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Effect of Cooking Methods on Anti-nutrient Content and Phenolic Acid Profiles of Groundnut Varieties Grown in Nigeria

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ABSTRACT

Phenolic compounds are secondary metabolites present in plant roots and seed which play a significant role as antioxidant and are produced as a means of protection against pathogen. Hence, in this study, phenolic compounds and the anti-nutritional factors of three cultivars of groundnuts (boro light, boro red and campala) grown in Nigeria were evaluated. The individual phenolic compounds were determined using Gas Chromatographic–Flame Ionization Detector (GC–FID) method. The data was analyzed by using one-way ANOVA with Duncan's post hoc test. Results of this study shows that antinutrients content of phenol was highest in sample A (30-130.3 mg/100 g), followed by sample C (11.70-101.0 mg/100 g) and sample B having the lowest (12.04-91.34 mg/100 g). Saponin content of sample C was the highest (4.89-5.29 mg/100 g), followed by sample B (1.39-13.59 mg/100 g) and sample A having the lowest (0.48-12.68 mg/100 g). Oxalate content was highest in sample B (5.08-27.78 mg/100 g), with sample A (3.24-11.44 mg/100 g) and C (5.05-10.25 mg/100 g) having the least respectively. Phytate content was highest in sample A (0.97-122.1 mg/100 g), followed by sample B (20.4-108 mg/100 g) and lowest by sample C (21.04-62.24 mg/100 g). Twenty-four phenolic compounds were identified in both fresh and roasted groundnut kernel. The boiled samples varied for p-hydroxy benzoic acid (15.77-17.49 mg/100 g); p-coumaric acid (3.69-4.07 mg/100 g); quercetin (0.96-1.186 mg/100 g), kaempferol (0.16-0.225 mg/100 g); chlorogenic acid (0.692-0.846 mg/100 g) as the major compounds while roasted samples varied for p-hydroxy benzoic acid (17.35-18.71 mg/100 g); p-coumaric acid (4.03-4.07 mg/100 g); quercetin (1.03-1.40 mg/100 g), kaempferol (0.185-0.276 mg/100 g); chlorogenic acid (0.83-0.86 mg/100 g). Phenolic compounds generated increased as roasting temperature and time increased and was significantly different from one another at $p \leq 0.05$. In this work roasting was found to have greater efficiency in the elimination or reduction of the levels of all antinutrients factors.

Key words: Boiling, roasting, saponin, oxalate, phytate, phenolic acids

INTRODUCTION

A consensus has been reached during the last couple of years that diet plays a major role in the development or control of chronic diseases such as cancer, coronary heart disease, obesity and diabetes. These assumptions were based on epidemiological

studies that show that fruits, vegetables, cereals and nuts provide the best protection against the development of diseases with little merit in recommending micronutrient (vitamins) supplements, for diseases prevention[1]. Nuts have been part of the human diet for a long time and valid as an alternative source of protein and lipid but recently many nuts have identified as a rich source of antioxidant and the most nutritionally concentrated kind of food available. Several studies indicate that frequent nut intake with 20-100 g day^{-1} will confer protective effect against cardiovascular disease[2]. Nuts are known to have favourable effect on cardiovascular disease through several mechanisms due to their fatty acids (low in saturated fatty acids and high in mono and polyunsaturated fatty acid), fibre or phytochemicals content or by a combination effect of compound presents studies connecting nut consumption and cardiovascular health disease have focused on fatty acid constituents; however there are other bioactive compounds that may confer additional protective effects. It was even observed that nut intake (walnut, coconut, hazelnut, groundnut and almond) had higher effect than the intake of the same fatty acids profile, confirming the effect of additional compounds such as phytosterols and phytochemicals. Several recent studies have shown that nut antioxidants have interesting biological effects that may be related to their favourable influence on cardiovascular disease[3]. Several studies have been carried out to determine the antioxidant potential of groundnut. These studies have clearly shown that the seeds are rich sources of antioxidants which have the capacity to scavenge free radicals when consumed in diet[1]. Generally, boiling is commonly used in folkloric medicine for the extraction of plants phytochemicals without any knowledge of their effect on the bioactive constituents of these plants. Hence, it is important to investigate the effect of boiling on the antioxidant properties of groundnut. Among the tropical nut consumed is groundnut (*Arachis hypogaea*) which plays an important role as an edible nut with the kernel which is valued as principal industrialized product. Groundnut is known to have several varieties which include Boro light, Boro red, Ela, Mokwa, Guta and Campala. The fatty acids and physico-chemical analysis of the oils of the varieties had been investigated[4]. Groundnut provides considerable amounts of mineral elements to supplement the dietary requirements of humans and farm animals. Groundnuts are one of those plant foods that are a dietary source of phytochemicals. Array of nutrients and phytochemicals play an important role in mechanism responsible for its putative health benefits. Despite the large available data on the antioxidant properties of peanuts, additional information are needed to ascertain the specific compounds that contribute to the antioxidant activity and to evaluate the application of peanut parts as natural antioxidant source. Generation of free radicals or Reactive Oxygen Species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress. Oxidative stress plays a role in heart diseases, neurodegenerative diseases, cancer and in the aging process. This concept is supported by increasing evidence that oxidative damage plays a role in the development of chronic, age-related degenerative diseases and that dietary antioxidants oppose this and lowers risk of disease. It has also been reported that the antioxidant constituents of plant materials delays or prevents oxidation of that substance and also provide protection from coronary heart disease, cancer and protect the body from damage caused by free radical induced oxidative stress. This study therefore focuses on discovering the effect of boiling and roasting on the phytochemicals constituents (saponin, tannins, oxalate, phytate, phenol) and phenolic acid present in the three groundnut varieties during boiling and roasting of the seeds. Studying the influence of boiling on the phytochemicals constituents of the seeds can contribute to the information on how to derive maximum health benefits from consumption of groundnut.

MATERIALS AND METHODS

Chemicals: 1,1-diphenyl-2-picryl-hydrazl (DPPH), 1-10 phenanthroline, trichloroacetic acid (TCA), potassium ferrocyanide, ferric chloride ($FeCl_3$), ethanol, diethyl ether, potassium permanganate, concentrated hydrogen chloride acid, ammonium thiocyanate, iron (III) Chloride, thiobabituric acid (TBA), sodium dodecyl sulfate (SDS) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used were of analytical grade and were obtained from standard chemical suppliers.

