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Research Paper

Effect of heat treatment on the antioxidant properties of *Tetrapleura tetraptera, Xylopia aethiopica* and *Piper guineense*

Nwaichi, E. O.

Department of Biochemistry, University of Port Harcourt, Port Harcourt, P. M. B. 5323 Port Harcourt, Rivers State, Nigeria.

E-mail: nodullm@yahoo.com

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Abstract. Phytoscreening and the effect of heat treatment on the antioxidant activity were studied in three medicinal plant parts used mostly by nursing mothers in Southern Nigeria; *Tetrapleura tetraptera, Piper guineense and Xylopia aethiopica*. Total phenols, flavonoids and vitamin C content of these spices were determined at different heating periods (0, 30, 60 and 90 min) at 100°C and the results showed significant phenol accumulation in tubers (1554. 54, 1555. 01 and 1550. 04 mgkg^{-1} DW) for *T. tetraptera, P. guineense* and *X. aethiopica* respectively at zero heat treatment. The antioxidant activity was determined by ferric ion reducing/antioxidant power (FRAP) assay. Results indicate a significant increases ($p \leq 0.05$) in FRAP values of *T*. *tetraptera* aqueous extracts at all monitored times. Similar trend was observed in heat-response for flavonoid content of measured spices. Increase in heat treatment time gave statistically significant decreases in observed levels of vitamin C for all spices studied. These suggest that heat treatment could have beneficial effect by improving antioxidant activity in consumers of these spices but prolonged treatment could have an inhibitory effect. The results recorded clearly indicate vitamin C and total phenolic content loss due to thermal treatment. Also, high antioxidant properties expressed by *T. tetraptera* expresses a potential to inhibit possible oxidation of LDL cholesterol.

Keywords: Antioxidant activity, heat treatment, Aridan plant, Ashanti pepper, guinea pepper, soup.

INTRODUCTION

The people of Southern Nigeria in their diverse culture and heritage are known for their traditional expertise in cooking hot and spicy National dishes of which preparations are incomplete without three major spices (*Tetrapleura tetraptera, Xylopia aethiopica,* and *Piper guineense).* Guinea pepper *(X. aethiopica),* Ashanti pepper *(P. guineese)* and Aridan plant *(T. tetraptera)* are valuable medicinal plants widely distributed in the West African rain forest (Takahashiet al., 2003; Burkill, 1995). Almost every morphological part of these plants is used in traditional medicine for managing various ailments including skin infections, Candidiasis, Dyspepsia, Cough and Fever (Agona and Muyinza, 2000) and for culinary uses. Recently, it is believed by the Ijaws (Nigeria) that it helps the uterine discharge and has a restorative effect after child delivery (Edlin et al., 2005). A study recently investigated their effects on some biochemical profile of mammals and reported their hypolipidemic and hypokalaemic (Nwaichi and Igbinobaro, 2012). Flavonoids have been considered to function as anti-oxidants and UV light filters in higher plants (McClure, 1986). This antioxidant activity was related to their protection against ascorbic acid oxidation. Vitamin C served standard antioxidant property essential in maintaining healthy connective tissues and the integrity of cell wall. While vitamin E serve as antioxidant that stabilize cell membrane by preventing oxidation of their unsaturated fatty acid compound (Ognjanović et al., 2003).Steam distillation is one of the most popular methods, for extracting oil from spices (DeLine et al.,

Table 1. Antioxidant activity and phenolic content of studied spices.

Sample	Total phenol (mg/kg)	Flavonoid content (%w/w)	Vitamin C (mg/kg)	FRAP (μmole TE/g dw)
T. tetrapleura	1554.54 ± 7.99^{i}	10.00 ± 0.87^{m}	5.40 ± 0.11^{x}	19.02 ± 0.95^{a}
P. guinneense	1555.01 ± 9.59^{i}	17.70 ± 0.22^{n}	4.90 ± 0.05^{y}	44.11 ± 2.00^{b}
X. aethiopica	1550.04 ± 8.81^{i}	22.00 ± 0.13^{o}	4.80 ± 0.09^{y}	24.80 ± 2.23^{c}

Columns with same superscripts are not significantly different at $P \leq 0.05$.

