fjrs.futa.edu.ng

FUTA Journal of Research in Sciences

ISSN: 2315-8239

FUTA Journal of Research in Sciences, 2015 (2): 201-209

EFFECTS OF COOKING ON PHENOLIC CONTENT AND ANTIOXIDANT PROPERTIES OF AFRICAN WALNUT *[TETRACARPIDIUM CONOPHORUM* (MÜLL. ARG.) HUTCH. & DALZIEL.] SEEDS

A.O. Ademiluyi*, G. Oboh, A.O. Ademosun, S.I. Oyeleye, I. I. Ejakpovi and O.B. Ogunsuyi
Functional Foods and Nutraceuticals Unit, Department of Biochemistry, Federal University of Technology, Akure, P.M.B. 704, Akure, 340001, Nigeria.
Corresponding authors:
*Email: ademiluyidayo@yahoo.co.uk**,** aoademiluyi@futa.edu.ng**;** Tel: +234(0)7031388544
Email: ayoademosun@yahoo.com;**Tel: +234(0)8033944653

ABSTRACT
African walnut *[Tetracarpidium conophorum* (Müll. Arg.) Hutch. & Dalziel.] seeds are predominant African delicacy with ethnomedical relevance. The shelled seeds are processed by cooking (boiling) to obtain the edible cotyledons. This study investigated the effect of cooking on the phenolic and vitamin C constituents as well as antioxidant properties of aqueous extracts of African walnut seeds cooked with (shelled) and without (unshelled) the shell. Walnut seeds were boiled with and without the shell for four hours and the edible cotyledon extracted into water. Results showed that shelled walnut extract had significantly ($P<0.05$) higher total phenol, total flavonoid and vitamin C contents than the unshelled walnut extract. Similarly, the shelled walnut extract possessed a significantly ($P<0.05$) higher reducing property, and there was also a significantly ($P<0.05$) higher 1, 1-diphenyl-2 picrylhydrazyl (DPPH) and hydroxyl (OH*) radicals scavenging abilities, as well as Fe^{2+} chelating ability than the unshelled walnut extract; there was however, no significant difference ($P>0.05$) in the 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{*+}$) radical scavenging abilities between the two extracts. The results of this study showed that cooking walnut with the shell could protect the antioxidant constituents from thermal decomposition and leaching thus culminating in the observed higher antioxidant activities over the unshelled walnut.
Keywords: African walnut, processed, cooking, antioxidants, phenolic acids, vitamin C

INTRODUCTION

African walnut [*Tetracarpidium conophorum* (Müll. Arg.) Hutch. & Dalziel.] is a tropical plant of the family Euphorbiaceae (Amaeze *et al*., 2011). It has wide distribution in southern part of Nigeria in particular and west and central African countries in general (Amaeze *et al*., 2011). The nuts produced from the plant have been consumed locally for decades with numerous nutritional and medicinal benefits. General method of processing of walnut prior to consumption involves prolonged cooking of the seeds by boiling in water with or without the shell. Cooking generally softens the cotyledons, increase its palatability, reduce the anti-nutrient contents and enhance nutritional constituents. Walnut contains bioactive compounds such as polyphenols, plant sterols, vitamin C and omega-3 fatty acids (Olabinrin *et al.,* 2010; Ihemeje *et al.,* 2012; Kanu *et al.,* 2015).

Consumption of walnuts has been shown to reduce serum cholesterol level as well as inhibit initiation and propagation of lipid peroxidation (Ihemeje *et al.*, 2012). Since the pathogenesis and progression of many diseases have been linked to free radical generation and oxidative stress (Sisein, 2014; Ademiluyi *et al.*, 2015), increasing body antioxidant status has been a major therapeutic approach. This could be achieved by increased consumption of antioxidant rich foods such as vegetables and fruits which have been shown to be rich antioxidant sources (Adefegha and Oboh, 2013)

