~27°C) until required for use (Figure 1).

Fermented wonderful kola seed flour

Wonderful kola seeds were sorted, dehulled, boiled for 1 hour as described by [15], wrapped in blanched banana leaves at room temperature for 72 h to ferment. The fermented seeds were oven dried at 50°C (Plus11 Sanyo Gallenkamp PLC, UK) for 20 hours, milled using a Philips laboratory blender (HR2811 model) and sieved using a 60 mm mesh sieve (British Standard). The wonderful kola flour was packed in plastic container sealed with aluminum foil and stored at room temperature (27°C) prior to analyses (Figure1).

CHEMICAL ANALYSES

Proximate Analyses Macronutrient analysis

Nutrient composition of the food sample was determined using the standard procedures of Association of Official Analytical Chemists [16]. Triplicate samples were used for moisture content in a hot-air circulating oven (Gallenkamp). Ash was determined by incineration (550°C) of known weights of the samples in a muffle furnace (Hotbox oven, Gallenkamp, UK, size 3) (Method No 930.05) [16]. Crude fat was determined by exhaustively extracting a known weight of sample in petroleum ether (boiling point, 40 to 60°C) using Tecator Soxtec (Model 2043(20430001), 69, Slandegarupgade, DK-3400, Hilleroed, Denmark) (Method No 930.09) [16]. Protein content ($N \times 6.25$) was determined by the micro-Kjeldahl method (Method No 978.04) [16]. Crude fiber was determined after digesting a known weight of fat-free sample in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide (Method No 930.10) [16]. Carbohydrate content was determined by difference, that is, addition of all the percentages of moisture, fat, crude protein, ash and crude fibre was subtracted from 100%

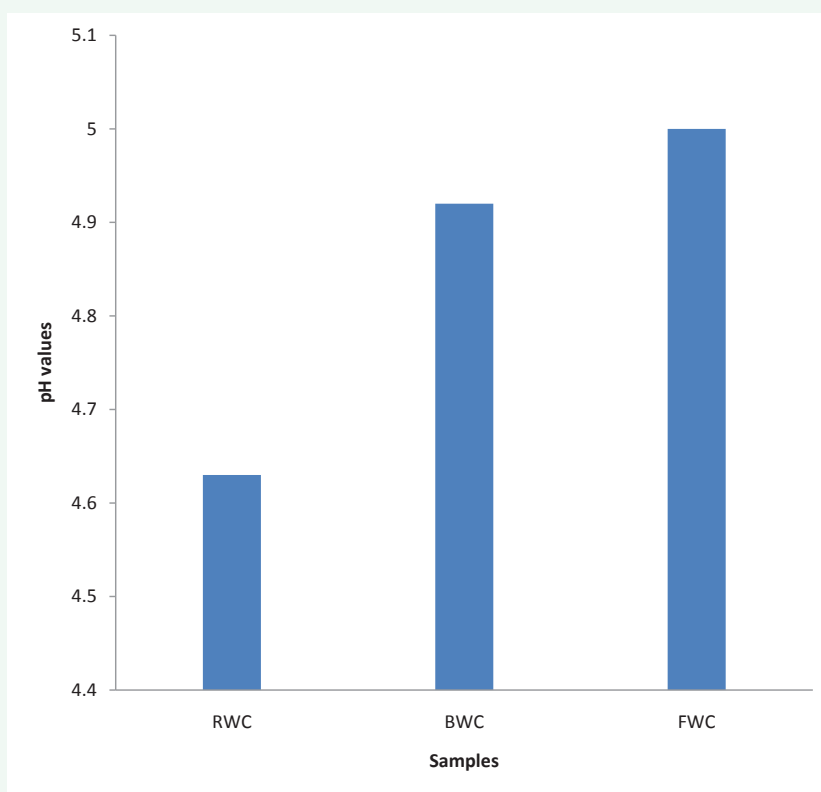


Figure 1 pH concentration of the raw, blanched and fermented wonderful kola

.This gave the amount of nitrogen free extract otherwise known as carbohydrate.

$\% \text{ carbohydrate} = 100 - (\% \text{Moisture} + \% \text{Fat} + \% \text{Ash} + \% \text{Crude fibre} + \% \text{Crude protein})$

The energy value of the samples were estimated [in kcal/g] by multiplying the percentages of crude protein, crude lipid and carbohydrate with the recommended factors (2.44, 8.37 and 3.57 respectively) as proposed by [17].

Mineral Analyses

The method described by Association of Official Analytical Chemists [16]. Was used for mineral analysis. Two grams (2 g) of each of the samples was digested with concentrated nitric acid and hydrogen peroxide, filtered and the filtrate in a 5 mL volumetric flask was loaded to Atomic Absorption Spectrophotometer, (model703 Perkin Elmes, Norwalk, CT, USA). Calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), sodium (Na), potassium (K) were determined at wavelengths 317.9 nm, 285.2 nm, 259.9 nm, 324.7 nm, and 213.9 nm respectively. Sodium [Na] and Potassium [K] were determined using flame emission photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK), and NaCl and KCl were used as the standards [16]. Phosphorus was determined using Vanado-molybdate method. The serially diluted phosphate standard solution was made acidic by addition of 2 ml nitric acid (2:1), 25ml of the Vanodo-molybdate reagent was added, the solution was diluted to the mark with distilled water, mixed thoroughly and allowed to stand for 10 minutes and the optical density was measured at 47mu. All values were expressed in mg/100g.

Determination of fatty acids

Fatty acids profile was determined using gas chromatography (GC) (PerkinElmer, Norwalk, USA) in accordance with [16]. Method number Ce 1b-89. Samples (10 g) were mixed with chloroform (100ml) for 2 min with the Ultra- Turrax followed by centrifuging at 2000 rpm for 5 min, filtered over a filter paper with anhydrous sodium sulphate and evaporated (20 ml) under a stream of nitrogen at 40°C. Fat (0.5 g) was dissolved in diethyl ether (2 ml), 0.5 ml of KOH in methanol (MeOH) solution was added to the dissolved fat solution. To the soap solution, water (2ml) and hexane (15 ml) were added, shaken thoroughly, decanted, and washed with water to remove residual hexane, dried using anhydrous sodium sulphate. Dried samples were transferred to a GC-auto sampler vial. Samples and standards were run on the gas chromatography (GC). Percentages of peak areas obtained were divided by the relative molecular weight of respective FAME to obtain moles percent of FA.