Seed collection and identification

Groundnut varieties: Boro light, Boro red and campala were purchased from a local market in Mile 12, Lagos State, Nigeria. The seeds were separated from undesirable materials such as stones, sand and plant parts. The seeds were thereafter washed, sun-dried and grinded into coarse powder by an electric blender and kept in clean plastic cans until use as the control (raw).

Processing techniques

Boiling: A set of three varieties groundnut kernels (200 g each) were boiled in distilled water (100°C) in a bean: water ratio of

1:10 (w/v) for 1 h. After boiling, the water was drained off and the boiled sample was mashed into paste using a ceramic mortar and then stored in as air tight container at 5°C until it was used.

Roasting: Another set of three varieties groundnut kernels (200 g each) were placed on frying pan were roasted using firewood for 1 h at about 300°C as practiced by the locals. Thereafter, the roasted kernels were pulverized to fine particles using the laboratory mill (SP) Supplies, PA, USA) and then stored in an airtight container at 4-6°C until it was used.

Preparation seed extracts: Powdered seeds were divided into three parts each weighing 20 g. The first part was placed into a beaker containing 400 mL of distilled water and left for 24 h to allow for extraction. Thereafter, the sample was decanted and filtered. The filtrate was then kept in the refrigerator and used as stock of raw extract of sample for all antioxidant determinations. The other two parts were poured into four beakers containing 400 mL of distilled water, sealed with an aluminum foil (closed system) and heated at boiling temperature (100°C). These were allowed to cool, decanted and extract was filtered using a Whatman filter paper. The filtrates were stored in the refrigerator and used as stock of boiled samples for all antioxidant determinations.

Determination of antinutritional factors

Determination of tannin content: This was determined by Folin Denis colorimetric method. Five grams of the groundnut samples (raw, boiled and roasted) were placed inside a volumetric flask and 50 mL of distilled water was dispensed inside the volumetric flask. The mixture was shaken for 30 min at room temperature and filtered to obtain the extract. A standard tannic acid solution was prepared, 2 mL of the standard solution and equal volume of distilled water were dispersed into a separate 50 mL volumetric flasks to serve as a standard and reagent blank respectively. Then 2 mL of each of the sample extracts was put in their respective labeled flask. The content of each flask was mixed with 35 mL distilled water and 1 mL of the Folin Denis reagent was added to each. This was followed by 2.5 mL of saturated Na_2CO_3 solution. Therefore, each flask was diluted to the 50 mL mark with distilled water and incubated for 90 min at room temperature. Their absorbance was measured at 760 nm in a spectrophotometer with the reagent blank at zero. The tannin content was calculated as shown below:

$$\text{Tannin (\%)} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{Vt}{Va} \quad (1)$$

Where:

W = Weight of sample
au = Absorbance of test sample
as = Absorbance of standard tanning solution
C = Concentration of standard tannin Solution
Vt = Total volume of extract
Va = Volume of extract analyzed

Determination of saponin content: This was done by the double solvent extraction gravimetric method[5]. Five grams of the sample was mixed with 50 mL of 20% aqueous ethanol solution and incubated for 12 h at a temperature of 55°C with constant agitation. After that, the mixture was filtered through Whatman No. 42 grades of filter paper. The residue was re-extracted with 50 mL of the ethanol solution for 30 min and the extracts weighed together. The combined extract was reduced to about 40 mL by evaporation and then transferred to a separating funnel and equal volume (40 mL) of diethyl ether was added to it. After mixing well, there was a partition and the other layer was discarded while the aqueous layer was reserved. This aqueous layer was re-extracted with the ether after which its pH was reduced to 45 with drop-wise addition of dilute NaOH solution. Saponin in the extract was taken up in successive extraction with 60 and 30 mL portion of normal butanol. The combine extract was washed with 5% NaCl solution and evaporated to dryness in a previously weighted evaporating dish. The saponin was then dried in the oven at 60°C (to remove any residual solvent) cooled in a desiccators and re-weighed. The saponin was determined and calculated as a percentage of the original samples.

$$\text{Saponin (\%)} = \frac{W_2 - W_1}{W} \times \frac{100}{1} \quad (2)$$

Where:

W = Weight of sample used
W_1 = Weight of empty evaporation dish
W_2 = Weight of dish + saponin extract

Determination of oxalate content: Oxalate was analyzed by treating the powdered samples with 0.75 M; H_2SO_4 stirred and filtered using Whatman No. 1 filter paper. The filtrates were then titrated hot (80-90°C) against standard potassium permanganate to a persistent faint pink colour[6].

Determination of phytic acid content: The modified procedure of AOAC[7] was used in determining phytic acid content. Two gram of ground groundnut samples (raw, boiled and roasted) was weighed in 250 mL conical flask. One hundrad milliliter of 2% concentrated hydrochloric acid was used to soak each sample into conical flask for 3 h. This was then filtered through a double layer of hardened filter paper. Fifty milliliter of each filtrate was placed in 250 mL beaker and 107 mL of distilled water was added in each case to give proper acidity. Ten milliliters (10 mL) of 0.3% ammonium thiocyanate solution was added into each solution as indicator. This was be titrated with standard iron (III) chloride solution which contained 0.00195 g iron per milliliter. The end point was slightly brownish yellow which persisted for 5 min. The percentage phytic acid will be calculated as below:

$$\text{Phytic acid (\%)} = \frac{X \times 1.19 \times 100}{2} \quad (3)$$

where, X = Titre value × 0.00195.

Determination of phenolic acid content

Phenolic extraction: Fifty milligrams of the three samples of groundnut varieties were extracted with 5 mL of 1 M NaOH for 16 h on a shaker at ambient temperatures. After extraction, the sample was centrifuged (5000×g), rinsed with water, centrifuged again and the supernatants were combined and placed in a disposable glass test tube and heated at 90°C for 2 h to release the conjugated phenolic compounds. The heated samples were cooled, titrated with 4 M HCl to pH<2.0, diluted 10 mL, with deionised water and centrifuged to remove the precipitate. The residue was extracted with 5 mL of 4 M NaOH, heated to 160°C in Teflon. After cooling, the mixture was filtered. Supernatant was collected and the residue washed with water (deionised). The supernatant were combined and adjusted to pH <2.0 with 4 M HCl. The filtrates were combined for further purification.