1995). In the same vein, almost all spices are hydrodistilled with the soup but over heating the plant and subsequent loss of the oil is implied as cooking times vary from one individual, home, locality, region and culture to the other. It is generally known that some natural compounds could be substantially lost during thermal processing (Azevedo-Meleiro and Rodriguez-Amaya, 2005). Therefore to address this, the objective of this research was to determine the effect of heat on the total phenolic content and antioxidant activities of the common spices used in the hot, spicy and pungent tasting soup made especially for nursing mothers in the Southern Nigeria.

MATERIALS AND METHODS

Materials

Freshly dried Guinea pepper, Ashanti pepper and Aridan pods were purchased from a fresh market in Uyo city, Nigeria. Samples were cleaned, washed with water, cut into small pieces and ground in a blender except for Aridan to mimick its peculiar recipe. Spices are added early when cooking for adequate infusion into food, hence the early addition (Adamson, 2004) of Aridan pods into boiling water.

Reagents

Ferric chloride hexahydrate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma), 2,4,6-tripyridyl-s-triazine (TPTZ), and potassium persulfate were purchased from Okotex Chemical distributor, Nigeria.

Heat treatment on total phenolic content and antioxidant activity

Samples of 5 ml of crude aqueous extract with a solid content between 0.1 and 0.15 dry weight per gram was added into a test tube and heated in a water bath at 100°C for 0, 30, 60 and 90 min. Samples were then cooled to room temperature before being evaluated for the total phenolic content and antioxidant activities by ferric ion reducing/ antioxidant power (FRAP) assay. Whole Aridan instead was placed in the boiling water for same timing and pods allowed in the water for the duration of the experiment to mimick its local recipe.

Determination of total phenolic content by King and Armstrong method

To 5 ml of sample, 5 ml of 0.1 M NaOH was added and warmed at 65°C and then allowed to cool. Then, 2.5 ml of 0.025 N Iodine was added and corked with foil paper. It was allowed to stand at room temperature for 30 to 40 min followed by addition of 0.5 ml of HCl. Titration was done using 0.1 ml $Na_2S_2O_3$ using starch as an indicator.

Determination of antioxidant activity

FRAP assay was carried out according to Benzie and Strain (Benzie and Strain, 1996).

Statistical analysis

Experimental data (triplicate determinations) were expressed graphically as mean ± standard deviation bars and also in a table. Test of significance was done by one-way analysis of variance (ANOVA) and mean comparisons were performed using Duncan's new multiple range test ($p \leq 0.05$).

RESULTS

Antioxidant profiling for fresh Guinea pepper, Ashanti pepper and Aridan pods revealed abundance of phenol, flavonoids and ascorbic acid in all species (Table 1).

DISCUSSION

Owing to increased surface area with blending for Guinea pepper and Ashanti pepper, higher concentrations up to 17.70 ± 0.22 and 22.00 ± 0.13 (flavonoids) were obtained for *P. guinneense* and *X. aethiopica*. Even with heat-associated decreases, flavonoid concentrations at the maximum heat far exceeded that at zero heat. These compounds possess the potential to scavenge and quench various radicals (oxygen-centered, carbon-centered, alkoxyl, peroxyl or phenoxyl radicals) and

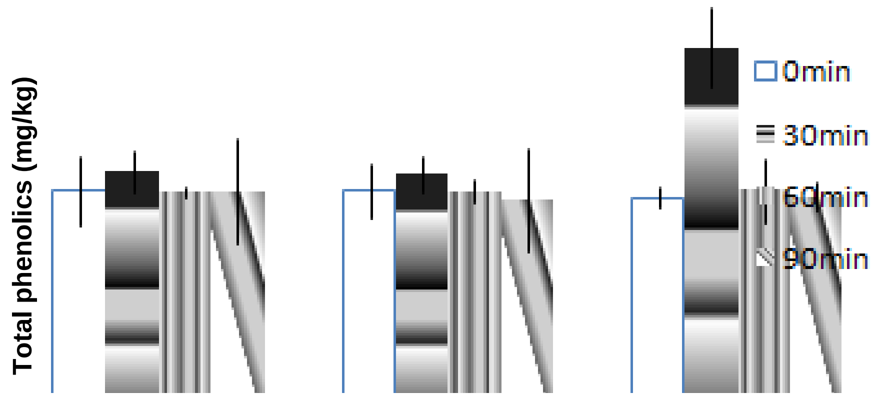

Figure 1. Effect of varying heat treatment on phenolic content of selected spices.

