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Chemical composition, antinutritional evaluation and phenolic compounds in gingerread plum (Neocarya Macrophylla) kernel

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Abstract:

In this study, the proximate composition, phenolic compounds, antinutritional factors and viscosity in gingerbread plum kernel (GPK) paste and flour from Niger and Guinea were determined. Results of the study have revealed high protein content in GPK paste and flour from Niger (18.41, 47.14 %) and Guinea (19.65, 53.71 %). Findings from the have also shown that Glu, Asp, Leu, Arg were the major amino acids in GPK while the dominant minerals were magnesium, potassium and calcium. On the other hand, minerals such as copper, iron and manganese were present in moderate amounts. Results from LC-MS analysis revealed that 3.O-caffeoylquinic acid, coumaric acid derivative, trisgalloyl (Hexahydroxydiphenoyl) glucose and 4.5-

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dicaffeoylquinic acid were the major compounds in GPK. It has further been found out that GPK is a rich source of water soluble vitamins especially biotin, thiamine, pyridoxine and riboflavin. Although the peak viscosity, final viscosity, breakdown and setback pasting properties of GPK from Guinea were higher than those from Niger, there were no significant differences and the pasting curves for the two kinds of GPK were observed to be overlapping. The findings from this study can have useful applications in genetic engineering and food processing technologies with improved nutritional value for human and animal nutrition.

Key words: gingerbread plum kernel, chemical composition, antinutritional factors, Phenolics compounds.

Graphical Abstract (GA)

(a) gingerbread plum kernel (GPK);
(b) Whole gingerbread plum kernel paste (WGPKP);
(c) Defatted gingerbread plum kernel flour (DGPKF).

1. INTRODUCTION

Gingerbread plum is an exceptionally under-explored source of plant based oils proteins that thrive in the arid and semiarid regions mainly in the Western part of Africa particulary Niger and Guinea as well as Panama Central America. A tree of family Chrysobalanaceae, gingerbread plum is also known by

two other names, *Neocarya* macrophylla (Sabine) *Prance* and *Parinari* macrophylla Sabine (Irvine, 1961) . The flesh is soft and yellowish when fresh, with a peculiar flavor sometimes likened with avocado. The endocarp contains one or two kernels (Arbonnier, 2004) that are of high nutritional value. A gingerbread plum kernel (GPK) from Niger contains 56.18 % oil and 18.41 % proteins while GPK from Guinea contains 60.60 % oil and 19.65 % proteins. These levels are comparable with those of almond (*Prunus dulcis L.*) (Ahrens et al., 2005) and peanut (Arachis hypogaea L.) seeds. GPK could be vital for human health since it is well known that the consumption of a plant-based diet, mainly vegetables, fruits and whole grains, is recommended as one, of the ways to lower the risk of some health problems in humans and animals (Cadenas and Packer, 1996). However, GPK like many other plants contain antinutritional factors whose major roles in the living plant include storage of nutrients and defense from destruction by insect pests and grazing animals (Harborne, 1989). When consumed by animals and humans, these antinutritional factors can be either beneficial or detrimental on human and animal health. Phytates for example, are important inhibitors of mineral absorption especially iron, calcium and phosphorus, whose biosynthesis occurs only in the aleurone and embryo but not in the starchy endosperm. While, tannins regulate the growth of the plant, it is generally known that they also inhibit the activity of digestive enzymes in the body of humans and animals there by lowering digestibility of nutrients, especially protein and carbohydrates (Reddy et al., 1985). However, research has shown that tannins also act as natural antioxidants and therefore play a positive role in the body as they neutralize free radical compounds (Shahidi and Naczk, 1989). Similarly, trypsin inhibitors interfere with the digestion of proteins and they have also been implicated in pancreatic disease. The reason for anti-nutritional factors in plants seems

to be as a way of storing nutrients or as a means of defense from destruction by insect pests and grazing animals (Harborne, 1989). From the available literature, it is evident that no research work has been carried out to determine the proximate composition, mineral content, phenolic content and antinutritional factors in GPK from Niger and Guinea. Therefore, the current work was carried out to determine the proximate composition, mineral content, phenolic content and antinutritional factors in GPK from Niger and Guinea.

2. MATERIALS AND METHODS

2.1 Sample preparation

Fresh whole GPK (*Neocarya macrophylla*) were obtained from two different locations (one from Birni N'Gaouré, southern region of Republic of Niger and the other from Gaoul in the Boke region, Republic of Guinea). The kernels were kept dried in desiccators at room temperature. The kernels were milled using a laboratory scale hammer miller. The resulting paste was dispersed in n-hexane at paste to n-hexane ratio of 1:5 (w/v) and stirred for 4 h at room temperature. The hexane was decanted and the defatted gingerbread plum kernel flour (DGPKF) was air dried for 24 h under a vacuum drier and stored at 5±1°C in sealed glass jars until use. Trypsin (E.C.3.4.21.4, trypsin >250 N.F. units/mg) Was obtained from Sigma Chemical Co. (St. Louis, MO, USA) while gallic acid was purchased from (Sinopharm chemical reagent Co., Ltd., Shanghai). All chemicals and reagents used in this study were of the highest grade commercially available.

2.1.1. Phenolic compounds extraction

The extracts from GPK were obtained by microwave assisted extraction (ETHOS 1, Milestone SRL, Sorisole, Italy) for 60 min, potency 500 W. The extraction of phenolic compounds from

GPK flour from Niger and Guinea was carried out with acetone: water (1:1, v:v) at 50 °C and flour to-solvent volume of 1:30 (w:v). The suspension was centrifuged (525 ×g at 4 °C for 20 min) in a Jouan CR-312 centrifuge (Thermo Electron Corporation, Madrid, Spain) and the supernatant was reserved. The pellets were re-extracted two more times and the supernatants were combined and stored at−80 °C until the analyses were carried out (Dorta et al., 2014).