Purification of extracted phenolic acids: An aliquot (5-15 mL) of the various supernatants was passed through a conditioned Varian (Varian Assoc., Habour City, CA) Bond Elut PPL (3 mL size with 200 mg packing) solid-phase extraction tube at ~5 mL min^{-1} attached to a Visiprep (Supelco, Bellefonte, PA). The tubes were then placed under a vacuum (-60 kPa) until the resin was thoroughly dried after which the PAs were eluted with 1 mL of ethyl acetate into gas chromatography autosampler vials. The PPL tubes were conditioned by first passing 2 mL of ethyl acetate followed by 2 mL water (pH <2.0). The phenolic acid standards used were purchased from Aldrich (Aldrich Chemical Co., Milwaukee, WI).

Determination of phenolic compounds: Ground groundnut samples consisting of raw, boiled and roasted (150 g each) was placed into 2 L round-bottom flask containing 1 L of boiling deionized water (pH = 5.6) and connected to combined steam distillation and extraction apparatus. The mixture was heated to boiling in a heating mantle (Electromantle, Stafford, UK). The extraction was allowed to proceed for 1 h using 30 mL *n*-pentane as the extraction solvent. The extract was neutralized with an aqueous solution of sodium carbonate, dried with anhydrous sodium sulfate and then gently concentrated by Vigreux-column (PLT Puchong, Malaysia) distillation to 1 mL by heating at 40°C in a water bath. The concentrated extract was then transferred into glass ampules, sealed and stored at cold room temperature (5°C). The volatile components in the extracts were analyzed using a Hewlett-Packard 6890 gas chromatograph equipped with a mass selective detector (HP 6890 GC/FID). The Gas Chromatography (GC) conditions were as follows: detector temperature set at 28°C; injector 20°C; column (HP-INNOwax–cross-linked polyethylene glycol, 30 m ¥ 0.32 mm i.d., 0.25 mm, HP-INNOwax, Agilent Technologies, Palo Alto, CA) set at conditions of temperature programming as follows: 50°C for 5 min and then the temperature was increased to 200°C for 5 min at 4°C min^{-1}. The temperature was then kept constant at 200°C for 5 min. The sample (1 mL) was injected into the GC using helium as carrier gas at a flow rate of 1.3 mL min^{-1}. Injections were conducted with a split ratio of 1:20. Fragmentation was performed by Electrothermal Impact, with ionization voltage at 70 eV and scan mode between 50 and 450 mass units, Quantification was carried out from peak areas of components.

Statistical analysis: Data are expressed as means±standard deviation (SD) from three replicate experiments. Statistical analyses were carried out using statistical program of SPSS version 17.0 for windows (SPSS Corporation, Chicago, IL). Significant differences among peanut genotypes were analyzed by using one-way (analysis of variance) ANOVA with Duncan's post hoc test. The criterion for statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Antinutritional content analysis groundnut varieties: Anti-nutritional factors are a chemical compounds synthesized

Table 1: Antinutritional content of raw groundnut varieties

Samples	Phenol	Saponin	Tannin	Oxalate	Phytate
	----------	----------	(mg/100 g)	----------	----------
A	130.30±4.36a	12.68±0.23a	85.61±0.36a	11.44±1.25a	122.10±1.68a
B	91.34±1.11b	13.59±0.19a	81.80±0.44b	27.28±1.25b	108.40±3.65b
C	101.00±0.77b	19.89±0.75b	97.62±0.56c	10.25±0.18a	62.24±1.68c

A: Boro light, B: Boro red; C: Campala cultivar; Values are Means±standard deviation of three replicates. Means with no common letters within a column significantly differ (p<0.05)

Table 2: Antinutritional content of boiled groundnut varieties

Samples	Phenol	Saponin	Tannin	Oxalate	Phytate
	----------	----------	(mg/100 g)	----------	----------
A	33.30±1.36a	1.28±0.11a	0.81±0.23a	3.24±1.25a	2.77±1.18a
B	12.34±1.44b	1.89±0.23a	1.60±0.32b	5.08±1.25b	22.14±2.65b
C	11.00±0.55b	5.29±0.66b	3.62±0.44c	5.05±0.18a	23.14±1.28c

A: Boro light, B: Boro red; C: Campala cultivar; Values are Means±standard deviation of three replicates. Means with no common letters within a column significantly differ (p<0.05)

Table 3: Antinutritional content of roasted groundnut varieties

Samples	Phenol	Saponin	Tannin	Oxalate	Phytate
	----------	----------	(mg/100 g)	----------	----------
A	30.30±4.36a	0.48±0.33a	0.65±0.16a	3.84±1.03a	0.97±1.18a
B	12.04±1.23b	1.39±0.10a	1.92±0.24b	6.18±1.20b	20.14±3.05b
C	11.70±0.77b	4.89±0.15b	4.02±0.36c	5.88±0.12a	21.04±1.04c

A: Boro light, B: Boro red; C: Campala cultivar; Values are Means±standard deviation of three replicates. Means with no common letters within a column significantly differ (p<0.05)