The effects of cooking on antioxidant content of different plant food sources have been well reported. Cooking has been shown to help remove anti-nutrient constituents, improve nutritional value and total antitoxin activity of plant food sources (Boari *et al*., 2013; Oulai *et al*., 2014). However, these observations have been shown to be time dependent and prolonged cooking time has been shown to promote thermal degradation of some plant antioxidant compounds; Barros *et al.* (2011) reported a decrease in vitamin C content in sweet chestnuts after cooking, while a study by Sahlin *et al.* (2004) showed that the total antioxidant activity of tomatoes were reduced upon boiling. These observations could be due to degradation of the constituent antioxidant compounds (Zhang and Hamauzu, 2004) as well as leaching away of water soluble antioxidants during boiling (Chipurura *et al*., 2010). Cooking walnut without the shell therefore, could further subject the edible cotyledons to increased thermal exposure and leaching; thus risking the loss of antioxidant constituents. Therefore, this study investigated the effects of cooking on total phenolic and vitamin C contents and antioxidant properties of African walnut seeds cooked with and without the shells.

MATERIAL AND METHODS

Materials

Plant Source and Identification

African walnut *[Tetracarpidium conophorum* (Mull. Arg.) Hutch. & Dalziel.] was obtained from Fditi market, Nigeria. Authentication and identification was carried out at the Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure, Nigeria.

Samples Preparation

The raw shelled walnut seeds were sorted and washed under running tap water to remove the dirt particles and spoiled nuts. Thereafter, it was divided into two (2) equal parts. One part was cooked with the shell (shelled walnut) while the other part was cooked without shell (unshelled wall nut) separately at the same temperature and for three hours using Tower aluminium cooking pot. The edible cotyledon of shelled walnut seeds were removed after cooking and placed in a clean container alongside with the edible cotyledon of the unshelled walnut seeds in a different clean container. Both samples were later sliced, using table knife into a clean tray and dried in oven to constant weight at 40^0C. After that, the dried samples were pulverized using kitchen electric grinding machine. Five grams of the grounded sample was later soaked in 50ml of distilled water for 24h, filtered and centrifuged. The supernatant was kept in tight clan container and stored in the refrigerator for subsequent analysis

Methods

Determination of total phenol content

The total phenol content was determined according to the method of Singleton et al. (1999). Briefly, appropriate dilutions of the extracts were oxidized with 2.5 ml 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by Meda *et al*. (2005). Briefly 0.5 mL of appropriately diluted extracts was mixed with 0.5 mL methanol, 50 µL 10% $AlCl_3$, 50 µL 1 M Potassium acetate and 1.4 mL water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was

subsequently measured at 415 nm in the UV-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom). The total flavonoid content was subsequently calculated using quercetin as standard.

Determination of vitamin C content

Vitamin C content in the extracts was determined using the AOAC (1990) method. Briefly, 75 µL DNPH (2 g dinitrophenyl hydrazine, 230mg thiourea and 270mg $CuSO4.5H_2O$ in 100mL of 5mol L^{-1} H_2SO_4) were added to 500 µL reaction mixture (300 µL of an appropriate dilution of the extracts with 100 µL 13.3% (TCA) and water).The reaction mixtures were subsequently incubated for 3 hrs at 37 °C, then 0.5mL of 65% H_2SO_4 (v/v) was added to the medium; their absorbance was measured at 520nm and the vitamin C content of the samples was subsequently calculated.

Determination of reducing property

The reducing property of the aqueous extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (1986). 2.5ml aliquot was mixed with 2.5 ml 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50□C for 20 min. and then 2.5 ml 10 % trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant property was subsequently calculated.

DPPH (1, 1-diphenyl–2 picrylhydrazyl) radical scavenging ability

The free radical scavenging ability of the extracts against 1, 1-diphenyl–2 picrylhydrazyl (DPPH) free radical was evaluated as described by Gyamfi *et al*. (1999). Briefly, appropriate dilution of the extracts (1 ml) was mixed with 1 ml, 0.4mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated as percentage of the control.

