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The Free Radical Scavenging Properties of Raw and Normal Cooked *Phaseolus vulgaris* and *Vigna unguiculata* Bean Extracts

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Abstract: *Introduction:* There are conflicting reports on the effects of convectional boiling/heating on the phytochemicals and antioxidants in seed legumes. Methods: In the present study, we determined the effect of boiling on total phenolics, Vitamin C and antioxidant potential by 2,2-diphenyl-1-picrylhydrazyl(DPPH) on *Phaseolus vulgaris* and *Vigna unguiculata* seed extracts. Results: The total phenolic content of boiled *Phaseolus vulgaris* increased compared to the raw extract while for *Vigna unguiculata*, the raw extract had a higher phenolic content than the boiled extract. The results showed that boiling reduced the Vitamin c contents of both extracts. The IC_{50} of the cooked *Phaseolus vulgaris* extract was 0.94mg/ml showing higher antioxidant activity relative to the raw extract. Conclusion: It can be concluded that boiling reduced the antioxidant potential of *Vigna unguiculata* seed extract while increasing the DPPH and phenolic content of *Phaseolus vulgaris*.

Key words: *Phaseolus vulgaris* · *Vigna unguiculata* · Boiling · Radical scavenging

INTRODUCTION

Free radicals and other reactive oxygen species (ROS) are continuously produced in our body through many pathways [1] and are important for the maintenance of normal physiological functions [2]. Free radicals are generally highly reactive and participate in reactions that can be deleterious to macromolecules such as DNA, proteins and lipids [3, 4]. There is an increasing focus in finding natural antioxidants from plants since they tend to protect human bodies from the attack of free radicals and retard the progress of many chronic diseases [5-7] and might provide lead for the development of novel drugs [8].

Many synthetic antioxidants have been used to retard the oxidation process. Synthetic antioxidants should be used with caution because of their potential health hazards [9]. Phenolics seems to be the most effective compounds that can be found in many fruits, seeds and herbs [10] and their metal-chelating and radical scavenging ability make the compounds to serve as effective antioxidants derived from natural plants [11]. Beans contain appreciable quantities of phenolic compounds [12, 13] that possess varying degrees of antioxidative properties. Antioxidant activities and phenolic compounds in raw legumes have been reported in many earlier results [14, 15]. Legumes must be cooked before consumption although, how processing methods affect the health enhancing phenolics and antioxidant activities still needs to be more systematically reviewed [16].

The present study was designed to investigate the antioxidative activities, including the radical scavenging effects, phenolic content and Vitamin C contents of methanolic extracts of cooked and raw *Phaseolus vulgaris* and *Vigna unguiculata*.

MATERIALS AND METHODS

Sample Collection: Fresh samples of the seeds of *Phaseolus vulgaris* and *Vigna unguiculata* were obtained from Wurukum market in Markurdi, Benue State, Nigeria. They were identified at the Botany section of the Department of Biological Sciences of Federal University of Agriculture Markurdi, Benue State.

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Extraction of Antioxidants from the Seeds: Dried seeds of *Phaseolus vulgaris and Vigna unguiculata* were finely ground into flour. The antioxidants were extracted from the seeds(raw and cooked) by the method adopted by Kumar *et al*. [17]. Dried seeds of *Phaseolus vulgaris* and *Vigna unguiculata* were each finely ground into flour and made to pass through 100-mesh sieve. For each sample 1.0g was extracted with 15ml of 70% aqueous acetone at 25°C in the dark over night. The mixture was centrifuged at 300 rpm for 10 minutes. The residues were re-extracted with 5ml of the 70% acetone. Both the extracts were combined and stored at 4°C in the dark for the assay of DPPH free radical-scavenging activity and total phenolic content.

DPPH Radical Scavenging Activity: The DPPH radical scavenging capacity of the extract were evaluated according to the method of Shimada *et al*. [18] as adopted by Kalava *et al*. [19] Different concentrations of sample(4ml) were mixed with 1ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2mM. The mixture was shaken vigorously and left to stand for 30 min and the absorbance was measured at 517nm. The percentage inhibition was calculated according to the formula:

$$[(Ao - A1)/Ao] \times 100$$

where Ao was the absorbance of the control and A1 was the absorbance of the sample.