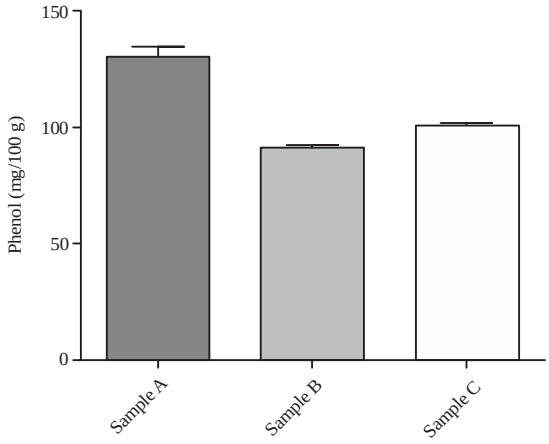

Fig. 1: Phenol content of groundnut varieties in mg/100 g

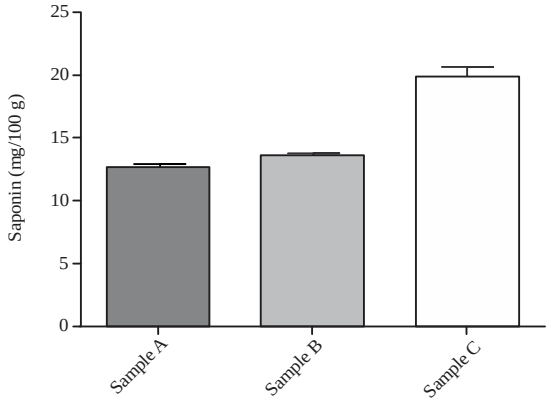

Fig. 2: Saponin content of groundnut varieties in mg/100 g

in natural food and/or feedstuffs by the normal metabolism of species and by different mechanisms (for example inactivation of some nutrients, diminution of the digestive process or metabolic utilization of food/feed) which exerts effect contrary to optimum nutrition. The phytochemical screening results of groundnut cultivars (Table 1-3, Fig. 1-5) showed that they contain oxalate, tannins, saponin and phenonlic compounds. The biological function of flavonoid includes protection against allergies, inflammation, platelets aggregation microbes, ulcer, vineses and tumors[8]. The Saponin constituent is responsible for the possession of hemolytic property. This can give the plant the traditional medicinal use as cholesterol binding agent. It also gives the plant leaves the bitter taste. Saponin has relationship with sex hormones like oxytocin. Oxytocin is a sex hormone involved in controlling the onset of labour in women and the subsequent release of milk[8]. The antinutrient content (phenol, oxalate, tannin, phytate and saponin) was significantly (p<0.05) higher in unprocessed samples compared to the processed sample (Table 1-4, Fig. 1-5). In this work roasting was found to have greater efficiency in the elimination or reduction of the levels of all antinutrients factors. Anti-nutritional factors are those substances found in most food substances which are poisonous to humans or in some ways limit the nutrient

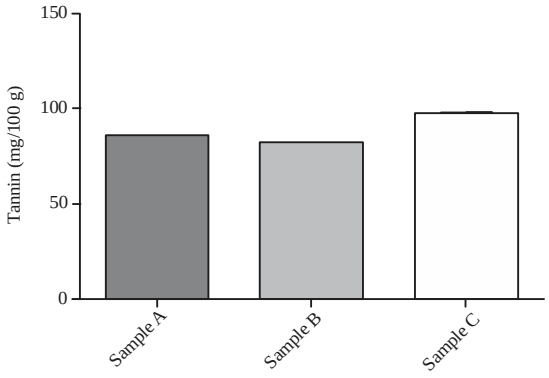

Fig. 3: Tannin content of groundnut varieties in mg/100 g

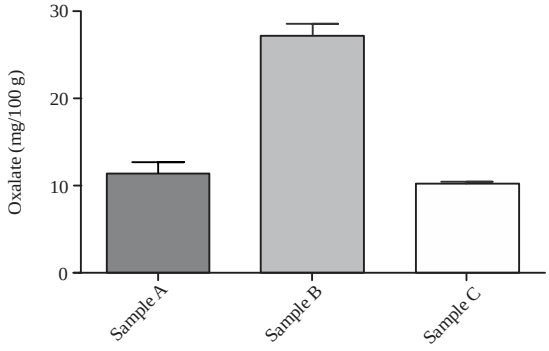

Fig. 4: Oxalate content of groundnut varieties in mg/100 g

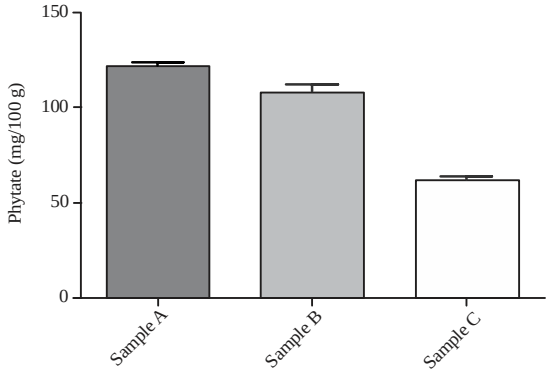

Fig. 5: Phytate content of groundnut varieties in mg/100 g

availability to the body. Plants evolved these substances to protect themselves and to prevent them from being eaten. However, if the diet is not varied, some of these toxins build up in the body to harmful levels[9]. Some vitamins in food may be destroyed by anti-nutritional substances. Aflatoxin in groundnut has been found to cause severe liver damage if eaten; they are heat stable and can be degraded by strong acid and alkaline treatment[10]. These anti-nutritional factors must be inactivated or removed, if values of food substances are to be fully maintained. Such chemical compounds, are frequently, but not exclusively associated with foods and feeding stuffs of plant origin. These anti-nutritional factors are also known as 'secondary metabolites' in plants and they have been shown to be highly biologically active. These secondary metabolites are secondary compound produced as side products of processes leading to the synthesis of primary metabolites. However, when used at low levels, phytate, lecithin, tannins, amylase inhibitors and saponin have also been shown to reduce the blood glucose and insulin responses to starchy foods and/or the plasma cholesterol and triglycerides. In addition, phytates, tannins, saponin, protease inhibitors, goitrogens and oxalates have been related to reduce cancer risks. This implies that anti-nutrients might not always harmful even though lack of nutritive value. Despite of this, the balance between beneficial and hazardous effects of plant bioactive and anti-nutrients rely on their concentration, chemical structure, time of exposure and interaction with other dietary components. Due to this, they can be considered as anti-nutritional factors with negative effects or non-nutritive compounds with positive effects on health. During thermal food processing involving heating or boiling, some chemical reactions may occur within the food components which brings about generation secondary antioxidants compounds in the form of Maillard Reaction Products (MRPs)[11]. In peanut roasting, soluble proteins and amino acids as a result of moisture losses may change and form Maillard derivatives such as pyrroles and furans which may have contributed to the increased in total phenolic compounds of roasted samples[12]. During thermal treatment, the brown colour of peanut formed increases due to sugar amino acid reactions, Millard browning which brings about the production of melanoidins[13]. The Millard reaction productions (MRPS) formed especially melanoidins, possess antioxidant activity which may scavenge oxygen radicals or chelating metals[14]. Therefore, heat increases the antioxidant capacity of peanut. The raw peanut skin color can be attributed to the presence of tannins and catechol-type compounds[13].