Amino acid analysis

Amino acid analysis was by Ion Exchange Chromatography (IEC) [18] using the Technicon Sequential Multisample (TSM) amino acid analyzer (Technicon Instruments Corporation, New York). The period of analysis was 76 min for each sample. The gas flow rate was 0.50 mL/min at 60 °C with reproducibility consistent within ± 3 min. The net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of two determinations. Nor-leucine was the internal standard. Tryptophan was not determined.

Determination of Hydrogen Ion Concentration (pH)

Two grammes (2 g) of the wonderful kola flour samples was weighed and mixed with 20 ml of distilled water and stirred thoroughly to form slurry. The digital pH meter (Crison Basic model 20) which was standardized with buffer 7.0 was used to determine the pH of the samples by dipping the electrode of the digital pH meter into the slurry of the wonderful kola sample. Triplicate determinations were made in all cases.

Determination of Total Titratable Acid

One gram (1g) of the wonderful kola sample was weighed into beaker and 10 ml of distilled water was added, phenolphthalein indicator was also added and mixed thoroughly. Ten-milliliter aliquots of the filtrate were titrated against 0.1 N NaOH standard solution. Triplicates were also made in all cases. Acidity was expressed as lactic acid based on the conversion of 1 ml of 0.1 N NaOH being equivalent to 9.008×10^{-3} g lactic acid [19].

Predicted Nutritional quality

Protein efficiency ratio (PER): Protein efficiency ratio of the raw, blanched and fermented *Moringa oleifera* leaves flour samples were calculated according to the equations developed by [20].

$$PER = 0.06320 [X_{10}] - 0.1539$$

Where X_{10} = Thr + Val + Met + Ile + Leu + Phe + Lys + His + Arg + Tyr

Essential Amino Acid Index (EAAI): Nutritional qualities were determined on the basis of the amino acid profiles. The Essential Amino Acid Index (EAAI) was calculated using the method of Labuda *et al.* [21] according to the equation below:

$$EAAI = \sqrt[n]{\frac{100a \times 100b \cdots 100j}{av \times bv \cdots jv}}$$

where:

n = number of essential amino acids, $a, b, \dots, j$ = represent the concentration of essential amino acids (lysine, tryptophan, isoleucine, valine, arginine, threonine, leucine, phenylalanine, histidine and the sum of methionine and cystine) in test sample and $av, bv, \dots, jv$ = content of the same amino acids in standard protein (%) (egg or casein) respectively.

Biological Value (BV): Biological Values were computed according to the methods of Oser [22], respectively. The following equation was used for BV determination.

$$BV = 1.09 (\text{EAA Index}) - 11.7.$$

Nutritional Index (NI): The nutritional index of the food samples was calculated using the formula below as described by Crisan and Sands [23].

$$\text{Nutritional index } [\%] = \frac{\text{EAAI} \times \% \text{ protein}}{100}$$

Calculation of Atherogenic Index (AI) and Lipid Preventive Score (LPS): The atherogenic index (AI) of the fatty acids in the formulated samples were calculated on the basis of the content of the middle chain fatty acids C12:0, C14:0 and C16:0, and the groups MUFA and PUFA as described by [24] with the following cut-off < 0.11 for low cardiovascular risk, 0.11- 0.21

for intermediate cardiovascular risk and > 0.21 for increased cardiovascular risk.

$$AI = \frac{C12:0 + 4 \times C14:0 + C16:0}{MUFA + PUFA}$$

And the Lipid Preventive Score (LPS) was calculated using the formula reported by [25]

$$LPS = \text{Total lipid} + 2 \times SFA - MUFA - 0.5 \times PUFA$$

Phytochemicals Composition of Wonderful Kola Seed Flour

Phytates were determined by the anion-exchange method as described by AOAC [26] method number 986.11 using phosphate as the standard. Oxalate content of the food samples was determined using the method of AOAC [16]. Tannin contents were determined by the modified vanillin-HCl methods described by Latta and Eskin [27]. Trypsin activity of the samples was determined using the method of Prokopet and Unlenbruck [28]. Saponin analysis was determined using Spectrophotometric method as described by Brunner [29]. Total Phenolic Compounds (TPC) was determined using the methods of Singleton *et al.* [30]. While total flavonoid was determined according to the method outlined by Harborne [31] and Alkaloid was determined by the method described by Harborne [31].

Determination of antidiabetic potentials of wonderful kola in rats

Wistar rats (*R. norvegicus*) weighing 125g to 150 g obtained from the central animal house of College of Medicine, University of Ibadan, Nigeria, were used for the study. The rats were fed ad

labitum with water and feed (Guinea feed), and were allowed to acclimatize to the environment for 4 days under the standard laboratory condition of temperature ($26 \pm 2^\circ\text{C}$) and light (12 hours day length) and were allowed free access to feed and water.

Induction of experimental rats with diabetes

The experimental rats were induced by administering single interperitoneal injection of freshly prepared Alloxan solution (150mg/kg body weight) after fasted overnight (access to water only) for 12 hours [32]. The serum glucose levels were determined after 72 hours of induction; diabetes was confirmed in alloxan induced rats showing fasting blood glucose levels > 140mg/dl. The experimental rats were divided into four groups, and three of the groups were fed with raw, blanched and fermented wonderful kola seed flour, while the fourth group (control) was treated with metformin (50mg/kg body weight) was for seven days.

Blood glucose measurement

The blood glucose of the rats was measured every day. Blood was collected from tail of fasting animals. Tail was embedded in 45°C water bath and about one millimeter of its end was cut and a drop of blood was used for blood glucose test with the help of Accu Check Active Glucometer (Roche Switzerland).

