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THE INFLUENCE OF COOKING METHODS ON THE ANTIOXIDANT STATUS OF *TETRAPLEURA TETRAPETRA*

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ABSTRACT

Africa is blessed with a rich array of local spices such as *Tetrapleura tetraptera.* The culinary uses of *T. tetraptera* are many. The seed of *Uhiokirihio* is majorly used in the preparation of 'Banga' (palm fruit) soup, meat pepper soup and other types of soup in the southern part of Nigeria. It is also commonly used in soups of nursing mothers to prevent post-partum contractions. The rich antioxidant activity of this spice has been reported. There is, however, a dearth of information on the effect of different cooking methods on its antioxidant activity. This study, therefore, evaluated the effect of cooking methods on the antioxidant status of the seeds of *Tetrapleura tetraptera*. The raw seeds of the spice were both toasted and boiled separately for 0, 5, 10, 15 and 20 minutes, respectively. The samples were analysed for anti-nutrients, vitamin contents and antioxidant properties. Anti-nutrient evaluation of the ethanolic (80% ethanol) extract revealed that both toasting and boiling time caused significant ($p<0.05$) variations in all the anti-nutrients studied. Total phenolics increased ($p<0.05$) from 20.80mg/100g to 28.53mg/100g for toasted samples and from 20.80mg/100g to 30.51mg/100g for boiled samples, respectively. Both cooking methods caused significant reduction in the phytate and tannin levels of the seeds. At the end of the cooking processes, tannin level was reduced by 62.07 % for boiling and 75.68 % for toasting treatment. The cooking methods led to significant reduction in both the vitamin C and β- carotene levels of the samples. Boiling for 20 min caused a 91.98% decrease in vitamin c and a 59.52 % decrease in β-carotene while toasting reduced these nutrients by 86.73 % and 39.88 %, respectively. Antioxidant activity as measured by DPPH scavenging activity and FRAP showed a significant rise with increase in cooking time. The DPPH activity of the toasted samples increased from 22.06µg/ml to 27.64 µg/ml while the boiled samples increased from 22.06µg/ml to 43.26µg/ml. It was observed that boiling led to a greater increase in total phenolics and antioxidant activity than toasting. It could, therefore, be concluded that cooking *T. tetraptera* seeds would improve its antioxidant properties.

Key words: *Tetrapleura tetrapetra*, spices, boiling, toasting, cooking, phytochemicals, antioxidant

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INTRODUCTION

Spices are products of plants which are used in various forms such as fresh, ripe, dried, broken or powdered mostly to contribute to colour, taste, aroma, flavour and pungency of food [1]. Spices, which include leaves (coriander, pepper mint), buds (clove), bulbs (garlic, onion), fruits (red chili, black pepper), stem (cinnamon), rhizomes (ginger) and other plant parts, have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods [2]. Spices are rich sources of health promoting phytochemicals.

Several epidemiological studies have established a link between phytochemicals and the range of biological activities that impart health benefits in humans. Such health benefits include the prevention of certain degenerative diseases like cancer and cardiovascular diseases [3]. Phytochemicals are bioactive compounds that confer these health benefits because they possess antioxidant activity. Their antioxidant activity is due to the presence of vitamins and pro-vitamins (such as ascorbic acid, tocopherols), terpenoids, phytoestrogens and carotenoids and in addition to that they are also rich in a wide variety of phenolic substances such as flavonoids, and alkaloids [4]. The antimicrobial activity, antiseptic and preservative effects of spices are also attributed to these compounds [5, 6].

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals in biological systems, leading to chain reactions that may damage cells. Antioxidants are therefore important to plants and animals to balance their oxidative state, counteract oxidative stress and reduce the detrimental effects of free radicals produced in the body. These natural antioxidants scavenge free radicals, enhance the immune system, prevent diseases and improve general health and life quality.

Phenolic compounds such as flavonoids, phenolic acids et cetera have a major role to play in the antioxidant effect of spices. The antioxidant effect of phenolic compounds is mainly due to their redox properties and is the result of various possible mechanisms such as free-radical scavenging activity, transition-metal chelating activity, and/or singlet-oxygen-quenching capacity [7]. They also play important roles in stabilizing lipid peroxidation and upregulation of antioxidant enzyme [8].

A great number of aromatics, spicy, medicinal and other plants like garlic, onion and ginger contain chemical compounds exhibiting antioxidant properties that play significant roles in lowering of cholesterol, prevention of certain diseases like cardiovascular diseases, liver damage, inflammatory diseases and diabetes [10]. It is not surprising that spices and herbs are at the top of the list of 100 products with the highest antioxidant content [11]. Their antioxidant activities are ten times higher than that of fruit and vegetables.