It also helps to protect the human body against cancer and also lower cholesterol level[15]. Saponins have been reported to be harmful due to their toxicity and haemolytic activity. However, there is enormous structural diversity within this chemical class and only a few are toxic[16]. They consist of a triterpene or steroid nucleus (the aglycone) with mono or oligosaccharides attached to this core. Most of the saponin occurs as insoluble complexes with 3-b-hydroxysteroids; which may also interact with bile acid and cholesterol to form large mixed micelles[17]. In addition, they form insoluble

saponin-mineral complexes with iron, zinc and calcium[18]. Saponins have reported to be nontoxic to man when consumed orally and have also been reported advantages of lowering plasma cholesterol concentrations in the body[19,20]. Also, recent evidence suggests that legume saponin may possess anti-cancer activity[16,19,20] and be beneficial for hyperlipidemia[16]. In addition, they reduce the risk of heart diseases in humans consuming a diet rich in food legumes containing saponin[21]. Epidemiological studies suggest that saponin may play a role in protection from cancer[16]. Research on colon cancer cells suggest that it is tehlipophilic saponin cores that may be responsible for the biological activity. Phytic acid (Inositol hexaphosphoric acid) forms insoluble salts with minerals such as calcium, iron, magnesium and zinc in food which inhibit their absorption into the blood stream[22]. Phytic acid is an important phosphorus storage form in plants known as phytates which regulate various cellular functions such as DNA repair, chromatin remodeling, endocytosis, nuclear messenger RNA export which are important for plant and seed development[23], as well as animal and human nutrition[24]. It is often regarded as an anti-nutrient because of strong mineral, protein and starch binding properties thereby decreasing their bioavailability[25]. Phytate has some potentially beneficial properties such as antioxidant activities, anti-cancer activities, ability to reduce cholesterol. Phytic acids delay postprandial glucose absorption reduce the bioavailability of toxic heavy metal such as cadmium and lead and exhibit antioxidant activity by chelating iron and copper (although phytic acid intake on improving antioxidant status *in vivo* remains unclear)[26]. Oxalate is attributed to its occurrence in plant oxalis (Wood sorel). The variation in oxalate content of fresh fruits and vegetables include genetic pre-harvest conditions of the plant, maturity of the edible product at harvest, post-harvest handling, storage and processing. Tannins has astringent properties, hastens the healing of wounds and inflamed mucous membrane. The presence of phenolic compounds in groundnut shows that the plant may have antimicrobial potential. This is because phenols and phenolic compounds have been extensively used in disinfections and remain the standards with which other bactericides are compared[27].

Phenolic acid analysis of groundnut cultivars: This study assessed the individual phenolic acid content of raw and roasted groundnut kernel using gas chromatography with flame ionization detector (GC-FID). A total of twenty-four phenolic compounds in the groundnut kernel were identified as shown in Table 4 to 9. The results showed that the roasted groundnut kernel has an average content of phenolic compounds higher than unroasted groundnut nuts. The phenolic compounds and contents are similar to those of other edible tree nuts such as pines, macadamia and almond nuts. Phenolic compounds are widely distributed in the plant.

Table 4: Total phenolic acid content of boiled boro light groundnut

S/N	Retention time (min)	Amount (mg/100 g)	Group name
1	9.693	9.52148e-1	1 Catechin
2	10.002	1.53156e-4	1 Protocatechuic acid
3	10.898	3.81971e-5	1 Vanillic acid
4	11.369	3.96645	1 P-coumaric acid
5	11.701	1.44405e-4	1 O-coumaric acid
6	12.832	16.95175	1 p-hydroxybenzoic acid
7	13.993	5.56703e-6	1 Gallic acid
8	14.503	1.56567e-6	1 Caffeic acid
9	15.102	2.00041e-1	1 Ferulic acid
10	15.253	1.69164e-6	1 Syringic acid
11	15.496	1.96202e-5	1 Piperic acid
12	16.226	8.08552e-6	1 Sinapinic acid
13	17.641	1.06966e-2	1 Resveratrol
14	19.100	5.08463e-5	1 Apigenin
15	19.488	1.67567e-6	1 Naringenin
16	20.397	1.70257e-1	1 Kaempferol
17	21.763	1.50862e-2	1 Luteolin
18	22.850	6.97258e-2	1 Epicatechin
19	23.077	6.06893e-7	1 Epigallocatechin
20	23.452	1.76036e-5	1 Gingerol
21	24.022	1.06392	1 Quercetin
22	24.271	5.62296e-5	1 Isorhamnetin
23	24.438	5.72697e-6	1 Myricetin
24	25.533	6.92097e-1	1 Chlorogenic acid

Table 5: Total phenolic acid content of boiled Campala groundnut cultivar

S/N	Retention time (min)	Amount (mg/100 g)	Group name
1	9.693	8.70005e-1	1 Catechin
2	10.008	1.84811e-4	1 Protocatechuic acid
3	10.897	4.71471e-5	1 Vanillic acid
4	11.370	3.69112	1 P-coumaric acid
5	11.702	1.00017e-4	1 O-coumaric acid
6	12.833	15.77208	1 p-hydroxybenzoic acid
7	14.160	4.51127e-6	1 Gallic acid
8	14.505	1.98828e-6	1 Caffeic acid
9	15.036	1.22400e-1	1 Ferulic acid
10	15.391	1.39748e-6	1 Syringic acid
11	15.495	1.90420e-5	1 Piperic acid
12	16.461	7.50904e-6	1 Sinapinic acid
13	17.642	7.09569e-3	1 Resveratrol
14	19.102	3.67013e-5	1 Apigenin
15	19.489	1.19001e-6	1 Naringenin
16	20.396	1.60045e-1	1 Kaempferol
17	21.954	1.29392e-2	1 Luteolin
18	22.695	4.01112e-2	1 Epicatechin
19	23.068	4.53827e-7	1 Epigallocatechin
20	23.344	1.37337e-5	1 Gingerol
21	23.956	9.61366e-1	1 Quercetin
22	24.325	3.71844e-5	1 Isorhamnetin
23	24.511	4.82415e-6	1 Myricetin
24	25.528	7.32124e-1	1 Chlorogenic acid