Determination of Total Phenolic Content: Total phenolic content of the *Phaseolus vulgaris and Vigna unguiculata* extracts was determined using Folin-Ciocalteu reagent following the method of Singleton and Rossi [20] as adopted by Kumar *et al*. [17]. Briefly, an aliquot(0.05ml) of 70% acetone *Phaseolus vulgaris and Vigna unguiculata* extracts were mixed with 0.5ml of Folin-Ciocalteu reagent and 0.5ml of 20% sodium carbonate and the final volume was made to 5ml with distilled water. The absorbance was taken at 700nm against distilled water as blank after incubation for 30min at room temperature. The total phenolic content was expressed as mg of garlic acid equivalents (GAE) per gram of dry extract.

Vitamin C Determination: Vitamin C was quantified by the standard 2,6-dichlorophenol-indole dye method. The total ascorbic acid was expressed in mg/100g.

Statistics: Data collected were subjected to analysis of variance (ANOVA) using the paired t-test statistics. The mean ± SD of each parameter was taken for each group. The probability value of $p < 0.05$ was considered significant. The analysis was carried out on SPSS for windows version 15.

RESULTS

Data for DPPH radical scavenging activity, total phenolic content (TPC) and ascorbic acid concentration of the raw and cooked *Phaseolus vulgaris and Vigna unguiculata* seeds extracts are as presented in Table 1 while Figure 1 shows the percentage DPPH inhibition of the raw and cooked *Phaseolus vulgaris and Vigna unguiculata* seeds extracts.

The extracts of the raw and cooked *Phaseolus vulgaris and Vigna unguiculata* demonstrated DPPH radical scavenging activity in a concentration dependent manner as shown in Figure 1. The IC_{50} of the cooked *Phaseolus vulgaris* was found to be 0.94mg/ml showing higher antioxidant activity than the raw *Phaseolus vulgaris* extract. The IC_{50} of raw *Vigna unguiculata* was also found to be 0.8mg/ml higher than the cooked *Vigna unguiculata* extract which was 1.4mg/ml. A positive DPPH test suggests that the extract is a potential free radical scavenger. The total phenolic content of the raw and cooked *Phaseolus vulgaris* seed extract were 1.64mgGAE and 4.42mgGAE while for raw and cooked *Vigna unguiculata* it was found to be 7.44 and 4.75 mgGAE respectively. The ascorbic acid content of the raw extracts were respectively higher than the cooked extracts.

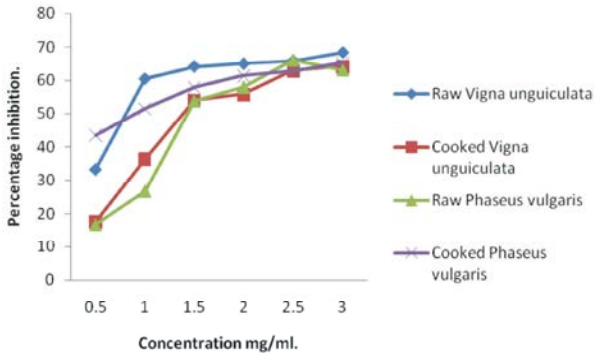

Fig. 1: The percentage DPPH inhibition of the raw and cooked *Phaseolus vulgaris and Vigna unguiculata* seeds extacts.

Table 1: Antioxidant activities of raw and cooked *Phaseolus vulgaris* and *Vigna unguiculata* seed extracts

Extract	DPPH IC_{50}(ml)	Total phenolic content (mgGAE/g).	Vitmin c (mg/100g).
Phaseolus vulgaris(raw)	1.45±0.23	1.64±3.66	136.4±0.21
Phaseolus vulgaris (boiled)	0.94±0.81	4.42±0.72	86.8±3.62
Vigna unguiculata(raw)	0.83±1.83	7.44±1.44	136.4±0.91
Vigna unguiculata(boiled)	1.43±0.31	4.75±0.88	99.2±0.69

Results are mean± SD of triplicate determinations.