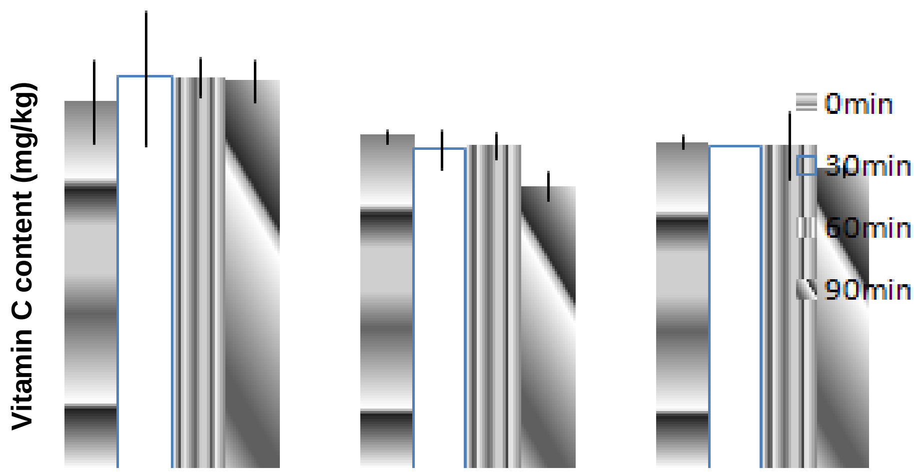

Figure 2. Effect of varying heat treament on vitamin C content of selected spices.

reactive oxygen species. Certain radical scavengers are not recyclable, while others are recycled through the intervention of a series of enzyme systems or other non-enzymatic antioxidant systems. The free radical-scavenging and antioxidant activity of plant flavonoids has been reviewed by Kandaswami and Middleton (1993). Also, these extracts decreased markedly the plasma cholesterol and triglyceride levels in treated Albino Wistar rats (Nwaichi and Igbinobaro, 2012) potentially for this property.

Heat treatment may activate certain protective compounds within the spices, or it may somehow prepare the compound for absorption at the cellular level. Vitamin C and phenols (Figure 1) were adversely impacted upon by heat beyond 60 min in content.

However, more antioxidants were unleashed with time at least on/before 60min. Decreases in vitamin C content beyond 1 h were in the magnitude of 0.8, 14 and 8% for *T. tetraptera, P. guinneense* and *X. aethiopica* (Figure 2).

This suggested that *T. tetraptera* is more heat-resistant. Grinding a spice greatly increases its surface area and so increases its oxidation and evaporation. This could explain the positive heat liability. In the first 30 min, flavonoid concentration increased with increasing heat (Figure 3).

The increases were 17%, 27%, and 16% in comparison to control for *T. tetraptera, P. guinneense* and *X. aethiopica*. Several flavonoids have been reported to inhibit either enzymatic or non-enzymatic lipid peroxidation. Increased LDL and especially oxidized LDL are recognized as risk factors in coronary artery disease (Davidson, 2009). A sharp relationship – a shielding effect between levels of flavonoids and hydrophilic Ascorbic acid (Figures 2 and 3) was observed; higher flavonoids implied higher ascorbic acid content. Harper (1969) explained this concept as they described several flavonoids serving as antioxidants for ascorbic acid. In their *in vitro* studies, flavonoids showed considerable capacity to retard the conversion of ascorbate to dehydroascorbate. One mechanism for this protection might involve the chelation of copper and other trace metals by flavonoids, resulting in the retardation of metal-catalyzed oxidation of ascorbic acid. Another protective mechanism is based on the ability of flavonoids to act as

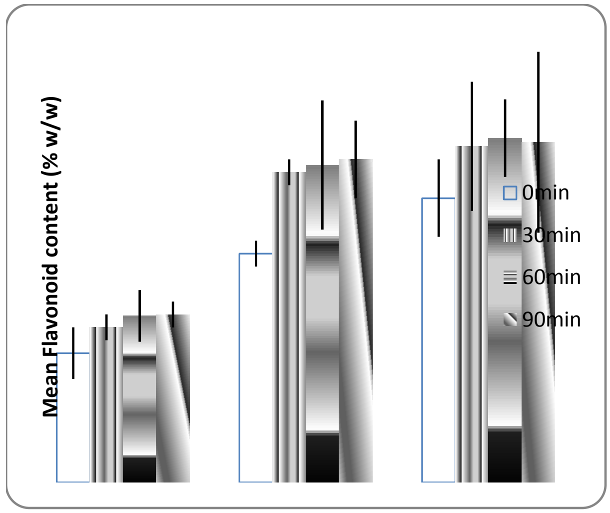

Fig 3. Effect of varying heat treament on Flavonoid content of selected Spices.