Statistical Analysis

The data were analysed using SPSS version 16.0. The mean and standard error of means (SEM) of the triplicate analyses were

calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means, while the means were separated using the new Duncan multiple range test at $p < 0.05$.

RESULTS AND DISCUSSION

Chemical composition of wonderful kola seeds flour

Proximate composition- The proximate composition of *Bulchholzia coriacea* seed flour samples is presented in Table 1. The moisture contents of the flour samples ranged from 7.99 ± 0.44 g/100g in fermented wonderful kola (FWC) to 10.12 ± 0.95 g/100g in raw wonderful kola (RWC) sample. It was observed in the present study that the moisture contents of processed samples, that is, blanched and fermented wonderful kola, were lower than that of raw wonderful kola sample. The value of moisture contents in the wonderful kola in the present study were similar to the report of other researchers [33,34]. The high moisture contents observed in these flour samples shows that the flour samples cannot be stored for long time in order to avoid spoilage. It is well known that the amount of moisture content in flour samples is a determinant of storage ability of the food sample. For instance, high moisture content in food sample facilitate the growth and multiplication of microorganisms, and that the activities of these microorganisms are the major factors responsible for the food spoilage [35]. Protein content of raw wonderful kola (RWC) flour (12.92 ± 3.53 g/100g) was lower than fermented wonderful kola (FWC) (15.41 ± 1.95 g/100g) and blanched wonderful kola (BWC) (14.20 ± 2.51 g/100g). The present study established that the protein content of fermented flour sample (RWC) was insignificantly higher than that of raw and blanched wonderful kola flour sample (>0.05). This observation could be attributed to the various stages of fermentation processing, which involved heating and microbial activities. It is evident that fermentation increased the protein content of fermented food products over unfermented products [36,37]. This was attributed to microbial activities, which involve using crude fiber and carbohydrate as sources of energy and synthesizing structural proteins that are integral part of the microbial cells [38]. This present result

agreed with other findings who reported on the increased in protein content of fermented food products [36]. In comparing the protein contents in this present study with other studies, the values agreed with the reports of Amaechi [39] for raw wonderful kola seeds (13.28%) and Akubugwo *et al.* [40] for *Solanum nigrum var virginicum* (17.63%). However, it was higher than the report of Ekop (2007) [41] for fluted pumpkin seeds (7.0%) and Odebunmi *et al.* (2009) [42] for *Cola nitida* (2.63%), *Cola nitida* (2.38%) and *Afromomum melegueta* (7.18%). Hence, the seed can serve as an alternative source of plant protein. The crude fibre content of the flour samples ranged between 2.51 ± 1.29 g/100g in FWC and 3.54 ± 1.64 in RWC, and the values were higher when compared with bitter kola (1.23%) [43]. Nutritional study has shown that adequate fiber intake render some health benefits like lowering the serum cholesterol level, risk of coronary heart diseases, hypertension, constipation and diabetes [44]. Therefore, the high fiber content in wonderful kola seeds are of nutritional and health benefits. Energy value of FWC (371.85 ± 3.48 kcal) was the highest when compared with RWC (354.82 ± 1.76 kcal) and BWC (363.71 ± 2.83 kcal), and the values were comparatively lower than *G. africanum* seeds (448.83kcal) [41] (Ekop, 2007) and *S. nigrum* seeds (403.54kcal) [40].

Mineral compositions: Mineral compositions of wonderful kola seeds are depicted in Table 2. The mineral contents of RWC, BWC and FWC ranged as follows: 0.003 ± 0.00 - 6.40 ± 0.10 mg/100g, 0.005 ± 0.00 - 4.65 ± 0.05 mg/100g and 0.002 ± 0.00 - 4.40 ± 0.10 mg/100g respectively. Potassium had highest concentration of the minerals and this agreed with the report of Aremu *et al.* [3], who established that potassium is the predominant mineral in plant products, whereas lead, cadmium and chromium were not detected in the flour samples. Comparatively, RWC had the highest values in Mg, Fe, Na, K, Mn and Co than FWC and BWC flour samples. The molar ratios of Na/K, Ca/P, Ca/Mg, Ca/K and Na/Mg ranged as follow: 0.39-0.44, 7.54-8.49, 0.74-1.28, 0.34-0.63 and 0.89-0.98 respectively. The Na/K and Ca/P molar ratios of wonderful kola were within the recommended ranged values of < 0.1 and > 2.0 respectively; and these indicated that the samples would facilitate bone and teeth formation in children, prevent osteoporosis in adults and high blood pressure in man. Scientific research has shown that calcium in combination with phosphorous, magnesium and manganese facilitate bone and teeth formation in children and bone maintenance in adult [45].

Amino Acid Profile of wonderful kola: The amino acid profile (mg/100g protein) of raw, blanched and fermented *Bulchholzia coriacea* seed flour is shown in Table 3. The amino acids of RWC, BWC and FWC had glutamic acid as the highest of amino acids with values of 15.43 ± 3.89 , 10.34 ± 0.39 and 13.84 ± 1.44 mg/100g of protein respectively; while tryptophan had the lowest concentration of amino acids with values ranged of 0.55 ± 0.01 mg/100g of protein in RWC to 0.79 ± 0.01 mg/100g of protein in FWC. The total amino acid of RWC had the highest concentration compared to BWC (73.31 mg/100g of protein) and FWC (68.96 mg/100g of protein) flour sample. The disparity in total amino acid profiles of the blanched and fermented flour sample compared with the raw sample could be attributed to the

Table 1: Proximate composition (g/100g dry weight matter) of raw, blanched and fermented *Bulchholzia coriacea* seed flour.

Parameters	RWC	FWC	BWC
Moisture	10.12 ± 0.95	7.99 ± 0.44	9.16 ± 0.41
Fiber	$3.54^a \pm 1.64$	$2.51^a \pm 1.29$	$2.54^a \pm 1.11$
Fat	$4.03^a \pm 1.07$	$4.78^a \pm 0.97$	$4.35^a \pm 1.05$
Ash	$2.66^a \pm 0.26$	$2.51^a \pm 0.48$	$2.56^a \pm 0.16$
Protein	$12.92^a \pm 3.53$	$15.41^a \pm 1.95$	$14.20^a \pm 2.51$
Carbohydrate	$66.91^a \pm 2.31$	$66.79^a \pm 3.30$	$67.19^a \pm 4.15$
Energy	$354.82^b \pm 1.76$	$371.85^a \pm 3.48$	$364.71^a \pm 2.83$

Means ($\pm$ SEM) with different superscripts in a row are significantly different at $P < 0.05$;

*FAO/WHO (1991).