$ABTS^{\cdot+}$ (2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging ability

The $ABTS^{\cdot+}$ (2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) scavenging ability of the extracts were determined according to the method described by Re *et al*. (1999). The $ABTS^{\cdot}$ was generated by reacting an (7 mmol/L) ABTS aqueous solution with $K_2S_2O_8$ (2.45 mmol/L, final concentration) in the dark for 16 h and adjusting the Absorbance at 734 nm to 0.700 with ethanol. 0.2 mL of appropriate dilution of the extract was added to 2.0 mL $ABTS^{\cdot+}$ solution and the absorbance were measured at 734 nm after 15 min. Trolox was used as standard and trolox equivalent antioxidant capacity (TEAC) was subsequently calculated.

Determination of Fe^{2+} chelating ability

The Fe^{2+} chelating ability of the sample extracts was determined using a modified method of Minotti and Aust (1987) with a slight modification by Puntel *et al*. (2005). Freshly prepared 500µM $FeSO_4$ (150 µl) was added to a reaction mixture containing 168 µl 0.1M Tris-HCl (pH 7.4), 218 µl saline and the extracts (0 - 100µl). The reaction mixture was incubated for 5min, before the addition of 13 µl 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe (II) chelating ability was subsequently calculated.

Fenton reaction (Inhibition of Degradation of deoxyribose)

The method of Halliwell and Gutteridge (1981) was used to determine the ability of the sample extracts to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose. The extract 0 to 100 µl was added to a reaction mixture containing 120 µl of 20 mM deoxyribose, 400 µl of 0.1 M phosphate buffer, 40 µl of 500 µM of $FeSO_4$, and the volume were made up to 800 µl with distilled water. The reaction mixture was incubated at 37°C for 30 min and the reaction was then stopped by the addition of 0.5 ml of 28% trichloroacetic acid. This was followed by addition of 0.4 ml of 0.6% thiobarbituric acid solution. The tubes were subsequently incubated

in boiling water for 20 min. The absorbance was measured at 532 nm in a spectrophotometer.

Data Analysis

The results of three replicates were pooled and expressed as mean ± standard deviation. All results were expressed in dry weight of sample basis. Student t-test, one-way analysis of variance (ANOVA) and least significance difference (LSD) were carried out (Zar, 1984). Significance was accepted at $p \leq 0.05$. EC_{50} (effective concentration of extract causing fifty percent activity) was determined using non-linear regression analysis.

RESULTS

The results of the total phenol, total flavonoid and vitamin C contents of the aqueous extract from shelled and unshelled walnuts are presented in Table 1. From the result, extract from shelled walnuts has significantly ($P<0.05$) higher total phenol (55.95 mgGAE/100g) and total flavonoid (32.50 mgQE/100g) contents than total phenol (46.43 mgGAE/100g) and total flavonoid (27.50 mgQE/100g) from unshelled walnut extract. Similar result was also recorded for vitamin C, where shell walnut extracts (23.83mg AAE/100g), has higher vitamin C content than unshelled walnut extract (14.17mg AAE/100g).

The reducing property of the shelled and unshelled walnut are presented in Table 2 as the ferric reducing antioxidant property (FRAP).The result showed that the shelled walnut had a significantly ($P<0.05$) reducing property (33.08mgAAE/100g) than the unshelled walnut (16.92mgAAE/100g). Also, the extract from shelled walnuts had higher DPPH scavenging ability (Fig. 1) at highest concentration (400mg/ml) than the unshelled walnut extract. This is further substantiated by the EC_{50} values (Table 3), where the shelled walnut extracts (EC_{50} = 8.99 mg/ml) showed significantly ($P<0.05$) higher radical scavenging ability than the unshelled walnut extracts (EC_{50} = 13.99mg/ml). However, there was no significant ($P>0.05$) difference in the ability of both shelled and unshelled walnut extracts to scavenge $ABTS^{\cdot+}$ free radical (Table 2).