Nigeria is blessed with a rich array of local spices such as *Monodora myristica* (Africa nutmeg), *Xylopia aethiopica* (Ethiopian pepper), *Syzygium aromaticum* (Tropical Cloves), *Piper guineense* (Black pepper), *Aframomum danielli* (Bastered melegueta),

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Afromomum melegueta (Alligator pepper/ grains of paradise), *Clerodendrum volubile* (Locally known as "Obenetete") and *Tetrapleura tetrapetra* among others. *T. tetrapetra* is known as 'Uhiokirihio' among the Igbos of Nigeria. The high antioxidant activity of *T. Tetrapetra* has been reported [12].

The economic and medicinal significance of *T. tetraptera* are numerous. The fruits have been widely used in Nigeria for manufacturing of seasoning spices, pomades and soaps due to its pleasant aroma characteristics [1] while it is used in Ghana as a vitamin source. An infusion of the whole fruit is usually used by convalescents for bathing in order to be relieved from feverish conditions [13]. The infusion is also used to relieve constipation and as an emetic. The plant has many other traditional medicinal uses such as in the management of convulsions, leprosy, inflammation and rheumatic pains. The soft parts of the fruit and the bark are known to contain sugars, tannins, traces of saponin and amino acids [14].

There is at present increasing interest both in the industry and in scientific research on spices and aromatic herbs because of their strong antioxidant and antimicrobial properties. Unfortunately, there is a dearth of information on the phytochemical compositions, antioxidant and antimicrobial properties of many Nigerian indigenous spices including *Tetrapleura tetrapetra*. Few studies carried out focused on the raw spices. It is known that food processing affects the phytochemical composition of herbs, spices, vegetables and fruits. It is, therefore, pertinent to study the effect of processing particularly cooking on the phytochemical and antioxidant properties of this spice. It is against this backdrop, therefore, that this research was designed to evaluate the effect of boiling and toasting time on the phytochemical and antioxidant status of a local Nigerian spice - *Tetrapleura tetrapetra.*

MATERIALS AND METHODS

Procurement of raw materials

The seeds of *Tetrapleura tetrapetra* were purchased from Orba market in Nsukka, Enugu State, Nigeria. The seeds were sorted, washed twice with clean water, dried in an oven at 60^{o}C for eight hours, kept in dark coloured tightly closed plastic container and stored at a temperature of -4^{o}C until analysis.

Boiling and toasting of the seeds

One kilogramme of the cleaned seeds was divided into two equal lots, one lot was for boiling treatment while the other was for toasting treatment. For the boiling treatment, the seeds (500 g) were added to 1mL of boiling water and boiled for 20 mins. Samples were removed (100 g) from the pot at the interval of five minutes. The seeds were allowed to cool for 30 mins, oven-dried at 60 ^{o}C until a constant weight was achieved. The seeds were then ground into powder using hand grinding machine, sieved through a clean cheese cloth and stored in airtight container at -4 ^{o}C.

For toasting treatment, the seeds were placed in hot (140^{o}C) saucepan, and toasted for 20 min with constant stirring. Samples were drawn (100 g) after every 5 mins, ground

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and sieved through a cheese cloth and stored as above. Raw (untreated) seeds served as control.

Preparation of spice extract for determination of total phenolic content and flavonoid content

Ground seed (10 g) was placed in a beaker with 50 ml 80% ethanol, allowed to stand at room temperature (28^{o}C ± 1) for 5 hrs on a shaker and thereafter was filtered using a Whatman filter paper No.2.

Determination of total phenol

Total phenol content was determined using Folin-Ciocalteu method [15]. The total phenol content of the various spice samples was determined by mixing 0.5 ml aliquot of freshly prepared sample extract with equal volume of distilled water, 0.5 ml Folin-Ciocalteu's reagent, and 2.5 ml of saturated solution of sodium carbonate (Na_2CO_3) were added. The absorbance was measured after 40 min at 760 nm. The total phenol content was subsequently calculated using gallic acid as standard.

Determination of total flavonoid content

The total flavonoid content of the spice extracts was determined using a slightly modified method reported by Meda *et al*. [16]. Briefly, 0.5ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 µl of 10% $AlCl_3$, 50 µl of 1 mol l-1 potassium acetate and 1.4 ml distilled water and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using quercetin as standard.

Determination of phytate content

Phytates were determined through phytic acid determination using the procedure described by Lucas and Markaka [17]. Two grammes of each sample were weighed into a 250 ml conical flask. Samples were soaked in 100 ml of 2% conc. HCl for 3 hrs and then filtered through a double layer Whatman number one filter paper. Fifty millimeters of each of the sample filtrate were placed in a 250 ml beaker and 100 ml of distilled water was added. To each sample, 10 ml of 0.3% Ammonium thiocyanate indicator solution was titrated then followed with standard iron chloride solution which contained 0.00195 g iron/ml. The end point was signified by the appearance of a brownish-yellow coloration that persisted for 5 min. The percentage phytic acid was calculated as follows:

$$\% \text{ Phytic acid} = y \times 1.19 \times 100 \text{ where, } y = \text{titre value} \times 0.00195 \text{ g}$$