2.2 Vitamin determination of gingerbread plum kernel flour (GPKF)

Vitamins were determined according to the method as described by (Erbaş et al., 2005) with slight modification. Three grams of sample were mixed with 5 mL *n*-hexane and 20 mL HPLC grade water. The mixture was first homogenized by vortexing and then centrifuged at 12,000 rpm for 30 min. The aqueous phase was filtered through filter paper and 0.45 µm membrane filter sequentially. The supernatant (10 µl) was injected into HPLC system (Agilent 1100 Technologies, USA) equipped with a UV Vis detector, which was set at 260 nm absorbance mode. Peaks were verified by adding the standard vitamins to samples and individual peak area were calculated in accordance with corresponding standard vitamins. Results were calculated on a dry weight basis.

2.3 Determination of amino acid composition of GPK paste and flour

GPK paste and flour digested with HCl (6 M) at 110 °C for 24 h under nitrogen atmosphere. Reversed phase high performance liquid chromatography (RP-HPLC) analysis was carried out using an Agilent 1100 (Agilent Technologies, Palo Alto, CA, USA) assembly system after precolumn derivatization with o-phthaldialdehyde (OPA). Each sample (1 µl) was injected on a Zorbax 80 A C18 column (4.6 i.d. ×180 mm, Agilent

Technologies, Palo Alto, CA, USA) at 40 °C with detection at 338 and 262 nm. Mobile phase Awas 7.35 mmol/l sodium acetate/triethylamine/tetra- hydrofuran (500:0.12:2.5, v/v/v), adjusted to pH 7.2 with acetic acid, while mobile phase B (pH 7.2) was 7.35 mmol/l sodium acetate/methanol/acetonitrile (1:2:2, v/v/v). The amino acid composition was expressed as g of amino acid per 100 g of protein.

2.4 Mineral determination of GPK paste and flour

For the analysis of mineral elements such as potassium, magnesium, calcium, phosphorous, iron, zinc, copper, sodium and manganese, samples were digested with pure HNO_3 in a microwave oven (MARS, CEM, USA). The oven temperature was initially set and held at 100 °C for 5 min, then increased and held at 150 °C for 10 min and finally increased and maintained at 170 °C for 10 min. The concentration of each element was determined with an atomic absorption spectrometer (Spectra AA 220, VARIAN, USA).

2.5 Antinutritional factors in GPK flour

2.5.1 Extraction and estimation of trypsin inhibitor

Trypsin inhibitor activity was determined by standard procedure reported by Kumar Dixit et al.,(2011). One gram sample of defatted GPK flour was extracted with 0.01 N NaOH (50 ml) for 4 h. After centrifugation at 10.000 rpm for 20 min, the supernatant was collected and tested trypsin inhibitor activity. To 2 mL of the sample, 2 mL of trypsin solution (prepared by dissolving 4 mg of the trypsin in 200 mL of 0.001 N HCl) was added to test tube and incubated at 37 ^{o}C for 10 min. BenzylD-arginine para-nitroanalide hydrochloride (BAPNA, 5 mL) was (prepared fresh by dissolving 0.08 g BAPNA in 2 mL of di-methyl sulfoxide and diluted to 200 mL with 50 mM Tris buffer pH 8.2 containing 20 mM calcium

chloride. The contents were warmed at 37 °C) and rapidly added to the test tube. The contents were stirred immediately on a vortex mixture incubated in water bath at 37 °C. The reaction was terminated after 10 min by addition of 1 ml of 30 % acetic acid. A sample blank was prepared by the same procedure, except that the trypsin solution was added after the reaction was terminated by addition of 30 % acetic acid. The absorbance was determined at 410 nm against the sample blank. The trypsin inhibitor content was determined by the following equation as reported by Kumar Dixit et al., (2011)

$$\text{Trypsin inhibitor content (mg/g)} = \frac{\text{Differential absorbance} \times \text{dilution factor}}{0.019 \times 1000}$$

Where 0.019 is the Absorbance of 1 μg of the pure trypsin.

2.5.2 Phytic Acid determination in GPK flour

The determination of phytic acid (PA) in wheat grains was based on precipitation of ferric phytate and measurement of iron (Fe) remaining in the supernatant (Wang et al., 2015). Phytate was extracted by adding 0.3 g GKP flour in 10 mL mixture solution of HCl (0.2 mol/L) and Na2SO4(10 %). The mixture was shaken for 2 h and the extracts were centrifuged at 10,000 rpm for 10 min, and 2.5 mL of supernatant was treated with 2 mL 0.2 % ferric solution ($FeCl_3$) in a boiling water bath for 30 min. The mixture was cooled and centrifuged at 10,000 rpm for 10 min. The supernatant was discarded, and then the precipitate was carefully washed twice with deionized water. Three mL NaOH (1.5 mol/L) were added to the precipitate, shaken for 5 min, and the solution was centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and 3 mL HCl (0.5 mol/L) were added to the precipitate and shaken until the precipitate was completely dissolved. The solution was transferred into a volumetric flask and brought up to 25

mL in order to measure the remaining Fe in the solution by AAS. Phytic Acid Sodium purchased from Sigma (St Louis, Missouri, USA) was used to test the PA recovery rate, and the PA addition test results indicated that the levels of recovery were between 96 and 102 %.

2.5.3 Determination of tannins in GPK flour

Two grams of the sample were extracted with 10 mL of 70 % aqueous acetone (v/v) for 24 h. The extracts were centrifuged at 3000 rpm for 20 min and the supernatant was tested for the tannins (G. Raja et al., 2012). Different aliquots of the supernatant were taken and the final volume was adjusted to 3ml by distilled water. The solution were vortexed and mixed with 1ml of 0.016 M $K_3Fe(CN)_6$, followed by 1mL of 0.02 M $FeCl_3$in 0.10 M HCl. The mixtures were vortexed again for 15 min. Five milliliters of stabilizer with a composition water: H_3PO_4:1 % gum Arabic (3:1:1, v: w: w) was added and the contents were vortexed. Absorbance was measured at 700 nm against blank. Standard curve was plotted using various concentrations of 0.001 M gallic acid.

2.5.4 Viscosity determination of GPK flour

Pasting characteristics were determined using a rapid visco-analyzer (RVA) (New port scientific, RVA TECMASTER, Australia). Three 3 g of each sample and 25 mL of distilled water were thoroughly mixed in a canister and fitted into the RVA as described by Amadou et al., (2014).