DISCUSSION

The proton free radical scavenging action has been known as an essential mechanism of antioxidation [20]. DPPH was used to determine the proton-radical scavenging property of raw and cooked methanolic extracts of *Phaseolus vulgaris and Vigna unguiculata* because it possesses a proton free radical and that shows a maximum absorption at 517nm. The purple colour of DPPH solution fades when it comes in contact with proton radical scavengers [21].

Antioxidants quench free radicals and convert them to colourless bleached products resulting in a reduction in absorbance by donation of electrons or by providing hydrogen atoms. Figure 1 shows the dose response curve for the radical-scavenging activities of the raw and cooked methanolic extracts of *Phaseolus vulgaris and Vigna unguiculata*. At a dose of 0.5-3.0 mg/ml, the extracts showed between 17.8-65.2% scavenging activity of DPPH radicals and the activities increased with the concentration of the extracts. The scavenging effect on DPPH radicals levelled off as the concentration further increased. The antioxidant activity of the extracts was demonstrated by a decrease in the absorbance band upon increasing the concentration of the extracts [22, 23].

After processing, the DPPH radical scavenging ability (DPPH IC_{50} values) of the boiled *Phaseolus vulgaris* extract was higher than the raw unprocessed *Phaseolus vulgaris* extract while for *Vigna unguiculata* the results show that boiling reduced the DPPH scavenging capacity of the extract. The results obtained from the *Vigna unguiculata* experiment was in line with that of Xu and Chang [15] who reported that after processing the DPPH scavenging capacities (DPPH values) of processed cool season food legumes were significantly reduced as compared to the respective original unprocessed cool season food legumes. Xu and Chang [15] suggested that the loss of DPPH was partly due to soluble antioxidants in leached water and heat effect. The report of Rocha-Guzman *et al*. [24] showed that the DPPH radical scavenging activity of cooked bean was found to be higher than that of crude beans which was in line with our result on *Phaseolus vulgaris.*

Both samples of raw *Phaseolus vulgaris* and *Vigna unguiculata* had higher concentrations of the antioxidant Vitamin C compared to the boiled extracts. Correspondly, Nagra and Jamil [25] reported 80% loss of Vitamin C after cooking turnip (*B. napus*) for 45 min. Also, Podsedek *et al*. [26] reported 64% and 84% recovery in two varieties of red cabbage after conventional cooking of chopped materials with both varieties, about 19% of the initial Vitamin C levels were recovered in the cooking water. Baardseth *et al*. [27] reported loss of Vitamin C in *Phaseolus vulgaris* using different processing methods in catering.

Polyphenolic compounds are associated with antioxidant activity and play role in stabilizing lipid oxidation. The phenolic compounds may contribute directly in antioxidative property. The antiradical potentials of flavonoids and phenols principally depend on the structural relationship between different parts of their chemical structure [28, 29]. The results from this study showed that phenolic content of the raw *Vigna unguiculata* was higher than the cooked extract while for *Phaseolus vulgaris* the cooked extracts contained high phenolic concentration than the raw extract. Although, there are hundreds of varieties of dry edible beans in the world, data on phenolics in cooked legumes are still limited. The report of Bressani and Elis [30] showed that about 30-40% of phenolics could be removed from common beans by cooking and discarding the cooking water. Beroga *et al*. [31] observed that boiling and cooking reduced total phenolic content in Mung bean by 75%. Large amount of the loss of phenolic components could be due to leaching of phenols into soaking and cooking water, as well as breaking down of phenolics during processessing. The report of Akilioglu *et al*. [32] demonstrates that soaking beans in water prior to cooking favoured the subsequent release of phenolics and flavonoids during cooking. Turkmen *et al*. [33] reported the concentration of total phenolics to increase to 114% (w/wDM) after boiling of raw green beans. Total phenolic contents in dry beans differ between studies [34]. This difference could be attributed to different factors which include but not limited to genotype, agronomic practices, maturity at harvest, post-harvest storage and climatic conditions and storage conditions [35].

CONCLUSION

It can be concluded that boiling reduced the antioxidant potential of *Vigna unguiculata* seed extract while increasing the DPPH and phenolic content of *Phaseolus vulgaris*.

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