Determination of Total Saponins

The method described by Obadoni and Ochuko [18] was used for saponin determination. A quantity of 20 g of each sample was placed into a conical flask and 100 ml of 20 % aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at 55 °C. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at 90 °C. The concentrate was transferred into a 250 ml

separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated, 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and saponin content was calculated.

$$\% \, saponin = \frac{weight \, of \, dried \, sample \, x \, 100}{weight \, of \, sample \, x \, 1}$$

Determination of Tannin

The method of Van-Burden and Robinson [19] was used with modification: 500 mg of the sample was weighed into a 50 mL plastic bottle. 50 mL of distilled water was added and shaken for 1 h in a mechanical orbital shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtered solution was pipetted into a test tube and mixed with 0.2 mL of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 720nm within 10min. A blank sample was prepared and read at the same wavelength. A standard was prepared using 0-5 µg/mL tannin acid and measured. Tannin content was calculated.

$$\% \, Tannin = \frac{absorbance \, of \, sample \, x \, average \, gradient \, factor \, x \, dilution \, factor \, x \, 100}{weight \, of \, sample \, x \, 10{,}000 \, x \, 1}$$

Determination of ascorbic acid (Vitamin C) content

The ascorbic acid content of the samples was determined by the method of AOAC [20]. Ground spice (1 g each) were homogenized with 50 ml of distilled water for 3 min, rested for 3h, re-homogenized for another 2 min and filtered through filter paper. A 15mL volume of metaphosphoric acid/acetic acid solution was added and the mixture was stirred and then transferred to a 250-mL conical flask. The residue in the beaker was washed with 30mL of deionized water and combined with the sample solution. Titration was immediately done using 2,6-dichloroindophenol until the first appearance of pink color that persists for about 5 seconds.

Ascorbic acid was used as a standard, and the concentration of ascorbic acid in the samples was calculated and expressed as mg/100 g of the dry spice sample.

mg ascorbic acid / 100 g = C x V x (F/W).

Where, C= mg ascorbic acid/ml,V= volume (ml) of 2,6-dichloroindophenol used, F= dilution factor, W= weight (g) of sample used.

Determination of β- Carotene

β-Carotene was determined according to the method of Nagata and Yamashita [21]. The dried ethanoic extract (20 mg) was vigorously shaken with 10ml of acetone – hexane mixture for 1min. The absorbance of the filtrate was measured at λ = 453, 505, and 663 nm.

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Contents of β- Carotene was calculated according to the following equation:

$$\beta\text{- Carotene (mg/100ml)} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453.}$$

Determination of free radical scavenging activity

The method described by Lee *et al*. [22] was used. The stable 1, 1-diphenyl-2-Picrylhydrazyl (DPPH) radical was used for determination of free radical scavenging activity of test samples. A 0.1 mm solution of DPPH in ethanol was prepared, 5 ml of the solution was added to 5 ml of methanol, and kept in the dark for 30 min at room temperature. After 30 min, the absorbance was recorded at 517 nm using UV/Vis's spectrophotometer against methanol as blank. Fifty milligrams of each of the extracts was dissolved in 100 ml of ethanol to get 500 µg/ml stock solutions. Lower concentrations (50, 100 and 150 µg/ml) were prepared by diluting serially with ethanol. A volume of extract was added to ethanolic solution of DPPH, and kept in the dark for 30 min at room temperature. After 30 min, the absorbance was recorded at 517 nm using UV/Vis spectrophotometer against ethanol as blank. Decreased absorbance of the reaction mixture indicates higher free radical scavenging property. The percent DPPH scavenging effect was calculated using the following equation:

$$Radical\ scavenging\ activity\ (\%) = [\frac{(A0 - A1)}{A0}]\ x\ 100$$

Where A0 is the absorbance of DPPH radical without sample extract and A1 is the absorbance of DPPH radical with sample extract.

Determination of reducing power

Evaluation of reducing power of the samples was determined using the Ferric thiocyanate (FTC) method of Yen and Chen [23]. One millilitre of 80 % methanolic sample extracts was mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6 and 1 % potassium ferricyanide. The mixture was incubated at 50 °C for 20 mins after which an equal volume of 1% trichloroacetic acid was added to the mixture, which was then centrifuged (Gallenkamp centrifuge, England) at 3000 rpm for 10 mins. The upper layer of the solution was mixed with distilled water and 0.1 % $FeCl_3$ in a ratio of 1:1:2 and the absorbance of the upper layer was measured at 700 nm using a spectrophotometer (Spectro 21D, Pec Medicals, USA). Increased absorbance of the reaction mixture indicated increase in reducing power.

Statistical Analysis

Results were presented as mean of triplicate analyses. Determination of significance was determined by analysis of variance (ANOVA) using SPSS version 20 software and mean separation was done using new Duncan's multiple range test. Significance was accepted at $p< 0.05$.