2.6 HPLC analysis

The HPLC–MS analysis was performed according to the procedure previously described by (Ribeiro et al. 2008) with some modifications. Chromatographic separation was performed on an Agilent 1200 series LC (Agilent Technologies, Waldbronn, Germany) consisting of a quaternary pump

(G1311A), a solvent degasser (G1322A), an autosampler (G1367B), a thermostat column compartment (G1316A) and diodearray detector (PDA) (G1315B) (Agilent Technologies, Waldbronn, Germany). Separation was carried out on a reverse phase C18 Hypersil ODS stainless steel column (250 mm × 4.6 mm, 5 µm) (Teknokroma, Barcelona, Spain) at a column temperature of 25 °C. The mobile phase consisted of 1%formic acid in Milli-Q-water (A) and 1% formic acid inacetonitrile (B). Separation was carried out in 70 min under the following conditions: 0 min, 100% A; 15 min, 75% A; 30 min, 70%A; 60 min, 20% A; 65 min, 0% A; and 70 min, 100% A. The column was equilibrated for 5 min prior to each analysis. The mobile phase flow rate was 1 mL/min and the injection volume was 10 µL. UV detection was carried out at 280 (Gallic and ellagic acid derivatives), 320 (xanthones) and 360 (Flavonols) nm.

2.7 Statistical analysis

All experiments were conducted in triplicate while the analysis was done with SPSS Inc. software (version 13.0). One-way analysis of variance (ANOVA) was used to determine significant differences between means, at p-value a = 0.05. Tukey's HSD was used to conduct multiple comparisons between means.

3. RESULTS AND DISCUSSIONS

3.1 Proximate Composition of GPK paste and flour

Results on proximate composition of GPK paste and flour are presented in Table 1. As shown in Table 1 the moisture contents of of GPK flour from Niger (11.94%) and Guinea (10.44 %) respectively and these values were comparable to the ones reported by other authors on other legumes (Aremu et al., 2006). The protein content was significantly increased in defatted paste (18.41; 19.65 % for GPK from Niger and Guinea

respectively) and flour (47.14 %; 53.71 % for GPK from Niger and Guinea respectively). On the other hand crude protein content in GPK was comparable with values obtained in other plant based foods such as soybeans, cowpeas, pigeon pea and Kersting's groundnut (Aremu et al., 2006). With respect to the paste, it was observed that there was a significant difference ($p<0.05$) in proximate composition was observed between GPK paste from Niger and Guinea. GPK paste from Guinea had higher protein, fat and ash content (19.65, 60.60 and 2.42 % respectively) than GPK paste from Niger (18.41, 56.18 and 2.82% respectively). Similarly GPK flour from Guinea had higher protein, fat and ash content (53.71, 6.59 and 11.36 % respectively) than GPK flour from Niger (47.14, 6.25 and 10.54 % respectively). In general it was observed that GPK paste from Guinea and Niger had higher fat content (60.60 %, 56.18 % respectively) but less protein (19.65 %, 18.41 % respectively) than values reported in other vegetable seeds such as Almond (47.50 %, 20.60 %), Cashew kernel (48.10 %, 21.20 %) and Peanut (49.50%, 24.5%) (Amza et al., 2014). The carbohydrate content was found to be higher in GPK samples from Niger than those from Guinea. From the results, GPK can be considered as an oil-rich legume seeds.

3.2 Mineral content analysis of GPK paste and flour

Results on the mineral contents of defatted GPK flour from Niger and Guinea are presented in **Table 1**. Results showed that defatted GPK flour had high levels of potassium, calcium, magnesium and phosphorus content but low levels of manganese and copper. However, it was observed that the mineral content was higher in GPK samples from Guinea than those from Niger. The amount of minerals contained in GPK were comparatively lower than those reported for raw cornflour (Radha et al., 2008) but were comparable with those reported for African yam bean (Aremu et al., 2006). The higher calcium

content of defatted GPK flour suggests that GPK could be a suitable alternative source of calcium supplements for vulnerable groups such as pregnant and lactating women, children and the elderly. Results further showed that defatted GPK flour also contained appreciable amounts of iron and therefore could be beneficial for mitigating the risks of numerous infections in human populations. Since defatted GPK flour contained high levels of zinc it can be assumed that the consumption of GPK could enhance the normal functioning of the immune system.

Table.1 Proximate and mineral composition of gingerbread plum kernel paste (GPKP) and flour (GPKF) from Niger and Guinea.

Component	GPKPN	GPKFN	GPKPG	GPKFG
Proximate composition				
Protein	18.41 ± 0.02^{a}	47.14 ± 0.03^{c}	19.65 ± 0.01^{b}	53.71 ± 0.03^{d}
Ash (%)	2.41 ± 0.04^{a}	6.25 ± 0.01^{b}	2.42 ± 0.03^{a}	6.59 ± 0.02^{c}
Fat (%)	56.18 ± 0.03^{c}	10.54 ± 0.03^{a}	60.60 ± 0.04^{d}	11.36 ± 0.02^{b}
Moisture (%)	2.82 ± 0.04^{a}	11.94 ± 0.03^{d}	3.17 ± 0.03^{b}	10.44 ± 0.04^{c}
Carbohydrates (%)	20.18 ± 0.12^{c}	24.13 ± 0.04^{d}	14.16 ± 0.08^{a}	17.89±0.03^{b}
Minerals				
Calcium	107±0.0.06^{a}	666.67±0.03^{c}	298.08±0.08^{b}	732.02±0.03^{d}
phosphorus	342.11±0.0.9^{a}	445.05±0.05^{b}	544.36±0.03^{c}	668.03±0.02^{d}
Magnesium	285.87±0.06^{a}	2098.60±0.03^{c}	316.72±0.05^{b}	2824.06±0.3^{d}
Sodium	1043.33±0.28^{a}	1443.21±1.8^{c}	1228.18±0.18^{b}	1552.21±0.18^{d}
Potasium	76.05±0.04^{b}	1240.16±0.14^{c}	56.41±0.03^{a}	1320.11±0.09^{d}
Iron	13.46±0.05^{a}	15.06±0.04^{b}	16.71±0.04^{c}	17.93±0.05^{d}
Copper	1.9±0.04^{b}	5.11±0.03^{d}	1.60±0.03^{a}	3.42±0.03^{c}
Zinc	2.54±0.04^{a}	4.26±0.05^{c}	3.53±0.04^{b}	6.44±0.05^{d}
Manganese	2.69±0.04^{a}	2.59±0.05^{a}	4.12±0.06^{c}	3.40±0.05^{b}

Means of three determinations±SD; Mean values in rows with different letters (a, b, c or d) were significantly different (Tukey's test); significance at ($p < 0.05$) (analysis of variance).