Furthermore, the walnuts (shelled and unshelled) extracts also chelate Fe^{2+} in a dose-dependent manner (20-100 mg/ml) (Fig. 2); however, the shelled walnuts (EC_{50} =0.09 mg/ml) extract had the highest chelating ability than the unshelled walnuts (EC_{50} = 0.14 mg/ml) (Table 3). In addition, Fig. 3, showed that both shelled and unshelled walnut extracts scavenge hydroxyl radicals (OH*) in a concentration dependent manner (50-200 mg/ml). The EC_{50} values (Table 3) however revealed that the shelled walnut (EC_{50} =0.13 mg/ml) had significantly ($P<0.05$) higher OH* scavenging ability than the unshelled walnut extract (EC_{50} =0.23 mg/ml).

Table 1: Total phenolic, total flavonoid and Vitamin C contents of shelled and unshelled walnut

Sample	Total Phenol (mgGAE/100g)	Total Flavonoid (mgQE/100g)	Vitamin C (mgAAE/100g)
Shelled walnut	55.95±0.01[b]	32.50±0.01[b]	23.83±0.01[b]
Unshelled walnut	46.43±0.01[a]	27.50±0.04[a]	14.17±0.01[a]

Values represent means ± standard deviation of triplicate readings.
Values with the different superscript letter down the same column are significantly different ($P<0.05$)

Table 2: The Ferric reducing antioxidant properties (FRAP) and $ABTS^{\cdot+}$ (2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging ability of shelled and unshelled walnut

Sample	FRAP (mgAAE/100g)	$ABTS^{+}$ (mmol/TEAC/100g)
Shelled walnut	33.08 ± 0.05^{b}	44.03 ± 0.01^{a}
Unshelled walnut	16.92 ± 0.02^{a}	44.05 ± 0.01^{a}

Values represent means ± standard deviation of triplicate readings.
Values with the different superscript letter down the same column are significantly different ($P<0.05$)

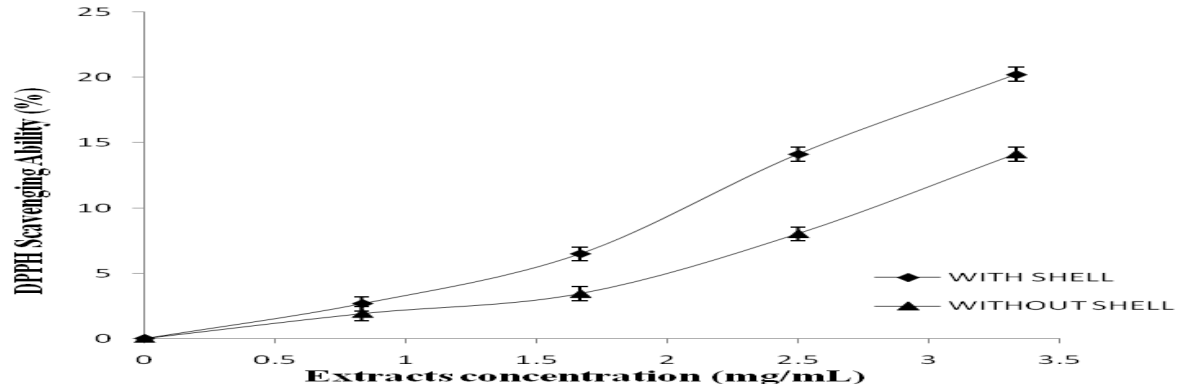

Fig. 1 DPPH (1, 1-diphenyl–2 picrylhydrazyl) radical scavenging ability of aqueous extracts from shelled and unshelled African walnut seeds.