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RESULTS AND DISCUSSION

Effect of cooking time on the total phenolic and flavonoid contents of *Tetrapleura tetraptera* seeds

The effect of cooking time on the total phenolic and flavonoid contents of *T. tetraptera* is presented in Figure 1. Both boiling and toasting time had significant influence on both the phenolic and flavonoid contents of the seed samples. The boiled samples had total phenolic content that increased from 25.84 mg/100g for the 5 mins boiling to 31.83 mg/100g for 20 mins. For the toasted samples, total phenols value ranged from 20.80 mg/100g to 28.58 mg/100g with T_{20} having the highest value and T_0 having the least value. A general increase in flavonoid content with boiling and toasting time was also observed. The flavonoid content increased from 19.78 mg/100g to 30.23 mg/100g for the boiled spice extracts and from 19.78 mg/100g to 22.24 mg/100g for T_0 and T_{20}, respectively. The phenolic composition observed in this study agrees with those reported by Uyoh *et al*. [24].

It has been shown that the bound phenolic compounds might be more easily released from plant tissues after heat treatment. This could be due to the cleavage of the esterified and glycosylated bond or by the formation of Maillard reaction products which are responsible for the increase in total phenolics after heating [25]. Total phenolics are usually stored in cellulose network of spices and can be released during thermal processing; individual phenolics may sometimes increase because heat can break supramolecular structures, releasing the phenolic sugar glycosidic bounds which react better with Folin- Ciocalteu reagent [26]. This observed increase agrees with many earlier reports where positive correlations were established between total phenolics, total flavonoids content and cooking time [27]. Mazzeo *et al*. [28], also reported that blanched samples of kale exhibited a higher total phenolic content compared to the fresh uncooked sample. The enhanced phenolic content of *Tetrapleura tetraptera* with cooking implies higher health benefits of the cooked spice.

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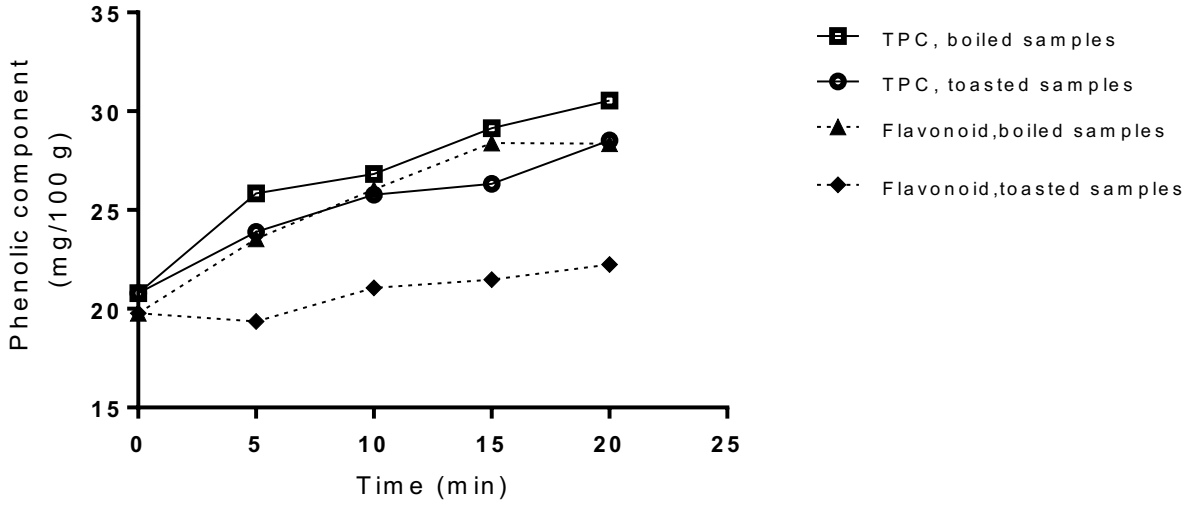

Figure 1: Effect of cooking time on the total phenolic and flavonoid contents of *T. tetraptera*

Effect of cooking time on the anti-nutritional contents of *Tetrapleura tetraptera* seeds

The effect of cooking time on the anti-nutritional contents of *Tetrapleura tetraptera* seeds is shown in Tables 1 and 2. Boiling caused significant ($p<0.05$) depletion in phytate and tannin contents of the spice samples from 0.327 % to 0.124 % and from 23.58 mg/100g to 18.53 mg/100g, respectively. A similar trend was observed for the toasting procedure. The phytate and tannin levels reduced from 0.372% to 0.061% and 23.59 mg/100g to 18.09 mg/100g, respectively. On the contrary, boiling and toasting time caused significant increases in saponin content (2.58 mg/100g to 5.58 mg/100g and 7.18 mg/100g), respectively.