Table 6: Total phenolic acid content of boiled boro red groundnut cultivar

S/N	Retention time (min)	Amount (mg/100 g)	Group name
1	9.693	1.15922	1 Catechin
2	10.110	2.05592e-4	1 Protocatechuic acid
3	10.897	7.03357e-5	1 Vanillic acid
4	11.370	4.07443	1 P-coumaric acid
5	11.703	1.79982e-4	1 O-coumaric acid
6	12.833	17.49077	1 p-hydroxybenzoic acid
7	14.160	6.37738e-6	1 Gallic acid
8	14.506	1.82743e-6	1 Caffeic acid
9	15.158	1.97486e-1	1 Ferulic acid
10	15.391	1.44972e-6	1 Syringic acid
11	15.495	2.19643e-5	1 Piperic acid
12	16.440	1.00865e-5	1 Sinapinic acid
13	17.642	1.07365e-2	1 Resveratrol
14	19.104	4.86413e-5	1 Apigenin
15	19.488	2.42304e-6	1 Naringenin
16	20.396	2.24493e-1	1 Kaempferol
17	21.830	1.94689e-2	1 Luteolin
18	22.696	1.43777e-1	1 Epicatechin
19	23.221	7.24459e-7	1 Epigallocatechin
20	23.339	1.65023e-5	1 Gingerol
21	23.955	1.18479	1 Quercetin
22	24.328	6.11989e-5	1 Isorhamnetin
23	24.504	5.80137e-6	1 Myricetin
24	25.528	8.46345e-1	1 Chlorogenic acid

Table 7: Total phenolic acid content of roasted boro light groundnut cultivar

S/N	Retention time (min)	Amount (mg/100 g)	Group name
1	9.690	1.01455	1 Catechin
2	10.001	2.05136e-4	1 Protocatechuic acid
3	10.893	4.42091e-5	1 Vanillic acid
4	11.367	4.02894	1 P-coumaric acid
5	11.696	1.51107e-4	1 O-coumaric acid
6	12.830	17.35032	1 p-hydroxybenzoic acid
7	14.063	5.70701e-6	1 Gallic acid
8	14.577	1.97405e-6	1 Caffeic acid
9	15.104	2.10934e-1	1 Ferulic acid
10	15.388	1.75989e-6	1 Syringic acid
11	15.493	2.31016e-5	1 Piperic acid
12	16.224	1.01099e-5	1 Sinapinic acid
13	17.639	1.05860e-2	1 Resveratrol
14	19.100	6.01342e-5	1 Apigenin
15	19.486	1.89008e-6	1 Naringenin
16	20.399	2.01892e-1	1 Kaempferol
17	21.794	1.65574e-2	1 Luteolin
18	22.832	7.80781e-2	1 Epicatechin
19	23.076	7.06471e-7	1 Epigallocatechin
20	23.326	1.88082e-5	1 Gingerol
21	23.952	1.24116	1 Quercetin
22	24.270	6.35019e-5	1 Isorhamnetin
23	24.437	5.82865e-6	1 Myricetin
24	25.530	8.31110e-1	1 Chlorogenic acid

Table 8: Total phenolic acid content of roasted boro red groundnut

S/N	Retention time (min)	Amount (mg/100 g)	Group name
1	9.691	9.38927e-1	1 Catechin
2	10.002	1.88509e-4	1 Protocatechuic acid
3	10.898	4.76240e-5	1 Vanillic acid
4	11.368	4.01123	1 P-coumaric acid
5	11.698	1.18966e-4	1 O-coumaric acid
6	12.830	16.42396	1 p-hydroxybenzoic acid
7	14.064	5.41434e-6	1 Gallic acid
8	14.502	2.07319e-6	1 Caffeic acid
9	15.103	1.64266e-1	1 Ferulic acid
10	15.252	1.40515e-6	1 Syringic acid
11	15.388	2.37918e-5	1 Piperic acid
12	16.241	9.08647e-6	1 Sinapinic acid
13	17.639	9.79038e-3	1 Resveratrol
14	19.099	5.16066e-5	1 Apigenin
15	19.486	1.38867e-6	1 Naringenin
16	20.396	1.85436e-1	1 Kaempferol
17	21.791	1.46777e-2	1 Luteolin
18	22.835	5.68762e-2	1 Epicatechin
19	23.075	5.18358e-7	1 Epigallocatechin
20	23.430	1.67911e-5	1 Gingerol
21	24.017	1.03197	1 Quercetin
22	24.272	4.79392e-5	1 Isorhamnetin
23	24.438	5.40926e-6	1 Myricetin
24	25.528	8.01997e-1	1 Chlorogenic acid

Table 9: Total phenolic acid content of roasted campala groundnut

S/N	Retention time (min)	Amount (mg/100 g)	Group name
1	9.690	1.21473	1 Catechin
2	9.999	2.44176e-4	1 Protocatechuic acid
3	10.792	7.10382e-5	1 Vanillic acid
4	11.367	4.35069	1 P-coumaric acid
5	11.694	1.95585e-4	1 O-coumaric acid
6	12.830	18.70622	1 p-hydroxybenzoic acid
7	14.062	6.88308e-6	1 Gallic acid
8	14.501	2.05741e-6	1 Caffeic acid
9	15.103	2.65041e-1	1 Ferulic acid
10	15.388	1.82882e-6	1 Syringic acid
11	15.496	2.87191e-5	1 Piperic acid
12	16.243	1.03393e-5	1 Sinapinic acid
13	17.696	1.38333e-2	1 Resveratrol
14	19.104	6.98402e-5	1 Apigenin
15	19.484	1.89798e-6	1 Naringenin
16	20.446	2.76085e-1	1 Kaempferol
17	21.801	2.27200e-2	1 Luteolin
18	22.840	1.50668e-1	1 Epicatechin
19	23.079	7.92026e-7	1 Epigallocatechin
20	23.436	1.86206e-5	1 Gingerol
21	24.022	1.40080	1 Quercetin
22	24.269	7.03796e-5	1 Isorhamnetin
23	24.435	6.15717e-6	1 Myricetin
24	25.539	8.65993e-1	1 Chlorogenic acid