Keynote

RWC = Raw wonderful kola seed flour

FWC = Fermented wonderful kola seed flour

BWC = Blanched wonderful kola seed flour

Table 2: Compositions of mineral (mg/100g) of raw, blanched and fermented *Bucholzia coriacea* seed flour.

Parameters	RWC	FWC	BWC
Ca	2.20 ^b ±0.10	2.75 ^a ±0.05	1.65 ^c ±0.05
Mg	2.95 ^a ±0.07	2.15 ^b ±0.05	1.85 ^c ±0.05
Fe	0.65 ^a ±0.05	0.49 ^b ±0.01	0.61 ^{ab} ±0.00
Na	2.76 ^a ±0.08	1.92 ^b ±0.01	1.83 ^b ±0.03
K	6.40 ^a ±0.10	4.40 ^b ±0.10	4.65 ^b ±0.05
Cu	0.25 ^a ±0.00	0.25 ^a ±0.002	0.13 ^b ±0.00
Mn	0.022 ^a ±0.00	0.009 ^b ±0.00	0.005 ^c ±0.00
Ni	0.003 ^{ab} ±0.00	0.002 ^b ±0.00	0.005 ^a ±0.00
Co	0.006 ^a ±0.00	0.003 ^b ±0.00	0.005 ^{ab} ±0.00
P	0.280 ^b ±0.01	0.365 ^a ±0.01	0.195 ^c ±0.01
Zn	0.003 ^b ±0.00	0.003 ^b ±0.00	0.005 ^a ±0.00
Pb	-	-	-
Cd	-	-	-
Cr	-	-	-
Na/K	0.43 ^a ±0.00	0.44 ^a ±0.01	0.39 ^b ±0.00
Ca/P	7.85 ^a ±0.07	7.54 ^a ±0.17	8.49 ^a ±0.39
Ca/Mg	0.74 ^c ±0.02	1.28 ^a ±0.00	0.89 ^b ±0.00
Ca/K	0.34 ^b ±0.01	0.63 ^a ±0.02	0.36 ^b ±0.01
Na/Mg	0.93 ^b ±0.00	0.89 ^b ±0.01	0.98 ^a ±0.01

Means (±SEM) with different superscripts in a row are significantly different at P<0.05;

Keynote

RWC = Raw wonderful kola seed flour

FWC = Fermented wonderful kola seed flour

BWC = Blanched wonderful kola seed flour

Table 3: Amino acid profile (mg/100g protein) of raw, blanched and fermented *Bucholzia coriacea* seed flour.

Parameters	RWC	FWC	BWC
Alanine	3.48±0.25 ^a	3.44±0.17 ^a	3.63±0.26 ^a
Arginine	2.88±0.72 ^a	3.69±0.31 ^a	4.29±0.05 ^a
Aspartic	4.10±0.16 ^a	6.23±0.02 ^a	7.37±0.30 ^a
Cysteine	1.85±0.32 ^a	2.04±0.81 ^a	1.87±0.30 ^a
Glutamic	15.43±3.89 ^a	13.84±1.44 ^a	10.34±0.39 ^a
Glycine	3.37±0.06 ^a	3.34±0.27 ^a	3.27±0.17 ^a
Histidine	2.62±0.11 ^a	2.24±0.01 ^a	3.01±0.82 ^a
Isoleucine	3.72±0.13 ^a	3.28±0.11 ^a	3.97±0.34 ^a
Leucine	7.15±0.59 ^a	6.50±0.41 ^a	8.95±0.79 ^a
Lysine	2.88±0.52 ^a	3.08±0.12 ^a	2.92±0.34 ^a
Methionine	1.92±0.63 ^a	1.78±0.04 ^a	1.33±0.49 ^a
Phenylalanine	4.48±0.26 ^a	3.57±0.30 ^a	4.20±0.02 ^a
Proline	5.44±0.13 ^a	4.59±0.29 ^a	3.43±0.29 ^b
Threonine	3.19±0.18 ^a	2.65±0.04 ^a	3.28±0.29 ^a
Tryptophan	0.55±0.01 ^c	0.79±0.01 ^a	0.60±0.03 ^b
Tyrosine	3.91±0.42 ^a	3.19±0.05 ^a	4.51±1.02 ^a
Serine	2.83±0.07 ^a	1.82±0.68 ^a	2.95±0.02 ^a
Valine	3.95±0.18 ^a	2.89±0.09 ^b	3.39±0.17 ^{ab}
ΣAmino acid (%)	73.66	68.96	73.31

Means (±SEM) with different superscripts in a row are significantly different at P<0.05;

Keynote

RWC = Raw wonderful kola seed flour

FWC = Fermented wonderful kola seed flour

BWC = Blanched wonderful kola seed flour

application of heat during the blanching that could denatured some of the protein in the flour sample or it could be due to the utilization of some amino acids by the growing microorganisms during the fermentation processing. However, this observation was in contrary to the report of Ochanda *et al.* [46], who reported an increased in the amino acid profile of blanched, germinated or fermented food products.