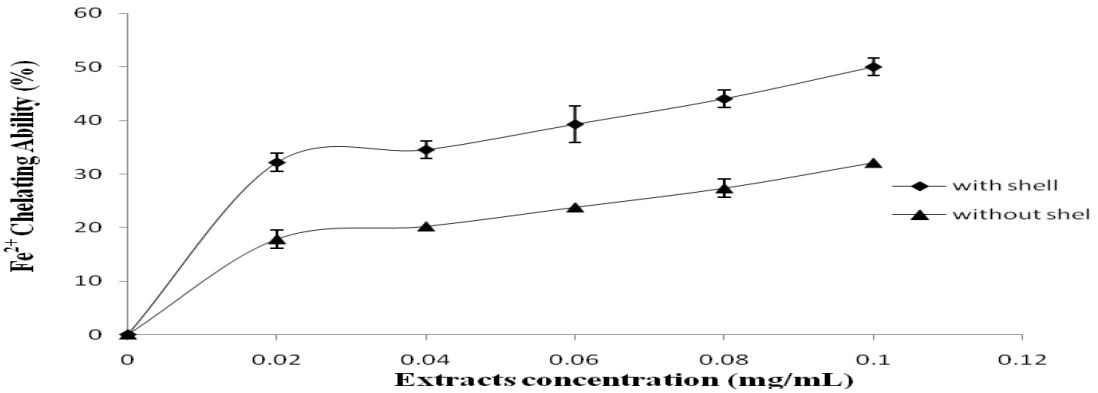

Fig. 2 Fe^{2+} chelating ability of aqueous extracts from shelled and unshelled African walnut seeds.

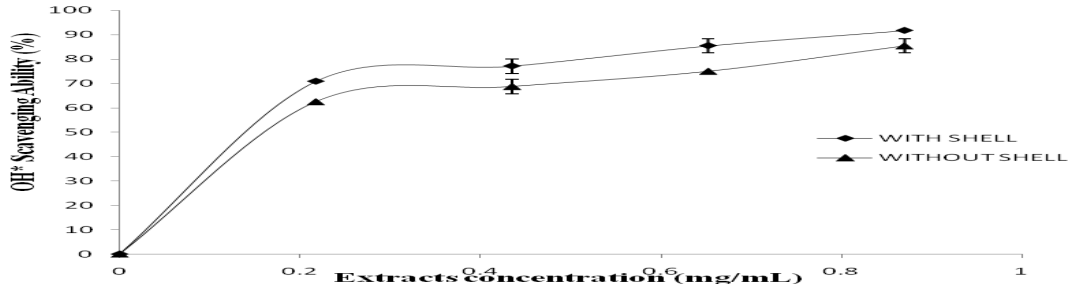

Fig. 3: OH* (hydroxyl) radical scavenging ability of aqueous extracts from shelled and unshelled African walnut seeds.

Table 3. EC_{50} values for DPPH (1, 1-diphenyl–2 picrylhydrazyl) and hydroxyl radical (OH*) radical scavenging abilities, as well as Fe^{2+} chelating ability of aqueous extracts from shelled and unshelled African walnut seeds.

	Sample	
	Shelled walnut	Unshelled walnut
	mg/ml	
DPPH* scavenging	8.99±0.02[a]	13.99±0.04[b]
Fe^{2+} chelation	0.09±0.05[a]	0.14±0.03[b]
OH* scavenging ability	0.13±0.01[a]	0.23±0.02[b]

Values represent mean± standard deviation of triplicate.
Values with the different superscript letters along the same row are significantly different (P<0.05)

DISCUSSION

Cooking is an essential and effective food processing method in order to obtain safe and high-quality food products (Adefegha and Oboh, 2011). In this study, the effect of cooking walnut with and without the shell on phenolic contents as well as their antioxidant properties was investigated. The relationship between health protective benefits of phenolic compounds and their antioxidant properties has been well established. Phenolic compounds have been reported to have antioxidant properties by scavenging free radicals, quenching reactive oxygen species (ROS) mediated reactions and also chelate metal ions (Mukherjee *et al.,* 2014; Ademiluyi *et al.,* 2015). This study has revealed that both shelled and unshelled walnut extracts are rich sources of phenolic compounds owing to their high total phenol and total flavonoid contents. However, the higher phenolic content observed in the extract from shelled walnut is hypothesised be due to protection offer by the shell to the cotyledon (edible portion) during cooking. Prolonged exposure of the edible portion of the unshelled walnut to hot water during cooking may be a possible factor responsible for the decrease in total phenol and flavonoid contents. The prolonged exposure to heat could have cause degradation of specific thermo labile flavonoids or leaching of phenolic compounds into the cooking water (Oboh, 2005). However, the shell would have offered protection to the cotyledon to prevent excessive thermal exposure and leaching of the constituent phenolics. Our findings are in agreement with previous studies which showed that thermal cooking processing including frying, roasting, boiling reduce the polyphenolic constituents of fruits and vegetables (Barros *et al., 2011;* Oulai *et al*., 2014). Vitamin C (L-ascorbic acid) is an important water soluble antioxidant, believed to protect against degenerative diseases caused by oxidative stress (Barros *et al.,* 2011*;* Ademiluyi *et al.,* 2015. It is capable of scavenging radical superoxide anions, the highly reactive