The observed reduction in phytic acid content of legume seeds during heat treatment may be partly due to the heat labile nature of phytic acid and the formation of insoluble complexes between phytate and other components [29]. Khattab and Arntfield [30] reported that in cooking, phytic acid combines with the calcium and magnesium in the seed to form insoluble calcium and magnesium phytates. Boiling caused less loss in phytate content than toasting and higher increase in saponin. This could be attributed to higher temperature of cooking in toasting which caused more loss of the heat labile phytate. Heat treatment such as cooking, blanching, roasting had been reported to reduce phytate level in several plant food stuffs [31].

The reduction of tannins with cooking is mainly due to the fact that those compounds in addition to their predominance in seed coats [16] are water soluble and consequently leach into the liquid medium. This explains the higher reduction caused by boiling (77.58 %) than toasting (75.68 %). These results agree with those of Mubarack [32] who found out that tannin content of mung bean seeds (*Phaseolus aureus L.*) was reduced after boiling (cooking) in tap water for 90 minutes, autoclaving at 121 ^{o}C for

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35 minutes, microwave cooking for 15 minutes and soaking for 12h, respectively. These results are also in harmony with those of Rehman and Shah [33] who stated that tannin content of black kidney bean, red kidney bean and white kidney bean were significantly reduced after ordinary cooking and pressure cooking at 121 ^{o}C for 20 minutes, respectively.

The tannin content of the raw seeds in this study agrees with the values observed in some accessories of the spice by Uyoh *et al.* [23] but the saponin values in this study is higher than those reported by Uyoh *et al*. [23].

The observed increase in saponin content was contrary to those reported by earlier researchers for instance Collinlaw *et al*. [34] who reported a decrease in saponin with boiling of vegetables and *T. leontopetaloides* tubers respectively. Odufuwa *et al*. [35], reported that while blanching reduced the saponin content of some vegetables, it increased it in others like *Crassocephalum rubens* and *Talinum triangulare.* The temperature used in this process may have optimized the release of saponins in those vegetables, increasing their quantification. Hydration may have also allowed water to penetrate in their interior, releasing more saponins by simple diffusion.

The effect of cooking time on the vitamin C and pro-Vitamin A contents of *T. tetraptera* seeds

The effect of cooking time on the vitamin C and pro-vitamin A of *T. tetraptera* seeds are as shown in Tables 3 and 4. The value of the vitamins decreased significantly ($p<0.05$) from 166.51 mg/ 100g ascorbic acid to 13.35 mg/100g ascorbic acid for vitamin C, and from 6.67 mg/ 100g to 2.70 mg/100g for pro vitamin A for the boiling procedure. Toasting also led to significant changes in the vitamin levels. Vitamin C content reduced drastically from 166.51 mg/100g ascorbic acid to 22.09 mg/100g with increasing toasting time. There was a significant ($p<0.05$) decrease in β-carotene content as a result of toasting for 5mins and further decrease occurred by extending the toasting time to 20mins. Boiling caused a more reduction in the vitamins than toasting, for example there was a 91.98 % and 86.73 % reduction in vitamin C due to boiling and toasting, respectively.

Vitamin C is water soluble, as such easily leached into the water and then degraded by heat, as a result of boiling. This explains the greater loss in vitamin C by the boiling procedure. The studies of Hamza *et al.* [36] collaborates the adverse effect of heat on vitamin C. Heat also leads to degradation of β-carotene, this degradation being higher in boiling method due to leaching into cooking water.

The effect of cooking time on the DPPH activity of *T. tetraptera* seeds

Figures 2 and 3 respectively show the results of the effect of boiling and roasting time on the DPPH activity of *T. tetraptera* seeds. The antioxidant activity of *T. teraptera* was seen to be increased with increase in boiling time as measured by DPPH assay. The DPPH concentration also led to a general increase in activity. At 50 µg/100ml concentration of DPPH, activity was elevated from 22.06 µg/ml to 43.26 µg/ml for the boiled samples and from 22.06 µg/ml to 27.64 µg/ml for the toasted samples. For boiling, there was increase in activity with time at all the levels of DPPH evaluated. For

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toasting, at higher DPPH concentration of 150 µg/100ml, there was an initial drop-in activity at 5 min then subsequent increase as toasting time increased. It was observed that boiling caused an increase in activity than the toasting.

The increase is suggested to be as a result of: (1) liberation of high amounts of antioxidant components due to the thermal destruction of cell walls and sub cellular compartments; (2) thermal chemical reaction may lead to the production of stronger radical-scavenging antioxidants; and/or (3) production of new non-nutrient antioxidants or the formation of novel compounds such as Maillard reaction products with antioxidant activity [37].

This increase in radical scavenging ability corresponds to the increase in the total phenol and flavonoid content as shown in Fig 1. This increase is despite the decrease in the levels of vitamin C (an antioxidant vitamin). This infers that phenolic could be the major antioxidant component in the seed. This assertion agrees with several results where correlation was established between the total phenol content of some plant foods and their antioxidant capacity [38].