Fatty Acid Profile of Raw, Blanched and Fermented *Moringa oleifera* Leave flour: The fatty acid compositions of raw, blanched and fermented wonderful kola flour are presented in Table 4. The predominant fatty acids in raw, blanched and fermented wonderful kola seed flour samples was palmitic acid with values of 30.28±0.00, 36.44±0.00 and 35.77±0.00 mg/100g respectively, while behenic acid was the lowest with values of 0.37±0.0, 0.32±0.00 and 0.28±0.00 mg/100g respectively. For polyunsaturated fatty acid, linoleic acid had the highest concentration with values of 33.27±0.0, 30.22±0.0 and 29.59±0.00 mg/100g in RWC, FWC and BWC respectively, while linolenic acid was the lowest with values of 9.71±0.0, 8.92±0.0 and 8.98±0.00 mg/100g respectively. For mono unsaturated fatty acid, oleic acid had the highest concentration with values ranged from 14.29±0.00 mg/100g in RWC to 12.54±0.00 mg/100g in BWC, while palmitoleic acid was the least with values ranged from 2.32±0.0 mg/100g in FWC to 2.72±0.00 mg/100g in RWC. Myristic and lauric acid were not detected in any of the flour samples. Finding has shown that lauric acid (C12:0) and myristic acid (C14:0) are related to human health issues, for instance, these fatty acids have been implicated to be responsible for raising bad

Table 4: Fatty acid composition (mg/100g) of raw, blanched and fermented *Bucholzia coriacea* seed flour.

Parameters	RWC	FWC	BWC
Caprylic Acid (C8:0)	-	-	-
Capric Acid (C10:0)	-	-	-
Lauric Acid (C12:0)	-	-	-
Myristic Acid (C14:0)	-	-	-
Palmitic Acid (C16:0)	30.28 ^c ± 0.0	35.77 ^b ± 0.0	36.44 ^a ± 0.0
Palmitoleic Acid (C16:1)	2.72 ^a ± 0.0	2.39 ^c ± 0.0	2.50 ^b ± 0.0
Margaric Acid (C17:0)	-	-	-
Stearic Acid (C18:0)	8.86 ^c ± 0.0	9.23 ^a ± 0.0	9.11 ^b ± 0.0
Oleic Acid (C18:1)	14.29 ^a ± 0.0	12.69 ^b ± 0.0	12.54 ^c ± 0.0
Linoleic Acid (C18:2)	33.27 ^a ± 0.0	30.22 ^b ± 0.0	29.59 ^c ± 0.0
Linolenic Acid (C18:3)	9.71 ^a ± 0.0	8.92 ^c ± 0.0	8.98 ^b ± 0.0
Arachidic Acid (C20:0)	0.50 ^b ± 0.0	0.50 ^b ± 0.0	0.51 ^a ± 0.0
Arachidonic Acid (C20:4)	-	-	-
Behenic Acid (C22:0)	0.37 ^a ± 0.0	0.28 ^c ± 0.0	0.32 ^b ± 0.0
Erucic Acid (C22:1)	-	-	-
Lignoceric Acid (C24:0)	-	-	-

Means (±SEM) with different superscripts in a row are significantly different at P<0.05;

Keynote

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cholesterol levels in the blood serum [47]. Nutritional study has established those polyunsaturated fatty acids are very common in vegetable oils and fish [48]. Unlike saturated fatty acids (>C16:0), inclusion of polyunsaturated or monounsaturated fatty acids in diets are recommended for preventing cardio-vascular diseases [49]. The polyunsaturated fatty acids are precursors of long chain *n*-3 PUFA in the eicosanoids biosynthesis, which are important in bio regulators of many cellular processes and immune system.

pH and Titratable Acidity (TA) of Raw, Blanched and Fermented Wonderful Kola

The pH and titratable acidity of raw, blanched and fermented wonderful kola were presented in Figure 1 and 2 respectively. The hydrogen ion concentration (pH) of FWC sample was pH = 5.00 and BWC was pH = 4.92; while that of RWC was pH = 4.63. This observation showed that wonderful kola seeds were acidic in nature; however, the acidity of the seed sample was reduced by the blanching and fermentation processing methods. The decreased in hydrogen concentration (pH) of the blanched and fermented samples could be attributed to the hydration of the wonderful kola seeds during the blanching and fermentation processing or it could also due to anti-microbial properties of the seed and thereby reduces the activities of the microorganisms in the production of lactic acid. The high acidity of the seeds could be responsible for its pungent taste. Studies have shown that wonderful kola has antimicrobial activities [50,51]. The titratable acidity values of the samples were 0.0378 mg/g in RWC, 0.0183 mg/g in BWC and 0.0261 mg/g in FWC. The high values of pH and TA that were observed in this present study proffers nutritional benefits by preventing the seed from the attack or proliferation of undesirable organisms [52].

Predicted Nutritional Status of Wonderful Kola seed flour

The predicted nutritional quality of raw, blanched and fermented *Bucholzia coriacea* seed flour is presented in Table 5. The sulphur amino acid and aromatic amino acid values ranged from 3.20 g/100g in BWC to 3.82 g/100g in FWC and 6.76 g/100g in FWC to 8.71 g/100g in BWC respectively. Percentage of total essential amino acid with histidine and arginine ranged between 52.61% in RWC and 58.98% in BWC, while percentage of essential amino acid without histidine and arginine was 44.19-49.02%. For non-essential amino acids, the percentage ranged from 49.02% in BWC to 55.19% in FWC. The values of essential amino acid index (EAAI) were 50.82%, 47.70% and 51.68% for RWC, FWC and BWC sample respectively. Comparatively, the total essential amino acid (TEAA) with histidine and arginine of wonderful kola flour samples were higher than 33.9 g/day of FAO/WHO [18] recommendation for young children. Similarly, the total essential amino acids of the flour samples were higher than FAO/WHO [18] recommendations for infants (39%), children (26%) and adults (11%). The predicted-biological value (P-BV) of the flour samples ranged between 40.29 – 44.63% in FWC and BWC respectively, while nutritional index of the flour samples were 6.36, 6.55 and 8.58% for BWC, FWC and BWC respectively. Essential amino acid index (EAAI) of BWC (51.68%) had the highest value compared with FWC (47.70%) and BWC (45.14%). Predicted-Protein Efficiency Ratio (P-PER) of wonderful kola seed flour samples were 1.95, 1.77 and 2.12 for RWC, FWC and BWC respectively. The predicted-biological values of RWC, BWC and FWC were 43.69%, 44.63% and 40.29% respectively. Nutritional index (NI) of the flour samples were 6.36, 6.55 and 8.58% for RWC, FWC and BWC