These compounds are known as important antioxidants because of their ability to donate hydrogen atom or an electron in order to form stable radical intermediate. They prevent the oxidation of various biological molecules. Several oilseeds and their byproducts have been reported containing phenolic compounds which are safe sources of natural antioxidants[26]. The identified phenolic compounds expressed as mg/100 g dry peanut seeds per cultivar are presented in

Table 4. The GC-FID study of *Arachis hypogeal* seed extract identified 24 phenolic compounds in three cultivars, with p-hydroxybenzoic acid, p-coumaric acid and quercetin, as the major compounds. Other compounds include caffeic acid, syringic acid, catechin, kaempferol and luteolin. Previous studies reported that predominant phenolics present in peanut kernel were *p*-hydroxy-benzoic and *p*-coumaric acid[28] and this also agreed with Win *et al.*[29]. After roasting, the amount of *p*-hydroxy-benzoic acid and *p*-coumaric acid were slightly increased however these values were not significantly different from each other. It has also been reported that peanut skin contains flavonoids such as epigallocatechin, epicatechin, catechin gallate, proanthocyanidins, including procyanidin B, phenolics acids such as chlorogenic, coumaric, caffeic and ferulic acids and the stilbene trans-resveratrol[30]. Resveratrol is one of the major stilbene phytoalexins that are produced by different parts of the peanuts. Substantial amounts of resveratrol was found in the leaves, roots and shells of peanuts but the levels noted to be lower in developing seed and seed coats of peanuts[31]. The maturity of peanut may also affected resveratrol content where it was reported that immature peanuts have higher levels of resveratrol than that of mature peanuts[32]. These results of this study is also in agreement with report of study carried out by Talcott *et al.*[28] who worked on peanut. Peanut kernels contain caffeic, ferulic and coumaric acids[30] and the syilbenes trand-reverantrol and its glucoside, transpiceid. Free p-coumaric acid was the predominant soluble polyphenolic that contributed to the antioxidant capacity of peanut kernels[26]. Phenolic compounds such as flavonoid and phenolic acids exhibit antioxidant and other specific properties. The potential health benefit of legume is attributed to the presence of secondary metabolite such as phenolics compound that possess antioxidant properties. Some phenolic compound can also reduce protein digestibility and mineral bioavailability. p-hydroxy benzoic acid is a popular antioxidant because of its low toxicity. It also has estrogenic activities both *in vitro* and *in vivo* and thus stimulates the growth of human breast cancer cell lines. Furthermore p-coumaric acid has antioxidant properties and is believed to reduce the risk of stomach cancer by reducing the formation of carcinogenic nitrosamines. Epigallocatechin is the most abundant catechin in tea; it's a polyphenol under basic research for its potential to affect human health and diseases. Epigallocatechin is used in many supplements. During food processing (heating or boiling), certain chemical reactions may occur among the food components and lead to generate secondary antioxidants compounds such as Maillard Reaction Products (MRPs). In peanut roasting, soluble proteins and amino acids are changed as a result of moisture losses and form Maillard derivatives, including pyroles and furans which may contribute to the increased in total phenolic compounds of roasted samples[28]. Recent research has shown that phenolic acids play a role in the capture of free radicals and also involved in other physiological mechanisms that stimulate the activity of antioxidant enzymes or as cellular signaling substances that activate and/or inhibit the expression of some enzymes related to the cancer process[26]. In the present study, roasting caused a significant increase of the phenolic content in the roasted groundnut even at higher roasting temperature and time. These results suggest that the phenolic compounds present in cashew nuts are not thermolabile and that the nut does not have a protect effect against heat. Such differences in data may be due to the different cultivars and different maturity/harvesting stages of peanuts used in the studies. The earlier studies have shown that luteolin content of peanut hulls increased with maturity[33]. Quercetin was reported to be the major flavonoid in peanut seed[34].

Some recommendations are nut consumption should be increased that are in return benefit for health such as reduced cardiovascular disease risk, are supported by epidemiological and clinical studies which revealed an inverse association between the frequency of nut consumption and weight increase[34]. In addition to the presence high fatty acid profile, high protein content, fiber, potassium, calcium, magnesium, tocopherol, phytosterols and other bioactive compounds have shown their multiple cardiovascular benefits. In this way, it is not possible to attribute the benefits to a single class of compounds and/or estimate which would be the recommendable intake.

CONCLUSION

A large variation in phenols and antioxidant capacity was found in the nuts evaluated in the present study. The results of the present study indicated that cooking procedures drastically reduced the level of antinutrients in groundnut varieties. However, this study has been able to show that boiling which incidentally is a common practice of traditional medical practitioners that may potentiates the antioxidant properties of groundnut aqueous extract. Therefore, boiled and roasted extracts of groundnut and their by-products may be useful for therapeutic purposes and as an inexpensive source of natural antioxidants which could also be explored as useful ingredients for functional foods. It can be applied in

other food industries as a natural antioxidant instead of synthetic antioxidants. Further biological studies are expected to show positive results.