GPKPN, GPKFN: gingerbread plum kernel paste and flour from Niger; GPKPG and GPKFG gingerbread plum kernel paste and Flour from Guinea.

3.3 Vitamins content in GPK flour

Result on vitamin content in GPK flour from Niger and Guinea are presented in Table 2 Results **showed that** biotin was the most abundant vitamin followed by thiamine, pyridoxine and riboflavin, respectively. Among the water-soluble vitamins, the B group including thiamin (B_1), riboflavin (B_2) and pyridoxine

(B_6) were the most abundant (Moreno and Salvado, 2000) . The amount of pyridoxine in GPK flour from Niger was lower than those reported for spices such as chili, cayenne, paprika and garlic (Leonard et al., 2001). The average amounts of biotin in GPK flour from Niger and Guinea were 235.47 mg/100g and 130.78 mg /100g respectively while the amounts of vitamin B_1were 13.14 mg/100g and 5.50 mg/100g respectively. The amounts of vitamin B2 in GPK flour from Niger and Guinea were 0.52 and 0.32 mg/100g respectively while those of vitamin B_6 in the same samples were 1.09 and 0.77 mg/100g respectively. In general, the amounts of vitamins determined in GPK flour from Niger were significantly ($p<0.05$) higher than those from Guinea. Therefore, the consumption of GPK could increase vitamin intake for the normal growth and maintenance of life. These vitamins could be taking through normal diet, enriched foods, multivitamin tablets, nutraceuticals and GPK related functional foods.

3.4 Antinutritional factors in GPK flour

Result on the content of antinutritional factors identified in defatted GPK flour are as listed in **Table 2. Results showed that** tannin content in defatted GPK flour from Niger and Guinea (1.92 g/100g and 1.82 g/100g respectively) were higher than those reported for acacia tortilis seed (0.98 g/100g) (Embaby and Rayan, 2016), and canola seeds (0.46 – 1.32 g/100g) (Khattab et al., 2010). Differences in tannin levels between GPK flour from Niger and Guinea were not significant. The level of phytic acid in GPK from Guinea (4.51 g/100g) was higher than those from Niger (3.19 g/100g). Generally phytic acid content in GPK was higher than values reported in different foods by various authors such as canola seed (2.16 – 3.57 g/100g), meals (2.07 – 3.62 g/100g), raw by Khattab et al., (2010), rice (0.382 g/100 g), cooked rice (0.1465 mg/100g), flat bread (0.395 /100 g), and wheat flour (0.519 g/100 g) by Roohani

et al., (2012). Results for the trypsin inhibitor activity of GPK as presented in Table 2 showed higher inhibition in GPK from Niger (24.49 mg/g) than GPK from Guinea (22.77 mg/g). The differences in the distribution of antinutritional factors between the two types could be attributed to variations in agro-climatic conditions (Martinez-Herrera et al., 2006) especially soil, seasonal variations and genetic factors (Brune et al., 1992). Trypsin inhibitor activity in GPK was lower than the values reported for soybean genotypes kalitur (91.84 mg/g) and hara soya (55.86 mg/g) by Kumar Dixit et al., (2011). Despite the negative roles exhibited by most antinutritional factors, others have beneficial effects when consumed at low concentrations. For example, phytic acid and tannins play a negative role by reducing the availability of certain nutrients in the body. However, the consumption of these antinutritional factors has proved to be beneficial in reducing the risk of cancer, blood glucose level, plasma cholesterol, triglycerides and improved insulin response to starchy foods.

Table 2 Vitamins and antinutritional factors of gingerbread plum kernel flour from Niger and Guinea

Component	GPKFN	GPKFG
Vitamin (mg/100g)		
Thiamin(B1)	13.16±0.03^{a}	5.56±0.05^{a}
Riboflavin(B2)	0.56±0.04^{a}	0.37±0.04^{b}
Pyridoxine(B6)	1.15±0.06^{b}	0.83±0.05^{a}
Biotin(H)	235.50±0.04^{b}	130.80±0.06^{b}
Antinutritional factors		
Trypsin inhibitor (mg/g)	24.49±0.06^{a}	22.77±0.07^{a}
Phytic acid (g/100 g)	3.19±0.11^{b}	4.51±0.06^{b}
Tannin (g/100g)	1.92±0.06^{b}	1.82±0.05^{a}

Means of three determinations±SD; Mean values in rows with different letters (a, b, c or d) were significantly different (Tukey's test); significance at ($p < 0.05$) (analysis of variance).

GPKFN: gingerbread plum kernel flour from Niger and GPKFG: gingerbread plum kernel flour Guinea.