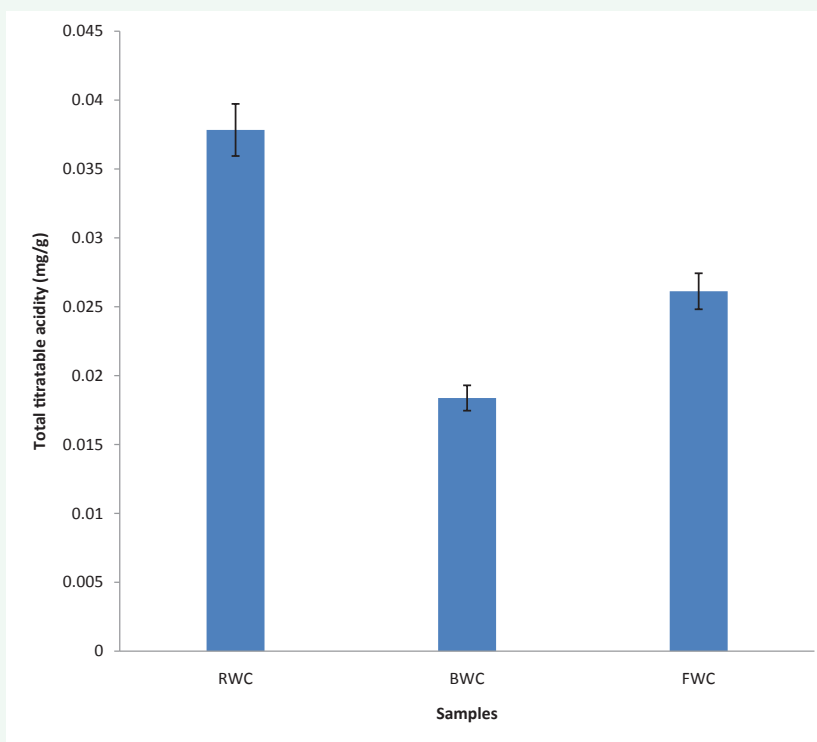


Figure 2 Titratable acidity of the raw, blanched and fermented wonderful kola.

Table 5: Predicted nutritional qualities of raw, blanched and fermented *Bucholzia coriacea* seed flour.

Parameters	RWC	FWC	BWC
Amino acids profile			
Σ Sulphur amino acid (Meth+Cyst) (g/100g)	3.77	3.82	3.20
Σ Aromatic amino acid (Phe+Tyr) (g/100g)	8.39	6.76	8.71
Σ EAA+His+Arg/TAA (%)	52.61	52.78	58.98
Σ EAA / Σ NEAA	0.82	0.79	0.96
(Σ NEAA/Σ AA)%	54.86	55.81	49.02
Σ EAA/TAA%	45.14	44.19	49.02
EAAI (%)	50.82	47.70	51.68
P-PER	1.95	1.77	2.12
P-BV (%)	43.69	40.29	44.63
Nutritional Index (%)	6.36	6.55	8.58
Fatty acids profile			
Σ SFA	40.01	45.75	46.38
Σ PUFA	42.98	39.14	38.57
Σ MUFA	17.01	15.01	15.04
Σ FA	100	99.97	99.99
(Σ SFA/Σ FA)%	40.01	45.76	46.38
(Σ PUFA/Σ FA)%	42.98	39.15	46.38
[(Σ PUFA + Σ MUFA) / Σ SFA]%	149.93	118.51	115.58
P:S	1.50	1.19	1.16
AI	0.504	0.648	0.679
LPS	141.52	156.89	158.43

Keynote

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BWC = Blanched wonderful kola seed flour

AI= Atherogenic index

LPS = Lipid Preventive Score

respectively. Comparatively, it was observed that BWC sample was higher in terms of EAAI, P-PER, P-BV and NI than in RWC and FWC flour samples. Nutritionally, a protein-based food material is said to be of good nutritional quality when its protein efficiency ratio is 2.7, biological values is >70%, essential amino acid index (EAAI) is >0.70 [22]. The nutritional outcome of wonderful kola in terms of essential amino acid index, protein efficiency ratio and biological value showed that the seeds contain appreciable amount of protein, however, the seeds cannot be used alone without complementing with other protein-based food materials.

The polyunsaturated/saturated fatty acid ratio (P:S), atherogenic index (AI) and lipid preventive score (LPS) of wonderful kola seed flour samples ranged from 1.16 in BWC to 1.50 in RWC, 0.504 in RWC to 0.679 in FWC and 141.52 in RWC to 158.43 in FWC respectively. In the present study, it was observed that the P:S ratio of the flour samples were high, which indicated that wonderful kola seeds contain large amount of polyunsaturated fatty acid when compared with unsaturated fatty acid. It is well known that intake of saturated fatty acids and trans fatty acids cause health problem in human, but polyunsaturated fatty acid intake prevent cardiovascular diseases (CVD) [53,54]. Therefore, the consumption of unsaturated fatty acids is

recommended to prevent cardiovascular disease and to increase the polyunsaturated/saturated fatty acid ratio to prevent the development of atherosclerosis [55]. However, studies have shown that polyunsaturated fatty acids (PUFA) are prone to oxidation in man, and thereby produces free-radicals, which is known to cause diseases like aging, cancer, diabetes mellitus, atherosclerosis, etc. [56]. The atherogenic index of the fatty acid in wonderful kola seed flour samples were found to be low, and this indicated that the consumption of wonderful kola seed will decrease the total cholesterol and low-density lipoprotein cholesterol (LDL-c) in human blood plasma [57]. Scientific study has shown that atherogenic indices are powerful indicators of the risk of heart diseases, the higher the value the higher the risk of developing cardiovascular disease and vice versa [58]. However, it is well established that food sample with lower atherogenic index values is liable to rancid of type taste due to the composition of middle chain fatty acids, that is, C12:0, C14:0 and C16:0, and the groups MUFA and PUFA, which is a potential disadvantage of food products with low atherogenic index [59]. Atherogenic index has been used to predict the risk of atherosclerosis and cardio-vascular disorders in human [60]. Atherosclerosis is a pathological disease of the arteries characterized with gradual accumulation of fatty streaks – containing foam cells in the arterial wall [61]. The lipid preventive score (LPS) serves as an estimate of the fatty acid content related to the risk of cardio-vascular disorders. The optimum balanced fatty acid content is obtained when lipid preventive score equals or closer to total lipid value [24]. The lipid preventive scores (LPS) in the present study were higher than total lipid, this indicated that fat in wonderful kola is mainly polyunsaturated fatty acid, hence, it is suitable for human consumption without health implications.