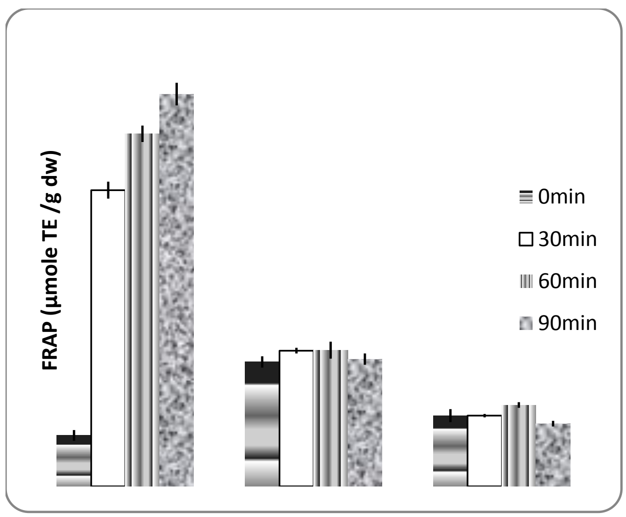

Fig 4. Effect of varying heat treatment on Antioxidant activity of selected Spices.

free radical acceptors since free radical formation is considered to be an all-important phase of ascorbate oxidation.

The total phenolic content and antioxidant activity of the studied spices are shown in Table 1. The results showed that the total phenolic content in *X. aethiopica* and *T. tetraptera* were highest at 30 min: 1664 ± 30 and 1570 ± 16 mg/kg DW, respectively, followed by 60 min:

1556 ± 14 and 1555 ± 4. Similarly, Xu et al. (2007) reported that the total amount of phenolic acids in huyou peel extract decreased after heat treatment, which indicated that some phenolic acids probably were destroyed by heat treatment and converted insoluble phenolic compounds to soluble phenolics. This indicated that the phenolic compounds of plants may be present in different bound forms depending on the species.

Antioxidant activity of chili extracts has been reported to be due to the existence of compounds, such as flavonoids (Miean and Mohamed, 2001) and ascorbic acid and tocopherol (Ching and Mohamed, 2001; Wojdylo et al., 2007). Though the total phenolic content in *T. tetraptera*, *P. guineense* and *X. aethiopica* extracts were not significantly different ($p \leq 0.05$), FRAP activity in *T. tetraptera* was higher than in *P. guineense* and *X. aethiopica* due to the breakdown of flavonoid glycosides to free flavonoids (Figure 4).

This suggested that the total phenolic content may not be a good indicator of antioxidant activity. However, the FRAP values of the *P. guineense* and *X. aethiopica* extracts were significantly increased by heating at 100°C for 60 min and significantly decreased ($p < 0.05$) when time increased to 90 min. Marked FRAP decreases beyond 60 min is attributable to the linear loss of ascorbic acid and some phenolic compounds. The FRAP assay measures the ability to reduce ferric tripyridyltriazine (Fe^{3+}-TPTZ) in samples to the ferrous form (Fe^{2+}-TPTZ). The loss of ascorbic acid was expected, as thermal treatment was known to accelerate the oxidation of ascorbic acid to dehydroascorbic acid, followed by hydrolysis to 3-diketogulonic acid and eventually polymerization to other nutritionally inactive components (Sujira et al., 2009; Kahkonen et al., 1999; Kim et al., 2006; Wangcharoen and Morasuk, 2007).

CONCLUSION

The abundance of phytonutrients, including antioxidants is interestingly significant. Also, the degenerative effect of prolonged cooking time on the antioxidant property of *T. tetraptera, P. guinneense* and *X. aethiopica* was well demonstrated in this study. Extracts of *T. tetraptera* gave constant high antioxidant activities, even with heat treatments. However, a long heating time reduced the total phenolic content and antioxidant activities for *P. guinneense* and *X. aethiopica* extracts. Therefore, to maintain the antioxidant profile and activities, the optimum heating time at cooking temperature for such soup is paramount. Moreso, high concentrations of antioxidants expressed by the study spices far outweigh their short-term taste sensations.

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