hydroxyl radical, hydrogen peroxides and the reactive nitrogen species (Halliwell and Gutteridge, 2007). However, vitamin C is thermo labile and easily denatured by heat (Ball, 2006). Also, being hydrophilic, it can be easily leached. The result of the vitamin C contents of the extracts from shelled and unshelled walnut fruits revealed that there was significantly ($P < 0.05$) decreased in the vitamin C contents of unshelled walnut extract compared to that of the shelled walnut extract. The loss in vitamin C contents in unshelled walnut extract may be due to the thermal denaturation of the vitamin C as a result of prolong exposure of the cotyledon to the hot boiling water. Similarly, reduction in vitamin C content observed in unshelled walnut extracts could be as a result of leaching of the vitamin C into the water used for boiling. Such would have been prevented by the presence of shell in the walnut cooked with shell and thus retain the vitamin C content. Our findings were further corroborated by the study of Barros *et al.* (2011) which reported that the vitamin C content of sweet chestnut were significantly reduced after different cooking methods (boiling and roasting). Antioxidant activity of polyphenols may be due to the reducing properties of their hydroxyl groups (Bendary *et al*., 2013). The result of the ferric reducing antioxidant properties of walnut extracts depicts that cooking walnut without shell decreased the FRAP of unshelled walnut extract. A similar trend was observed for this effect on phenolic contents and Vitamin C. The result of this study is in agreement with earlier report by Adefegha and Oboh (2011) where cooking was reported to increase the reducing property of green leafy vegetables than the raw form. However, prolonged cooking especially without shell in the case of unshelled walnut may be responsible for the decrease in its ability to reduce Fe^{3+} to Fe^{2+}. The age-long link between oxidative stress and pathogenicity of many degenerative diseases has led to the hypothesis that dietary antioxidants such as those present in walnut may offer preventive and protective measures (Kanu *et al.,* 2015 by preventing free radical-induced damage to biomolecules such as DNA protein and lipids and thus arrest oxidative stress (Ademiluyi *et al*., 2015). The free radical (DPPH and ABTS*) scavenging ability of both aqueous walnut fruit extracts (shelled and unshelled) is an indication that walnut is an excellent dietary source of antioxidants. The shelled walnut extract (with higher EC_{50} value) significantly ($P<0.05$) scavenged DPPH radicals more than the unshelled walnut extracts, a result that correlates with the total phenol, total flavonoid and vitamin C contents. This observation agrees with previous findings that phenolic content of plant extracts correlates with their DPPH free radical scavenging activity (Adefegha and Oboh, 2011; Ademiluyi *et al.*, 2015). The correlation between the free radical scavenging ability of the walnut extracts and their total phenol, total flavonoid and vitamin C contents signifies that these bioactive compounds are largely responsible for their antioxidant properties. We however conclude that the removal of shell prior to cooking could exposed the antioxidant constituents of the unshelled walnut fruit, especially phenolics and vitamin C to increased thermal degradation and a concomitant reduction in their free radicals (DPPH and $ABTS^{\cdot *}$) scavenging activities. Iron can become dangerous when Fe^{2+} reacts with hydrogen peroxide (H_2O_2) via the fenton reaction (Valko *et al*., 2005) to produce the highly reactive hydroxyl radical (OH*) which can induce and propagate the lipid peroxidation chain reaction (Valko *et al*., 2005). Therefore, the need for possible removal of excessive iron accumulation in the body is of importance. Interestingly, both extracts (shelled and unshelled) chelated Fe^{2+} in a concentration-dependent manner. However, judging by the EC_{50} values, extract from shelled walnut exhibited a higher Fe^{2+}chelating ability than unshelled walnut. This observation is further strengthened by the fact that the shelled walnut extract (with higher EC_{50} value) significantly ($P<0.05$) scavenged OH* radicals more than the unshelled walnut extracts. It is noteworthy that the trend in Fe^{2+} chelating and OH* scavenging ability of these extracts correlates with their respective free radical (DPPH and ABTS*) scavenging abilities with the shelled walnut extract showing stronger effects. Hence, it is suggestive that the removal of shell prior to cooking exposed the antioxidant constituents of