3.5. Amino acid composition of GPK paste and flour

Eighteen kinds of amino acids were identified in GPK of which nine were essential (Histidine, Threonine, Valine, Methionine, Phenylalanine, Isoleucine, Leucine, Lysine, Tryptophan) while the rest were non essential (Tyrosine, Cysteine's, Aspartic acid, Glutamic acid, Serine, Glycine, Arginine, Alanine and Proline. The major constituent amino acids were Aspartic acid, Glutamic acid, Argine and Leucine. As shown in Table 3, the amounts of glutamic acid that were identified in GPK paste and flour from Niger (22.66, 23.87 g/100g) and Guinea (25.47, 23.35 g/100g) were higher than the other amino acids. These amounts were also higher than those reported for wheat (13.76 g/100 g), corn (14 g/100 g), sorghum (13.98 g/100 g) and soybean (13.71 g/100 g) by Galla et al., (2012) Other amino acids including valine, serine, glycine, lysine and phenylalanine were identified in amounts ranging from 4.94 to 5.85 g/100 g. Amino acids such as histidine, threonine, isoleucine, proline and tyrosine were identified in low amounts ranging from 2.12 to 4.26 g/100 g. Similarly, other kinds of amino acids such as methionine and cysteine's were also identified in very low amounts. The amounts of methionine in GPK paste and flour from Niger (1.54, 1.24g/100g) and Guinea (1.98 and 1.46 g/100g) were found to be low. However, it is well established that methionine is an efficient scavenger of almost all oxidizing molecules under physiological conditions. On the other hand, the amounts of cysteine's identified in GPK paste and flour from Niger samples (1.52, 1.62 g/100g) and Guinea (1.21, 1.17 g/100g) were low yet cysteine's is necessary for GSH synthesis (Amza et al., 2014). Nevertheless GPK paste and flour contained higher amounts of the essential amino acids: valine, leucine, threonine, lysine, isoleusine, phenylalanine and tyrosine than the reference protein. Additionally, there was no significant difference between the isoleucine and lysine contents in GPK and the FAO/WHO/UNU reference protein (Table 5). These results are

in accordance with those previously reported by Gwatidzo et al., (2013) on the manketti flour. The methionine and histidine contents of GPK were found to have fallen short of the requirements by FAO/WHO/UNU (1985) reference protein with the statistical test showing significant difference. Proteins are considered nutritive if the levels of their essential amino acids are higher than the reference levels required by at least for pre-school children (2–5 years). FAO/WHO/UNU (1985) recommends that preschool children need a minimum of 34, 35, 25, 28, 66, 63 and 58 mg/g protein of threonine, valine, (methionine + cysteine's), ileucine, leucine, (phenylalanine + tyrosine) and lysine, respectively by Horax et al., (2010) in their protein diet.

Table 3 Amino acid patterns of gingerbread plum kernel paste and flour from Niger and Guinea (g/100 g protein)

Amino acid	GPKFN	GPKFG	GPKPN	GPKPG
essential amino acid (EAA)				
Histidine	2.33±0.02^{b}	2.44±0.02^{c}	2.12±0.01^{a}	2.48±0.01^{d}
Threonine	2.45±0.02^{b}	2.38±0.1^{a}	2.49±0.2^{c}	2.54±0.1^{d}
Valine	5.03±0.06^{a}	5.24±0.02^{b}	5.35±0.02^{d}	5.31±0.01^{c}
Methionine	1.24±0.01^{a}	1.46±0.02^{b}	1.54±0.01^{c}	1.98±0.03^{d}
Phenylalanine	5.23±0.01^{b}	5.15±0.3^{a}	5.38±0.01^{c}	5.33±0.02^{c}
Isoleucine	3.96±0.02^{a}	4.04±0.03^{b}	4.26±0.03^{c}	4.21±0.02^{c}
Leucine	7.61±0.01^{b}	7.42±0.02^{a}	7.63±0.02^{b}	7.59±0.01^{b}
Lysine	4.41±0.08^{a}	5.75±0.13^{b}	4.83±0.03^{c}	4.72±0.03^{b}
Tryptophan	1.43±0.02^{c}	1.48±0.02^{c}	1.05±0.03^{a}	1.22±0.03^{b}
Nonessential amino acids				
Tyrosine	3.38±0.03^{d}	2.58±0.2^{a}	2.76±0.01^{c}	2.64±0.01^{b}
Cysteine-s	1.62±0.03^{c}	1.17±0.03^{a}	1.52±0.02^{b}	1.21±0.01^{a}
Aspartic acid	8.86±0.02^{c}	8.60±0.02^{a}	8.68±0.02^{b}	8.72±0.01^{b}
Glutamic acid	23.87±0.03^{c}	25.47±0.03^{d}	22.66±0.03^{a}	23.35±0.3^{b}
Serine	5.19±0.02^{d}	4.92±0.01^{b}	4.85±0.01^{a}	5.01±0.01^{c}
Glycine	5.25±0.02^{c}	4.94±0.01^{a}	5.85±0.03^{d}	5.04±0.02^{b}
Arginine	12.03±0.04^{c}	11.66±0.02^{a}	12.11±0.02^{d}	11.80±0.02^{b}
Alanine	3.98±0.02^{b}	3.87±0.1^{a}	4.12±0.03^{c}	3.92±0.04^{a}
Proline	3.45±0.01^{a}	3.98±0.04^{b}	4.01±0.03^{b}	4.22±0.03^{c}

Means of three determinations±SD; Mean values in rows with different letters (a, b, c or d) were significantly different (Tukey's test); significance at ($p < 0.05$) (analysis of variance).

GPKPN and GPKFN: gingerbread plum kernel paste and flour from Niger; GPKPG and GPKFG: flour and kernel paste from Guinea.

3.6 Viscosity

Results of the pasting property of the flour samples are presented in Figure 1. The RVA viscoamylogram of the GPK sample from Guinea had higher peak viscosity, final viscosity, breakdown and setback pasting properties than that from Niger. The overlapping of pasting curves for both GPK Niger and Guinea implies that there were no significant differences between the two kinds of samples. Some researchers have previously reported decreasing peak viscosity, trough viscosity, final viscosity, breakdown and setback when samples have been subjected to moisture and heat. On the other hand, pasting temperature can be increased depending on the treatment conditions (RODRÍGUEZ-DAMIAN et al., 2013, Puncha-Arnon and Uttapap, 2013). In all the samples, it was further observed that there was no increase in peak viscosity, final bonding speed and even the synchronization holding force in all samples. This can be attributed to the effect of high protein content on the viscosity.