Phytochemicals composition of Raw, Blanched and Fermented wonderful kola seeds flour

The Phytochemical/ antinutritional factors of raw, blanched and fermented wonderful kola seeds flour are presented in Table 6. The phytochemicals/ antinutrient concentrations in blanched and fermented wonderful kola samples were significantly lower in oxalate, trypsin, saponin, alkaloid and flavonoid when compared with raw wonderful kola sample. This observation could be due to the effects of blanching and fermentation processing techniques on these phytochemicals. In comparing with other seed, it was observed that the saponin, alkaloid and flavonoid in wonderful kola seeds were lower when compared with raw *Garcinia kola* [43]. Study has shown that food processing techniques, particularly; heating, germination and fermentation techniques usually reduce concentration of antinutritional factors in food products [62]. Phytochemicals, which are synthesized for defense and other biological functions in plants [63], have multiple health beneficial effects at low dosage [63]. Phytic acid has antioxidant, anticancer, hypocholesterolemic and hypolipidemic effects, prevent oxidative browning in fruits and vegetables by inhibiting polyphenol oxidase; while saponins reduce the absorption of certain nutrients like glucose and cholesterol at the gut, and thereby prevent diabetes and cardiovascular diseases [63]. Despite all these health benefits, finding has shown that intake of phytochemicals at high dosage are detrimental to human health [64]. For instance, phytate and oxalate chelate with cation metals

Table 6: Phytochemicals (mg/100g) and phytate/(Ca, Fe, & Zn) molar ratios of raw, blanched and fermented *Bucholzia coriacea* seed flour.

Parameters	RWC	FWC	BWC	*Critical values
Oxalate	0.405 ^a ±0.00	0.360 ^b ±0.00	0.315 ^c ±0.00	0.25g/100g
Phytate	2.685 ^b ±0.03	2.815 ^a ±0.01	2.530 ^c ±0.00	5-6g/100g
Tannin	0.685 ^c ±0.01	0.980 ^a ±0.00	0.705 ^b ±0.01	3.0mg/100g
Trypsin	0.860 ^a ±0.00	0.789 ^c ±0.00	0.800 ^b ±0.00	0.25g/100g
Saponin	0.077 ^a ±0.00	0.039 ^c ±0.00	0.059 ^b ±0.00	-
Alkaloid	0.213 ^a ±0.00	0.177 ^c ±0.00	0.194 ^b ±0.00	-
Flavonoid	0.004 ^a ±0.00	0.002 ^c ±0.00	0.003 ^b ±0.00	-
Phytate/(Ca, Fe, & Zn) molar ratios (mol/kg)				
Phytate:Zinc	88.144	92.412	49.833	>15.0
Phytate:Calcium	0.074	0.062	0.093	>0.24
(Ca)(Phytate):zinc	4.848	6.353	2.056	>200
Phytate:Iron	0.350	0.483	0.355	>1.0

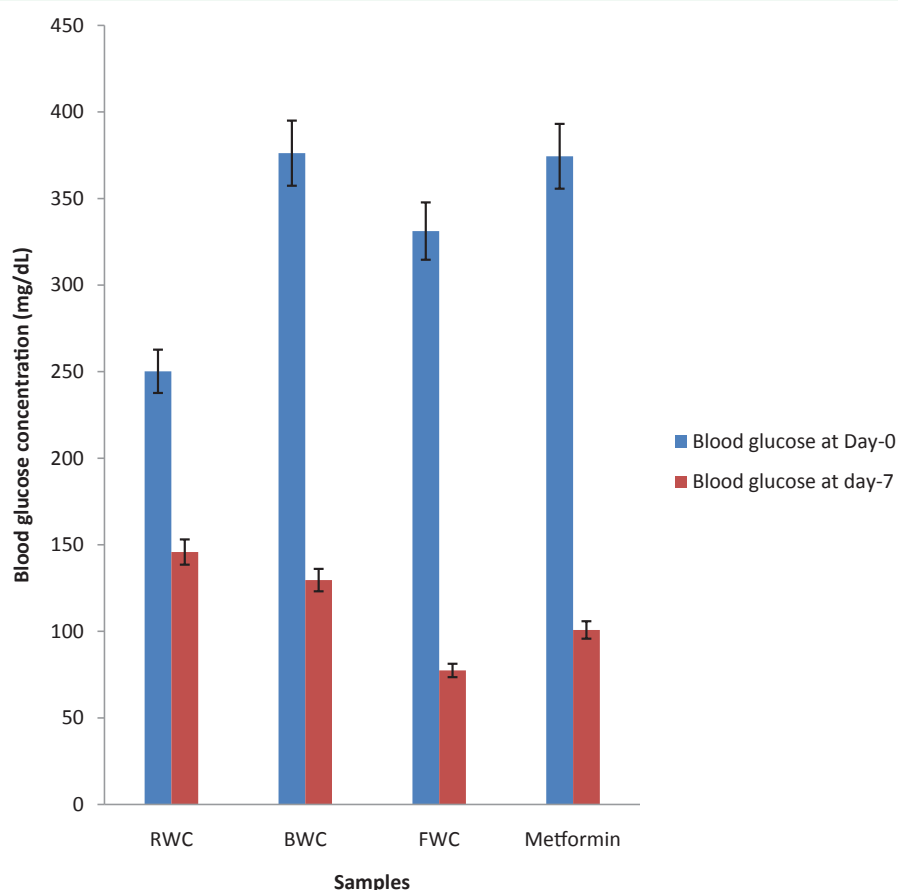
Means (±SEM) with different superscripts in a row are significantly different at P<0.05;