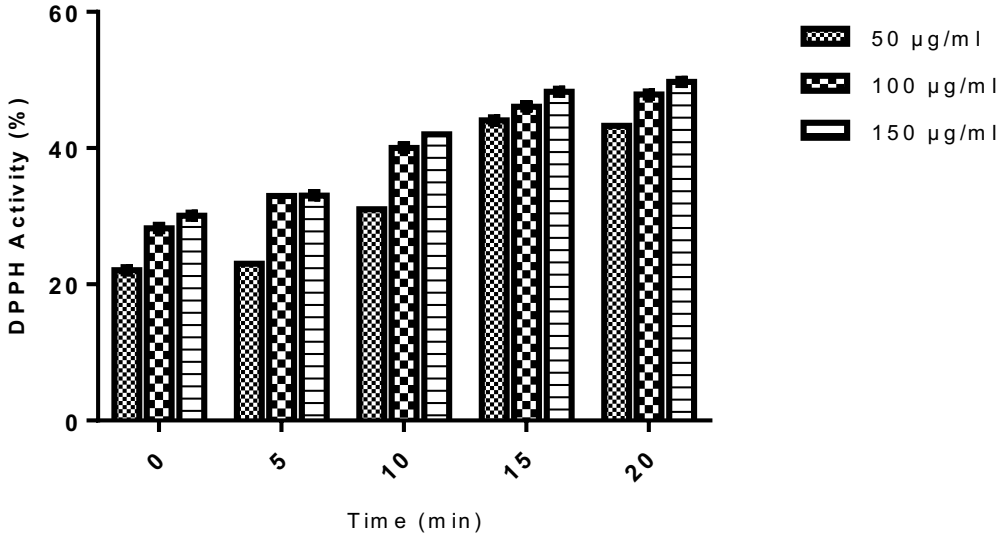

Figure 2: Effect of boiling time (mins) on DPPH activity of *T. tetraptera*

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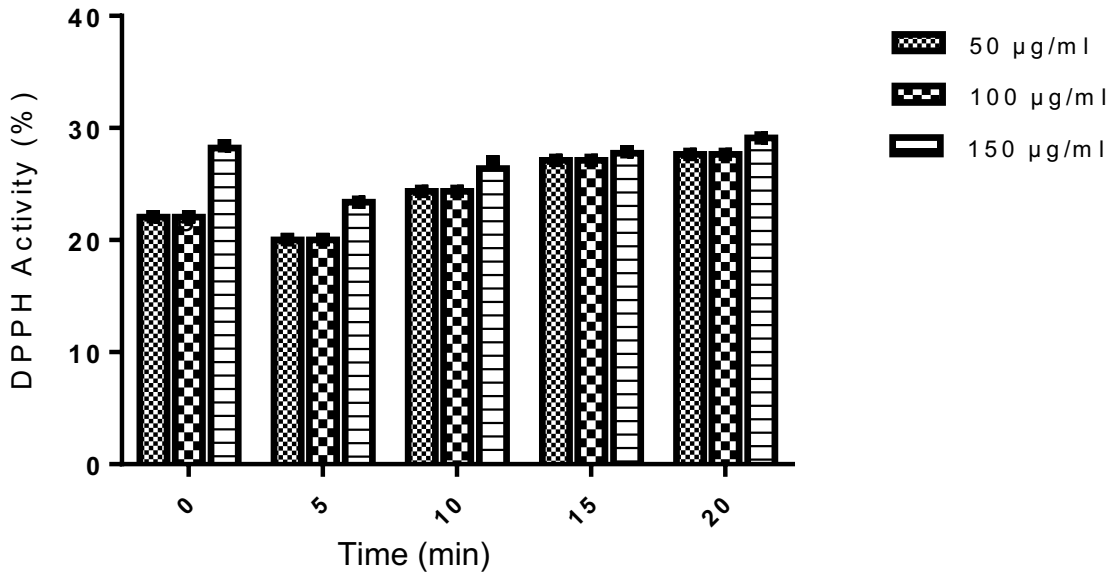

Figure 3: Effect of toasting time (mins) on DPPH activity of *T. tetraptera*

Effect of cooking time on the ferric reducing power of *Tetrapleura tetraptera* seeds
The effect of cooking time on the ferric reducing power of *Tetrapleura tetraptera* seeds is shown in figures 4 and 5. It could be observed the reducing power was positively influenced by both cooking time and ferric chloride concentration. The ferric reducing activity at 50µg/100ml concentration of *T. tetraptera* increased significantly from 0.14 µg/100ml to 0.69µg/100ml and from 0.14 µg/100ml to 0.47µg/100ml for boiled and toasted samples respectively. Boiling treatment resulted in higher increase in reducing power than toasting treatment. This could be as a result of higher increase in phenolic compounds due to boiling. Among the samples evaluated, B_{20} had the highest reducing power of 0.69 µg/ml.