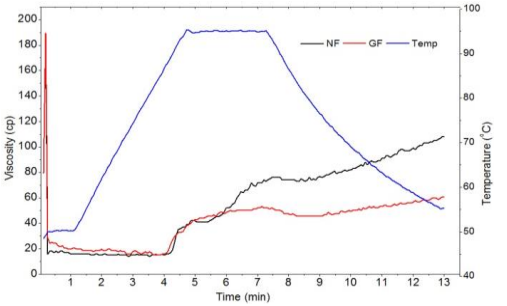

Figure 1. RVA (rapid visco analyzer) analysis of gingerbread plum kernel flour

3.7 Phenolic compound of the GPK methanolic extract

In this study, fourteen phenolic compounds were separated and tentatively identified from GPK from Niger and Guinea. An overview of all the characterized compounds in the GPK by-product extracts by HPLC-ESI-QTOF-MS is given in Figures 2 and **Table 4**. The tentative assessment of the compounds is

summarized along with their retention time, observed *m/z* and MS/ MS fragments. Major phenolic compounds that were separated and identified included: Coumaric acid derivative, 3.O-caffeoylquinic acid, 4.5-Dicaffeoylquinic acid and Trisgalloyl (Hexahydroxydiphenoyl) glucose. Other phenolic compounds were: Caffeic acid Hexoside, Epicatechin-3-O-gallate, Quercetin O-malonylhexosyl rhamnoside, Methyl-3-O-Caffeoyl quinic acid and Quercetin-pentoside-hexoside. Minor phenolic compounds that were identified include; Galloylquinic acid, Dihydro-o-coumaric acid, Protocatechuic acid-O-Hexoside derivative, 6-O-P-Coumaroyl-ß-glucose and Methyl genistein.

Mass spectra of peak **1** displayed a parent ion at *m/z* 341 and two fragment ions with one peak at *m/z* 179 for caffeic acid formed through the loss of a hexose moiety, and the other at *m/z* 135 for decarboxylated caffeic acid formed after elimination of both hexose and CO2. From the analysis, we concluded that the compound could be caffeoyl hexoside. Peak **10** was identified as quercetin-pentoside-hexoside characterized by a parent ion at *m/z* 595 and fragment ions at *m/z* 433 and 301 that were obtained through a sequential loss of hexose [M−H − hexose] and pentose [M − H − hexose − pentose] moieties (Chen et al., 2012). The compound at peak **2** was detected at m / z 439 in the extracted ion chromatogram and tentatively assigned as Epicatechin-3-O-gallate. It had fragments at *m/z* 289 and *m/z* 169. Peak **3** [M–H] at *m/z* 695 released a majority of MS2 fragments at *m/z* 651 [M–H-44]. The fragments could be formed through the loss of CO_2, coherent with the existence of a non-substituted carboxyl. Other fragments identified at *m/z* 609 [M-H-86] and 447 ([M-H-86-162]) could be formed through the loss of malonyl and malonylhexosyl residues, respectively. On the other hand, the fragment at *m/z* 301 could be formed through the loss of a rhamnosyl residue. The presence of fragments derived from the alternative loss of the malonylhexosyl and the rhamnosyl moieties implies that these

fragments were located at different positions on the aglycone. On the other hand, this might also imply that the two sugars constituted a disaccharide. In that case the fragment at *m/z* 447 could be formed through structural rearrangement following the loss of the internal malonylhexosyl residue and further linkage of the terminal rhamnose to the aglycone, as reported by Dias et al., (2013).

ESI-MS spectrum for peak 4 indicated deprotonated molecule at *m/z* 951, which after fragmentation registered a base peak at *m/z* 907, which could be associated with the loss of a carboxyl group. This result is consistent with the presence of unesterified carboxyl group, while the fragment ion at *m/z* 783 indicates the presence of an additional galloyl residue. We therefore concluded that this compound could be trisgalloyl hexahydroxydiphenoyl glucoside since the properties were comparable with data for trisgalloyl hexahydroxydiphenoyl glucoside as reported by Negri and Tabach, (2013). The phenolic compound 4.5-O-Dicaffeoylquinic acid at peak **5** with [M H] at *m/z* 515 was also identified in GPK. Peak **6** of the ion chromatogram at *m/z* 367 was temporarily assigned as caffeoylquinates methyl while peak at *m/z* 161 was identified as [acid-H_2O-H caffeic +]$^-$. Similarly, secondary peak MS^2 at *m/z* 135 was identified as [CO_2 caffeic acid H +]$^-$compound as reported by Jayasinghe et al., (2012)**.** The mass spectrum of galloylquinic acid at peak 7 (mw 344) showed the quasimolecular ion [M-H] at *m/z* 343 and another signal at m/z 169 due to the loss of quinic acid (Abu-Reidah et al., 2015) . Peak **8** showed an almost molecular ion m/z 327 [M-H]$^-$ and a fragment m/z 163 [M-H-hexose]$^-$. The fragment ion [MH-hexose 44] corresponding to the loss of -CO_2 group was also detected. Dihydro-o-coumaric acid 2-O-glucoside was identified by UV-vis spectrum data and MS SM (Ieri et al., 2012). Compound **9** showed a [M-H] $^-$ at *m/z* 315 and displayed the same fragmentation pattern as protocatechuic acid-O-hexoside

(Rivera-Pastrana et al., 2010). Peak **11** ([M–H]$^-$ at *m/z* 353) was identified as 3-O-caffeoylquinic acid, yielding the base peak at *m/z* 191 and the ion at *m/z* 179. This compound was identified as comedic acid derivative. Peak **12** was identified as with a typical fragment at *m/z* 541 (Spinola et al., 2015). The compound at peak **13** was detected at *m/z* 341 in the extracted ion chromatogram and tentatively it was assigned as caffeic acid-O-hexoside in accordance with previous findings by Gouveia and Castilho., (2010). Finally, compound **14** was identified as genistein since the stems presented a pseudo molecular ion [M-H]$^-$ at *m/z* 283 releasing a fragment ion at *m/z* 268 possibly due to the loss of a CH_3 group.