the unshelled walnut fruit, especially phenolics and vitamin C to increased thermal degradation and this significantly lead to reduction in Fe^{2+} chelating and OH* scavenging abilities observed in the unshelled walnut extracts.

CONCLUSION

This study has been able to establish that walnuts cooked with the shell showed higher antioxidant phytoconstituent and properties than in those cooked without the shell. Cooking walnuts with the shell can therefore, protect the constituent antioxidants, especially phenolics and vitamin C from thermal degradation and thus increasing their antioxidant and free radical scavenging abilities over walnuts cooked without the shell. These observed higher antioxidant constituents in shelled walnut extracts are believed to be responsible for higher reducing power, free radical scavenging and Fe^{2+} chelating abilities. It is hence, recommended to cook African walnut fruits with the shell to enhance its health protective properties.

REFERENCES

Adefegha, S.A., and Oboh, G. (2013). Phytochemistry and mode of action of some tropical spices in the management of type-2 diabetes and hypertension African Journal of Pharmaceutical and Pharmacology 7:32-346.

Adefegha, S.A., and Oboh, G. (2011). Enhancement of total phenolics and antioxidant properties of some tropical green leafy vegetables by steam cooking. Journal of. Food Processing and Preservation 35: 15-622.

Ademiluyi, A.O., Oboh, G., Aragbaiye, F.P., Oyeleye, S.I., and Ogunsuyi, O.B. (2015). Antioxidant properties and in vitro α-amylase and α-glucosidase inhibitory properties of phenolics constituents from different varieties of *Corchorus* spp. Journal of Taibah University Medical Science 10: 278-287.

Amaeze, O.U., Ayoola, G.A., Sofidiya, M.O., Adepoju-Bello, A.A., Adegoke, A.O., and Coker,H.A.B. (2011). Evaluation of antioxidant activity of *Tetracarpidium conophorum* (Müll. Arg) Hutch & Dalziel leaves. Oxid. Med. Cell Longev. 2011: 1-7.

AOAC. (1990). Official methods of analysis. 15th ed. Washington DC: Association of Official AnalyticalChemists

Ball, G.F.M. (2006). Vitamin C in vitamin in foods. analysis, bioavailability, and stability. Boca Raton: CRC Press. pp. 289–305.

Barros, A.I., Nunes, F.M., Gonçalves, B., Bennett, R.N.., and Silva, A.P. (2011). Effect of cooking on total vitamin C contents and antioxidant activity of sweet chestnuts (*Castanea sativa* Mill.). Food Chemistry 128: 165-172.

Bendary, E., Francis, R.R., Ali, H.M.G., Sarwat, M.I., and El-Hady, S. (2013). Antioxidant and structure–activity relationships (SARs) of some phenolic and anilines compounds. Annals Journal of Agricultural Sciences 58: 173-181.

Boari, F., Cefola, M., Di-Gioia, F., Pace, B., Serio, F., and Cantore, V. (2013). Effect of cooking methods on antioxidant activity and nitrate content of selected wild Mediterranean plants. International Journal of Food. Science Nutrition 64: 870-876.