Processing *T. tetraptera* seeds by heating caused a significant increase in antioxidant values, which is in accordance with previous findings that indicate that heating enhances antioxidant properties of naturally occurring compounds by the formation of novel compounds such as Maillard reaction products that have antioxidant activity. It has been shown that the thermal processing of sweet corn, tomato, and other vegetables increase antioxidant activity, perhaps as a result of Maillard reaction products [39].

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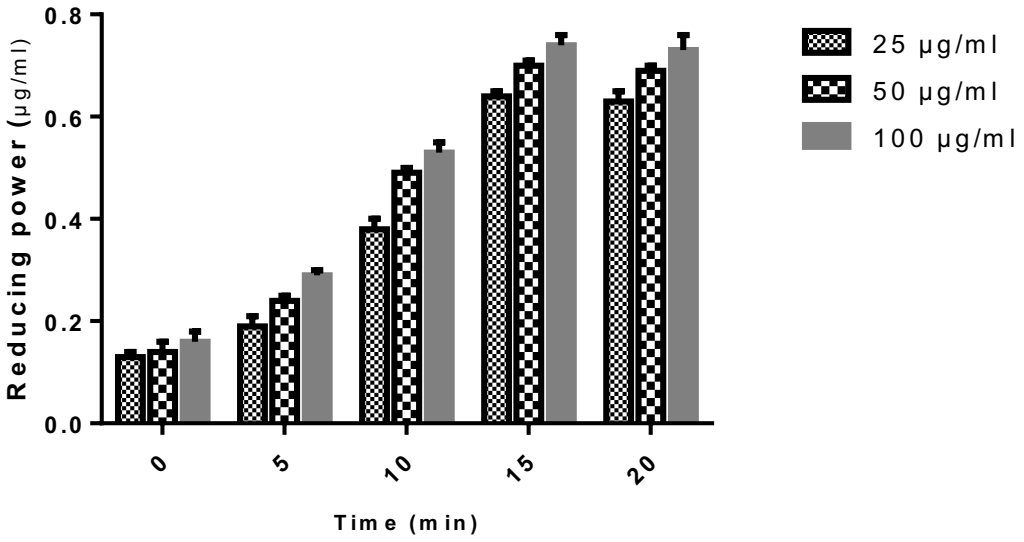

Figure 4: Effect of boiling time (mins) on ferric reducing power of *T. tetraptera*

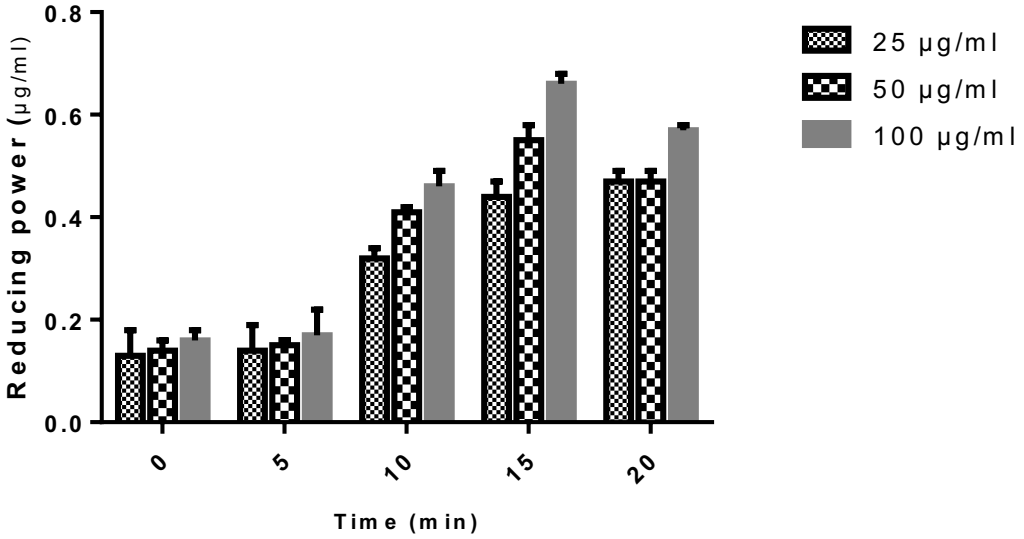

Figure 5: Effect of toasting time (mins) on ferric reducing power of *T. tetraptera*

CONCLUSION

The results from this study showed that *Tetrapleura tetraptera* seeds are rich sources of health promoting phytochemicals. Cooking led to general increases in the phenolic content, flavonoid as well as the antioxidant status of the spice. On the contrary, cooking caused significant losses in the vitamin C and ß-carotene content. The phytate and tannin content of the seeds were also reduced by cooking. This study, therefore, indicates that cooking of this spice, particularly boiling can enhance its antioxidant

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potential. This is contrary to the common belief that the antioxidant activities in cooked spices are lower than those in the raw spices.