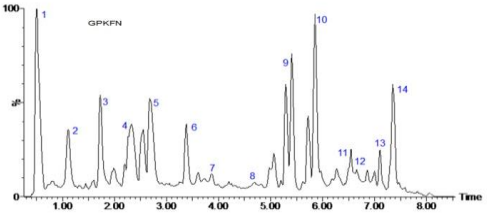

Figure 2. HPLC–MS spectra of Gingerbread plum kernel flour from Niger (GPKFN)

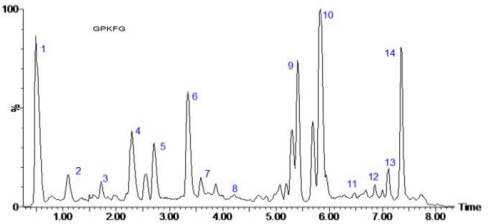

Figure 3. HPLC–MS spectra of Gingerbread plum kernel flour from Guinea (GPKFG)

Table 4: Retention time (RT), wavelengths of maximum absorption in the visible region (λmax), mass spectral data, identification and percentage of phenolic acids and flavonoids in Gingerbread plum kernel flour

Peak	RT (min)	Compounds	Niger Area %	Guinea Area %	λmax (nm)	MW [M-H]- (m/z)	MS2 (m/z)
1	0.46	Caffeic acid Hexoside	3.43	2.6	200	[341]	215,179,135
2	1.3	Epicatechin-3-O-gallate	2.74	3.57	269	[439]	407, 289, 271, 169, 125
3	1.87	Quercetin O-malonylhexosyl rhamnoside	3.84	3.53	273	[695]	651, 609, 447,301
4	2.21	Trisgalloyl(Hexahydroxydiphenoyl)glucose	14.72	6.48	270	[951]	907, 783
5	2.61	4.5-Dicaffeoylquinic acid	12.63	6.25	200	[515]	353, 203, 191,179, 173
6	3.31	Methyl-3-O-Caffeoyl quinic acid	1.33	4.77	237	[367]	335, 193, 161, 135, 133
7	3.98	Galloylquinic acid	0.22	ND	277	[343]	191, 169.2
8	4.43	Dihydro-o-coumaric acid	0.44	0.55	263	[328]	327, 165, 121
9	5.32	Protocatechuic acid-O-Hexoside derivative	0.43	0.38	320	[315]	269, 223, 161, 193
10	6.06	Quercetin-pentoside-hexoside	1.81	4.71	272	[595]	433[M-Hexose], 301
11	6.5	3.O-caffeoylquinic acid	32.9	14.22	272	[353]	191, 179, 173, 135
12	6.64	Coumaric acid derivative	25.06	52.46	272	[541]	325, 205, 163
13	7.17	6-O-Caffeoyl-α-glucose	0.28	0.16	272	[341]	179, 161, 135
14	7.27	Methyl genistein	0.17	0.32	272	[283]	268

4 CONCLUSIONS

The findings in this study have shown that gingerbread plum kernel contains substantial amounts of essential nutrients such as fats, proteins, vitamins, minerals and phenolic compounds that can beneficial effects in improving human health. The results have also shown that GPK has higher levels of antinutritional phytic acid and tannin but lower levels of trypsin inhibitors than other edible plant seeds. The findings of this study can have useful applications in genetic engineering and food production meant for enhancing human and animal health. It is recommended that further investigations should be conducted to determine if the content of tannin, phytic acid and trypsin inhibitors in GPK could pose a harmful effect when incorporated into animal feed.

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REFERENCES

1 ABU-REIDAH, I. M., ALI-SHTAYEH, M. S., JAMOUS, R. M., ARRAEZ-ROMAN, D. and SEGURA-CARRETERO, A. 2015. HPLC-DAD-ESI-MS/MS screening of bioactive components from Rhus coriaria L. (Sumac) fruits. *Food Chem,* 166, 179-91.

2 AHRENS, S., VENKATACHALAM, M., MISTRY, A. M., LAPSLEY, K. and SATHE, S. K. 2005. Almond (Prunus dulcis L.) protein quality. *Plant foods for human nutrition,* 60, 123-128.

3 AMADOU, I., GOUNGA, M. E., SHI, Y.-H. and LE, G.-W. 2014. Fermentation and heat-moisture treatment induced changes on the physicochemical properties of foxtail millet (Setaria italica) flour. *Food and Bioproducts Processing,* 92, 38-45.

4 AMZA, T., AMADOU, I., BALLA, A. and ZHOU, H. 2014. Antioxidant capacity of hydrolyzed protein fractions obtained from an under-explored seed protein: Gingerbread plum (Neocarya macrophylla). *Journal of Food Science and Technology*, *52*, 2770.

5 ARBONNIER, M. 2004. *Trees, shrubs and lianas of West African dry zones*, Editions Quae.

6 AREMU, M., OLONISAKIN, A., ATOLAYE, B. and OGBU, C. 2006. Some nutritional and functional studies of Prosopis africana. *Electron. J. Environ. Agric. Food Chem,* 5, 1640-1648.

7 BRUNE, M., ROSSANDER-HULT N, L., HALLBERG, L., GLEERUP, A. and SANDBERG, A.-S. 1992. Iron absorption from bread in humans: inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. *The Journal of nutrition,* 122, 442-449.

8 CADENAS, E. and PACKER, L. 1996. *Handbook of antioxidants*, Marcel Dekker Inc.

9 CHEN, H. J., INBARAJ, B. S. and CHEN, B. H. 2012. Determination of phenolic acids and flavonoids in Taraxacum formosanum Kitam by liquid chromatography-tandem mass spectrometry coupled with a post-column derivatization technique. *Int J Mol Sci,* 13, 260-85.

10 DIAS, M. I., BARROS, L., DUENAS, M., PEREIRA, E., CARVALHO, A. M., ALVES, R. C., OLIVEIRA, M. B., SANTOS-BUELGA, C. and FERREIRA, I. C. 2013. Chemical composition of wild and commercial Achillea millefolium L. and bioactivity of the methanolic extract, infusion and decoction. *Food Chem,* 141, 4152-60.

11 DORTA, E., GONZ LEZ, M., LOBO, M. G., S NCHEZ-MORENO, C. and DE ANCOS, B. 2014. Screening of phenolic compounds in by-product extracts from mangoes (Mangifera indica L.) by HPLC-ESI-QTOF-MS and multivariate analysis for use as a food ingredient. *Food Research International,* 57, 51-60.

12 EMBABY, H. E. and RAYAN, A. M. 2016. Chemical composition and nutritional evaluation of the seeds of Acacia tortilis (Forssk.) Hayne ssp. raddiana. *Food Chem,* 200, 62-8.

13 ERBAŞ, M., CERTEL, M. and USLU, M. K. 2005. Some chemical properties of white lupin seeds (Lupinus albus L.). *Food Chemistry,* 89, 341-345.