Chipurura, B., Muchuweti, M., and Manditseraa, F. (2010). Effects of Thermal Treatment on the Phenolic Content and Antioxidant Activity of Some Vegetables. Asian Journal of Clinical Nutrition 2: 93-100.

Gyamfi, M.A., Yonamine, M., and Aniya, Y. (1999). Free-radical scavenging action of medicinal herbs from Ghana:*Thonningia sanguinea* on experimentally-induced liver injuries. General Pharmacology: The Vascular System. 32(6): 661–667.

Halliwell, B., and Gutteridge, J.M.C. (2007). Free radicals in biology and medicine (4th edition), Oxford University Press, Oxford.

Halliwell, B., and Gutterdge, J.M.C. (1981). Fomation of a thiobarbituric-acid- reactive substance from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. FEBS letters 128: 347-352.

Ihemeje, A., Okorie, S.U., and Ekwe, C.C. (2012). Effects of processing methods onbiochemical, functional and antinutritional properties of African walnut (*Tetracarpidium*

conophorum). Journal of Biological Science and Bioconservation 4: 55-64.

Kanu, A. M., Kalu, J. E., and Okorie, A. C. (2015). Nutritional and Health Values Of African Walnut (*Tetracarpidium conophorum*). Intetrnationa Journal of Scientific & Technology Research 4 (09): 215- 220

Meda, A., Lamien, C.E., Romito, M., Millogo, J., and Nacoulma, O.G. (2005). Determination of the total phenolic, flavonoid and praline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry 91: 571–577.

Minotti, G., and Aust, S.D. (1987). An investigation into the mechanism of citrate-Fe^{2+}-dependent lipid peroxidation. Free Radical Biology and Medicine 3: 379–387.

Mukherjee, P.K., Chaudhary, S.K., Maity, N., Nema, N.K., Bahdra, S., and Saha, B.P. (2014) *Ocimum sactum* L, a potential angiotensin converting enzyme (ACE) inhibitor useful in hypertension. Indian Journal Natural Product Resource 5:83-87.

Oboh, G. (2005). Effect of blanching on the antioxidant property of some tropical green leafy vegetables. Lebensm.-Wiss. Technology 38: 513–517.

Olabinri, B. M., Eniyansoro, O. O., Okoronkwo, C. O., Olabinri, P. F., and Olaleye, M. T. (2010). Evaluation of chelating ability of aqueous extract of Tetracarpidium conophorum (African walnut) in vitro. International Journal of Applied Research in Natural Products 3(3): 13-18.

Oulai, P.D., Zoue, L.T.., Bedikou, M.E., Megnanou, R.M., and Niamke, S.L. (2014). Impact of cooking on nutritive and antioxidant characteristics of leafy vegetables consumed in northern Côte d'ivoire. International Journal of Plant, Animal and Environmental Science 4: 576- 585.

Oyaizu, M. (1986). Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition 44: 307–315.

Puntel, R.L., Nogueira, C.W., and Rocha, J.B.T. (2005). Krebs Cycle Intermediates modulate T hiobarbituric Acid Reactive Species (TBARS) production in Rat brain in vitro. Neurochemistry Research 30: 225–35.

Re, R., Pellegrini N, Proteggente A, Pannala A, Yang M. and Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radical Biology and Medicine 26: 1231–1237.

Sahlin, E., Savage, G.P., and Lister, C.E. (2004). Investigation of the antioxidant properties of tomatoes after processing. Journal of Food Composition and Analysis 17: 635–647.

Singleton, V.L., Orthofer, R., and Lamuela-Raventós, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology 299: 152–178.

Sisein, E.A. (2014). Biochemistry of free radicals and antioxidants. Scholars Academic Journal of Biosciences 2: 110-118.

Valko, M., Morris, H., and Cronin, M.T.D., (2005). Metals Toxicity and Oxidative Stress. Current Medicinal Chemistry 12: 1161-1208.

Zar, J.H. (1984). Biostatistical Analysis. Prentice-Hall, Inc., Upper Saddle River, NJ, p.620.

Zhang, D., and Hamauzu, Y. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chemistry 88: 503-509