To derive maximum health benefits from the antioxidant effects of this spice, it could be more effective to cook the spice before consumption. Further research is, however, recommended to ascertain the individual phenolic components of this spice and their health effects.

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Table 1: Effect of boiling time on the anti-nutrient content of *Tetrapleura tetraptera* seeds

Anti-nutrient	RS	B_5	B_{10}	B_{15}	B20	%Change
Phytate (%)	$0.372^{e}\pm$ 0.003	$0.296^{d}\pm$ 0.002	$0.170^{c}\pm$ 0.004	$0.133^{b}\pm$ 0.005	$0.124^{a}\pm$ 0.002	-0.248
Saponin (%)	$2.58^{e}\pm$ 0.15	$4.35^{d}\pm$ 0.21	$4.87^{c}\pm$ 0.13	$5.25^{b}\pm$ 0.02	$5.58^{a}\pm$ 0.12	53.76
Tannin (mg/100g)	$23.58^{c}\pm$ 0.31	$19.86^{c}\pm$ 0.68	$19.09^{c}\pm$ 0.08	$18.86^{b}\pm$ 0.19	$18.53^{a}\pm$ 0.23	-77.58

RS – Raw seed, B_5 – seeds boiled for 5 min, B_{10} – seeds boiled for 10 min, B_{15} – seeds boiled for 15 min, B_{20} – seeds boiled for 20 min. Mean values in the same row bearing different superscripts are significantly different ($p<0.05$)

Table 2: Effect of toasting time on the anti-nutrient content of *Tetrapleura tetraptera* seeds

Anti-nutrient	RS	T_5	T_{10}	T_{15}	T_{20}	%Change
Phytate (%)	0.372^{e} ± 0.002	0.187^{d} ± 0.005	$0.107^{c}\pm$ 0.002	0.090^{b} ± 0.003	0.061^{a} ± 0.002	- 0.31
Saponin (%)	$2.59^{d}\pm$ 0.15	3.66^{c} ± 0.34	6.52^{b} ± 0.18	$6.75^{b}\pm$ 0.24	$7.18^{a}\pm$ 0.13	4.59
Tannin (mg/100g)	23.59^{c} ± 0.32	22.58^{c} ± 0.41	$21.14^{b}\pm$ 0.63	$20.14^{b}\pm$ 0.11	$18.09^{a}\pm$ 1.00	-75.68

RS – Raw seed, T – seeds boiled for 5 min, T_{10} – seeds boiled for 10 min, T_{15} – seeds boiled for 15 min, T_{20} – seeds boiled for 20 min. Mean values in the same row bearing different superscripts are significantly different ($p<0.05$)

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Table 3: Effect of boiling time (mins) on vitamin content of *T. tetraptera* seeds

Vitamins	RS	B_5	B_{10}	B_{15}	B_{20}	% Change
Vitamin C (mg/100g ascorbic acid)	$166.51^{e}\pm$ 0.29	$23.29^{d}\pm$ 0.04	$18.31^{c}\pm$ 0.02	$16.67^{b}\pm$ 0.04	$13.35^{a}\pm$ 0.02	-91.98
Pro-vit. A (mg/100g) β- carotene	$6.67^{e}\pm$ 0.02	$6.05^{d}\pm$ 0.02	$4.68^{c}\pm$ 0.02	$4.28^{b}\pm$ 0.04	$2.70^{a}\pm$ 0.02	-59.52

RS – Raw seed, B_5 – seeds boiled for 5 min, B_{10} – seeds boiled for 10 min, B_{15} – seeds boiled for 15 min, B_{20} – seeds boiled for 20 min. Mean values in the same row bearing different superscripts are significantly different ($p<0.05$)

Table 4: Effect of toasting time (mins) on vitamin content of *T. tetraptera* seeds

Vitamin	RS	T_5	T_{10}	T_{15}	T_{20}	%Change
Vitamin C (mg/100gascorbic Acid)	$166.51^{a}\pm$ 0.29	$139.97^{b}\pm$ 0.003	$79.96^{c}\pm$ 0.40	$26.71^{d}\pm$ 0.35	$22.09^{e}\pm$ 0.31	-86.73
Pro-vit. A (mg/100g) (β - carotene)	$6.67^{e}\pm$ 0.03	$6.11^{d}\pm$ 0.04	$5.82^{c}\pm$ 0.04	$4.52^{b}\pm$ 0.37	$4.01^{a}\pm$ 0.03	-39.88

RS – Raw seed, T – seeds boiled for 5 min, T_{10} – seeds boiled for 10 min, T_{15} – seeds boiled for 15 min, T_{20} – seeds boiled for 20 min. Mean values in the same row bearing different superscripts are significantly different ($p<0.05$)

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