14 G. RAJA, IVVALA. ANAND SHAKER, INAMPUDI. SAILAJA, R. SWAMINATHAN, S. SALEEM BASHA, KONDAVEETI. SURESH and BABU. 2012. Phytochemical Screening and Reducing Power Assay of Nut Extracts of Juglans regia L. *International Journal of Chemical and Life sciences,* 2234, 8638.

15 GALLA, N. R., PAMIDIGHANTAM, P. R. and AKULA, S. 2012. Chemical, amino acid and fatty acid composition of Sterculia urens L. seed. *Food Hydrocolloids,* 28, 320-324.

16 GOUVEIA, S. C. and CASTILHO, P. C. 2010. Characterization of phenolic compounds in Helichrysum melaleucum by high-performance liquid chromatography with on-line ultraviolet and mass spectrometry detection. *Rapid Communications in Mass Spectrometry,* 24, 1851-1868.

17 GWATIDZO, L., BOTHA, B. M. and MCCRINDLE, R. I. 2013. Determination of amino acid contents of manketti seeds (Schinziophyton rautanenii) by pre-column derivatisation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate and RP-HPLC. *Food Chem,* 141, 2163-9.

18 HARBORNE, J. 1989. Biosynthesis and function of anti-nutritional factors in plants. *Aspects of Applied Biology (UK).*

19 HORAX, R., HETTIARACHCHY, N., KANNAN, A. and CHEN, P. 2010. Proximate composition and amino acid and mineral contents of Mormordica charantia L. pericarp and seeds at different maturity stages. *Food Chemistry,* 122, 1111-1115.

20 IERI, F., PINELLI, P. and ROMANI, A. 2012. Simultaneous determination of anthocyanins, coumarins and phenolic acids in fruits, kernels and liqueur of Prunus mahaleb L. *Food Chem,* 135, 2157-62.

21 IRVINE, F. R. 1961. Woody plants of Ghana with special reference to their uses. *Woody Plants of Ghana with Special Reference to their Uses.*

22 JAYASINGHE, L., LAKDUSINGHE, M., HARA, N. and FUJIMOTO, Y. 2012. Phenolic constituents from the fruit juice of Flacourtia inermis. *Natural product research,* 26, 278-281.

23 KHATTAB, R., GOLDBERG, E., LIN, L. and THIYAM, U. 2010. Quantitative analysis and free-radical-scavenging activity of chlorophyll, phytic acid, and condensed tannins in canola. *Food Chemistry,* 122, 1266-1272.

24 KUMAR DIXIT, A., KUMAR, V., RANI, A., MANJAYA, J. G. and BHATNAGAR, D. 2011. Effect of gamma irradiation on lipoxygenases, trypsin inhibitor, raffinose family oligosaccharides and nutritional factors of different seed coat colored soybean (Glycine max L.). *Radiation Physics and Chemistry,* 80, 597-603.

25 LEONARD, S. W., HARDIN, K. and LEKLEM, J. E. 2001. Vitamin B-6 content of spices. *Journal of Food Composition and Analysis,* 14, 163-167.

26 MARTINEZ-HERRERA, J., SIDDHURAJU, P., FRANCIS, G., DAVILA-ORTIZ, G. and BECKER, K. 2006. Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of Jatropha curcas L. from Mexico. *Food chemistry,* 96, 80-89.

27 MORENO, P. and SALVADO, V. 2000. Determination of eight water-and fat-soluble vitamins in multi-vitamin pharmaceutical formulations by high-performance liquid chromatography. *Journal of chromatography A,* 870, 207-215.

28 NEGRI, G. and TABACH, R. 2013. Saponins, tannins and flavonols found in hydroethanolic extract from Periandra dulcis roots. *Revista Brasileira de Farmacognosia,* 23, 851-860.

29 PUNCHA-ARNON, S. and UTTAPAP, D. 2013. Rice starch vs. rice flour: Differences in their properties when modified by heat–moisture treatment. *Carbohydrate polymers,* 91, 85-91.

30 RADHA, C., KUMAR, P. R. and PRAKASH, V. 2008. Preparation and characterization of a protein hydrolysate from an oilseed flour mixture. *Food chemistry,* 106, 1166-1174.

31 REDDY, N., PIERSON, M., SATHE, S. and SALUNKHE, D. 1985. Dry bean tannins: a review of nutritional

implications. *Journal of the American Oil Chemists Society,* 62, 541-549.

32 RIVERA-PASTRANA, D. M., YAHIA, E. M. and GONZ LEZ-AGUILAR, G. A. 2010. Phenolic and carotenoid profiles of papaya fruit (Carica papaya L.) and their contents under low temperature storage. *Journal of the Science of Food and Agriculture,* 90, 2358-2365.

33 RODRÍGUEZ-DAMIAN, A., DE LA ROSA-MILLÁN, J., AGAMA-ACEVEDO, E., OSORIO-DÍAZ, P. and BELLO-PÉREZ, L. 2013. Effect of different thermal treatments and storage on starch digestibility and physicochemical characteristics of unripe banana flour. *Journal of Food Processing and Preservation,* 37, 987-998.

34 ROOHANI, N., HURRELL, R., WEGMUELLER, R. and SCHULIN, R. 2012. Zinc and phytic acid in major foods consumed by a rural and a suburban population in central Iran. *Journal of Food Composition and Analysis,* 28, 8-15.

35 SHAHIDI, F. and NACZK, M. 1989. Effect of processing on the content of condensed tannins in rapeseed meals. *Journal of Food Science,* 54, 1082-1083.

36 SPINOLA, V., PINTO, J. and CASTILHO, P. C. 2015. Identification and quantification of phenolic compounds of selected fruits from Madeira Island by HPLC-DAD-ESI-MS(n) and screening for their antioxidant activity. *Food Chem,* 173, 14-30.

37 WANG, Z., LIU, Q., PAN, F., YUAN, L. and YIN, X. 2015. Effects of increasing rates of zinc fertilization on phytic acid and phytic acid/zinc molar ratio in zinc bio-fortified wheat. *Field Crops Research,* 184, 58-64.