Keynote

RWC = Raw wonderful kola seed flour

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**Figure 3** Blood glucose concentration (mg/dL) of rats fed with wonderful kola seed flour samples

Keynote

RWC = Raw wonderful kola seed flour

FWC = Fermented wonderful kola seed flour

BWC = Blanched wonderful kola seed flour

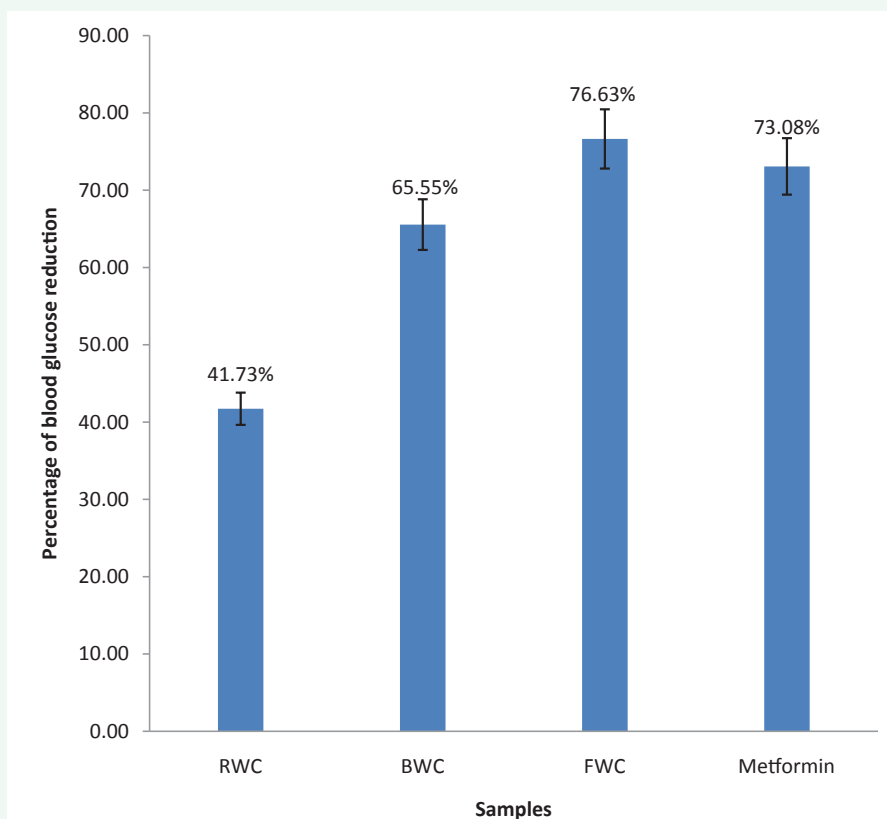


Figure 4 Percentage reduction of blood glucose of rats fed with the wonderful kola seed flour samples

Keynote

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like Cu^{2+} , Zn^{2+} , Co^{2+} , Mn^{2+} , Fe^{2+} , Ca^{2+} etc. and protein to form stable complexes, thereby reducing their availability in the body and aiding the formation of kidney stones [65], while saponin has hemolytic activity by reacting with the sterols of erythrocyte membrane [66].

Calculated phytate:zinc, calcium:phytate, $[\text{Ca}]/[\text{Phytate}]/[\text{Zn}]$ and phytate: Iron molar ratios are presented in Table 6. The calculated ranged values of phytate: zinc were from 49.833 in BWC to 92.412 in FWC, phytate/calcium from 0.062 in FWC to 0.092 in BWC and (Ca) (Phytate):zinc from 2.056 in BWC to 6.353 in FWC, while phytate: Iron ranged was 0.35 – 0.483. These values were below the critical level except for phytate: zinc molar ratios, which were far above the critical level. However, finding has shown that the calculated $[\text{Ca}]/[\text{Phytate}]/[\text{Zn}]$ molar ratio is considered a better index for predicting zinc bioavailability compared with the phytate: zinc ratio because of the calcium to phytate interaction [67]. Hence, the calculated $[\text{Ca}]/[\text{Phytate}]/[\text{Zn}]$ molar ratio were below the critical level (0.5mol/kg), an indication of bioavailability of dietary zinc [68]. Zinc deficiency in humans can lead to poor foetal growth, cognitive development and reduction in milk production during lactation [69]. Marginal zinc deficiency is associated with plant-based diets, especially those diets rich in phytate, an inhibitor of zinc absorption [70].

Anti-diabetic activity of wonderful kola seed

Blood glucose concentrations (mg/dL) of rats fed with raw, blanching and fermented wonderful kola seed flour samples are presented in Figure 3 and 4. Percentage reduction of blood glucose of diabetic-induced rats fed with FWC samples had the highest blood glucose reduction, while those of rats fed with RWC sample had the lowest blood glucose reduction. This observation showed that anti-diabetic activity of wonderful kola was significantly influenced by blanching and fermentation processing techniques. Comparatively, the anti-diabetic activity of wonderful kola seed was similar to that of metformin (an anti-diabetic agent). Hence, the seed has anti-diabetic potential. Diabetes is the largest endocrine disease associated with increased morbidity and mortality rate worldwide [71], and it is characterized by elevated blood glucose level-hyperglycemia, due to a deficient insulin secretion by pancreas or an inefficient insulin action in body tissues [72]. The total number of people with diabetes is increasing in developing countries [73] (Shaw *et al.*, 2010), and this is due to changing in lifestyle like nutritional habits modification, *i.e.*, increased intake of saturated fats, refined sugars and alcohol, and reduced intakes of plant-based foods and physical activity [74].

CONCLUSION

The study established the nutritional profile of *Bucholzia coriacea* seed flour after subjected into blanching and fermentation. The findings showed that blanched *Bucholzia coriacea* flour sample had the highest essential amino acid, appreciable amount of minerals, biological value, protein efficiency ratio, atherogenicity index, lipid preventive score, tolerable phytochemicals and anti-diabetic potentials compared with the raw and fermented samples. Hence, blanching significantly improved nutritional efficacy of *Bucholzia coriacea* seed.

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