REFERENCES

1. Alasalvar, C., F. Shahidi, C.M. Liyanapathirana and T. Ohshima, 2003. Turkish Tombul hazelnut (*Corylus avellana* L.) Compositional characteristics. J. Agric. Food Chem., 51: 3790-3796.
2. Abe, L.T., F.M. Lajolo and M.I. Genovese 2010. Comparison of phenol content and antioxidant capacity of nuts. Clienc. Tecnol. Aliment., Compinas, 30: 254-259.
3. Oboh, G., 2005. Effect of blanching on the antioxidant property of some tropical green leafy vegetables. LWT-Food Sci. Technol., 38: 513–517.
4. Anyasor, G.N., K.O. Ogunwenmo, O.A. Oyelana, D. Ajayi and J. Dangana, 2009. Chemical analyses of Groundnut (*Arachis hypogaea*) oil. Pak. J. Nutr., 8: 269-272.
5. Peng, J.P. and H. Kobayasli, 1995. Novel furastonol glycosides from allium anacros tenon. Planta Med., 6: 58-61.
6. Hodgkinson, A., 1977. Oxalic acid in biology and medicine, Academic Press London.
7. AOAC., 2005. Official Methods of Analysis, 15th Edn. Association of Official Analytical Chemists, Washington DC.
8. Oulai, P.D., L.T. Zoue, M.E. Bedikou, R.M. Megnanou and S.L. Niamke, 2014. Impact of cooking on nutritive and antioxidant characteristics of leafy vegetables consumed in northern Côte d'ivoire. Int. J. Plant Anim. Environ. Sci., 4: 576- 585.
9. Ihemeje, A., S.U. Okorie and C.C. Ekwe, 2012. Effects of processing methods on biochemical, functional and antinutritional properties of African walnut (*Tetracarpidium conophorum*). J. Biol. Sci. Bioconservation, 4: 55-64.
10. Sahlin, E., G.P. Savage and C.E. Lister, 2004. Investigation of the antioxidant properties of tomatoes after processing. J. Food Compos. Anal., 17: 635–647.
11. Dittrich, R., F. El-Massry, K. Kunz, F. Rinaldi, C.C. Peich, M.W. Beckmann and M. Pischetstrieder, 2003. Millard reaction products inhibit oxidation of human low- density lipoproteins *in vitro*. J. Agric. Food Chem., 51: 3900-3904.
12. Yanagimoto, K., K. Lee, H. Ochi and T. Shibamoto, 2002. Antioxidative activity of heterocyclic compounds found in coffee volatiles produced by the Maillard reaction. J. Agric. Food Chem., 50: 5480-5484.
13. Sobolev, V.S. and R.J. Cole, 2004. Note on utilization of peanut seed testa. J. Sci. Food Agric., 84: 105-111.
14. Yilmaz, Y. and R. Toledo, 2005. Antioxidant activity of water soluble Maillard reaction products. Food Chem., 93: 273-278.
15. Shi, J., K. Arunasalam, D. Yeung, Y. Kakuda, G. Mittal and Y. Jiang, 2004. Saponins from edible legumes: Chemistry, processing and health benefits. J. Med. Food, 7: 67-78.
16. Oakenfull, D. and G.S. Sidhu, 1989. Saponin. In: Toxicants of Plant Origin. Cheeke, P. (Eds.), Boca Raton, FL: CRC.
17. Milgate, J., and D.C.K. Roberts, 1995. The nutritional and biological significance of saponin. Nutr. Res., 75: 1223-1249.
18. Chang, W.W., C.Y. Yu, T.W. Lin, P.R. Wang and Y.C. Tsai, 2006. Soya saponin decreases I the expression of alpha2, 3-linked sialic acid on the cell surface and suppresses the metastatic potential of B16F10 melanoma cells. Biochem. Biophys. Res. Commun., 341: 614-619.
19. Topping, D.L., G.B. Stover, G.D. Calvert and R.A. Meller, 1980. Effects of dietary saponin on fecalbile acids and neutral sterols, plasma lipids and lipoprotein turnover in the pig. Am. J. Clin. Nutr., 33: 783-786.
20. Merck Index, 1976. 9th Edn., M. Merck, Rahway, pp: 8120.
21. Griel, A.E., B. Eissenstat, V. Juturu, G. Hsieh and P.M. Kris-Etherton, 2004. Improved diet quality with peanut consumption. J. Am. College Nutr., 23: 660-668.
22. Zhou, J.R. and J.W. Erdman, Jr., 1995. Phytic acid in health and disease. Crit. Rev. Food Sci. Nutr., 35: 495-508.
23. Barros, A.I., F.M. Nunes, B. Gonçalves, R.N. Bennett and A.P. Silva, 2011. Effect of cooking on total vitamin C contents and antioxidant activity of sweet chestnuts (*Castanea sativa* Mill.). Food Chem., 128: 165-172.
24. Weaver, C.M. and S. Kannan, 2002. Phytate and Mineral Bioavailability. In: Food Phytates, Sathe and S.K. Reddy (Eds.), Boca Raton: CRC., pp: 21 1-224.
25. Minihane, A.N. and Rimbach, 2002. Gerald Iron absorption and the iron binding and anti-oxidant: Properties of phytic acid. Int. J. Food Sci. Technol., 37: 741-748.
26. Kim, K., R. Tsao, R. Yang and S.W. Cui, 2006. Phenolic acid profiles and antioxidant activities of wheat bran extract and the effect of hydrolysis conditions. J. Food Chem., 95: 466-473.
27. Yu, J., M. Ahmedia and I. Goktepe, 2005. Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. Food Chem., 9: 199-206.
28. Talcott, S.T., S. Passeretti, C.E. Duncan and D.W. Gorbet, 2005. Polyphenolic content and sensory properties of normal and high oleic acid peanuts. Food Chem., 90: 379-388.
29. Win, M.M., A.A. Hamid, B.S. Baharin, F. Anwar, M.C. Sabu and M.S. Pak-Dek, 2011. Phenolic compounds and antioxidant activity of peanut's skin, hull, raw kernel and roasted kernel flour. Pak. J. Bot., 43: 1635-1642.
30. Francisco, M.L. D.L. and A.V.A. Resurreccion, 2009. Development of a reversed-phase high performance liquid chromatography (RP-HPLC) procedure for the simultaneous determination of phenolic compounds in peanut skin extracts. Food Chem., 117: 356-363.

31. Chung, L.M., M.R. Park, J.C. Chun and SJ. Yun, 2003. Resveratrol accumulation and resveratrol synthesis gene expression in response to a biotic stresses and hormones in peanut plants. Plant Sci., 164: 103- 109.
32. Yanagimoto, K., K. Lee, H. Ochi and T. Shibamoto, 2002. Antioxidative activity of heterocyclic compounds found in coffee volatiles produced by the Maillard reaction. J. Agric. Food Chem., 50: 5480-5484.
33. Yu, J., M. Ahmedna and I. Goktepe, 2005. Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. Food Chem., 90: 199-206.
34. Mattes, R.D., P.M. Kris-Etherton and G.D. Foster, 2008. Impact of peanuts and tree nuts on body weight and healthy weight loss in adults. J. Nutr., 138: 1741S-1745S.