

EFFECT OF HEAT TREATMENT ON ANTIOXIDANT ACTIVITY OF SOME SPICES

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ABSTRACT:

Spices show potential health benefits as they possess antioxidant activity. The study was to determine the effect of cooking on the antioxidant activity of some selected spices. The total phenol content of five spices (Onion, Garlic, Ginger, Turmeric, and Basil) was determined at different heating periods (1h and 2 h) at 100^oc. Although these dietary spice are resistant to thermal denaturation, interestingly, in the case of onion shows reduction in all the tested activities and others shows different variation in all the activity. Total antioxidant activity was measured, based on the reduction of Mo (VI) to Mo (v) by the extract and subsequent formation of green phosphate / Mo (v) complex at acid pH. The extracts were found to have different level of antioxidant properties at different heating time. Considering all the activities, turmeric has good activity amongst the five spice materials screened for their antioxidant properties. Lowest activity was found in onion. In addition to the antioxidant activity of these spices, the total phenolic compounds, flavonoids were measured in the extracts. A correlation between the antioxidant activity and total phenolic content was observed.

KEYWORDS: Free radical scavenger, cooking, bioactivity and total phenol, total flavonoid

INTRODUCTION

Free radicals, such as superoxide radical (O₂⁻), hydroxyl radical (OH[·]) and non-free radical, such as H₂O₂ and singlet oxygen (¹O₂), e.t.c.), are produced in the body, primarily as a result of metabolism. These radicals cause chronic diseases such as cancer, cardiovascular diseases, diabetes, cataract, etc Halliwell *et al.* (1992); Aruoma (1994). Free radicals create chain reactions, which cause cell membrane damage, DNA mutation, lipid and protein damages, and immune cell damage and cell death. Natural antioxidants are known to scavenge free radicals, enhance the immune system prevent diseases and improve general health and life quality. Epidemiological studies show an inverse correlation between cardiovascular disease risk and dietary antioxidant consumption Waring (2001). Well known antioxidants include a number of enzymes (superoxide, catalase, glutathione peroxidase, etc) vitamin C, vitamin E, carotenoids, phenolic compounds, etc. Phenolic compounds are the major bioactive compounds found in spices. Phenolic compounds act as antioxidant to scavenge reactive oxygen species and to chelate metals.

Bioactive components of spices such as *Curcumin* (turmeric), *Zingerone* (ginger), *Allicin* (garlic) inhibit lipid peroxidation Lokesh (1992) and Noguchi *et al.* (1994). Curcumin acts as an anticarcinogen and anti-mutagenic agent Nagabhushau and Bhide (1997). A great number of aromatic, spicy, medicinal and other plants contain chemicals compounds exhibiting antioxidant properties. Numerous studies were carried out on some of these plants, *e.g.* rosemary, sage and oregano, which resulted in a development of natural antioxidant formulations for food, cosmetic and other application. Therefore, the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidant, functional foods and nutraceuticals.

Spices are usually consumed after thermal cooking. Therefore antioxidant activity of spices may be affected by thermal cooking. As far as our literature survey could ascertain, scarce information was available on the effect of heat treatment on the *in vitro* antioxidative activities of spices. Therefore, the aim of this study was to investigate the *in vitro* antioxidant capacities of the methanol extracts of spices before heating and the changes of antioxidant activity after heating for 1 and 2 hours. The antioxidant activity was examined for all the fives spices (Garlic, Onion, Turmeric, Ginger and Basil) using different antioxidant

assays such as total antioxidant activity and free radical scavenging. Furthermore, the total phenolic content, and total flavonoids contents were also measured from the spices extracts and the correlation with the antioxidant activities were ascertained.

MATERIALS AND METHODS:

Five spices were collected from Spice Programme of National Horticultural Research Institute, Ibadan. The spices were Ginger (*Zingiber officinale* L), Turmeric (*Curcuma longa* L), Onion (*Allium Cepa*), Garlic (*Allium Sativum*), Basil (*Ocimum gratissimum*)

Heat Treatment:

The skin of ginger and turmeric were peeled with knife, the outer layer of onion and garlic were removed and the basil was washed and chopped. Each spice was pounded with mortar and pestle to have a thoroughly mined and fine powder spices. Each spice (1 g) was put in a light – capped test tube which was placed in a boiling water bath and heated at 100°C for 1 and 2 hour to prevent oxidation and loss of active components by evaporation. One treatment was also done without heating as a control experiment.

Extraction:

After heat treatment, the tube was allowed to cool, and then the bioactive compounds were extracted with 20 ml of methanol by shaking for 20 min and centrifuging at 2,000 g for 20 min. The supernatant was used to determine the antioxidant activity, total phenol and total flavonoid content were measured using this extract solution. Three measurements were performed for each spice sample, and the results were expressed as the mean value \pm SD.

ANALYSIS PROCEDURE

Determination of total phenolic component

Total soluble phenolic with methanol extract was determined with Folin Ciocalteu reagent, according to the method of Spanos and Wrolstad (1990).

Determination of the total flavonoid content

Total flavonoid content was determined by using a method described by Sakanaka and Okada (2005).

Determination of total antioxidant capacity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.* (1999).

DPPH radical scavenging activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al.* (2001).

Statistical Analysis

All data were recorded as means \pm SD and analyzed by SAS (2003). One – way analysis of variance (ANOVA) and Duncan comparisons were carried out to test any significant differences between raw and cooked vegetables.

RESULTS AND DISCUSSION

The Effect of heat treatment on total phenolic and total flavonoid content of spices

Spice phenolics constitute one of the major groups of compounds acting as primary antioxidant or free radical scavengers. Therefore, it is worthwhile to determine their total amount in the spices chosen for the study. Flavonoids as one of the most diverse and widespread group of natural compounds, are likely to be the most important natural phenolics Agrawal (1989). These compounds possess a wide spectrum of chemical and biological activities including radical scavenging properties. Hence, flavonoids are phenolic compounds which are very effective antioxidants. The total phenolic content differed among the different

spices and each plant contained lower total flavonoid content than the phenolic content (Table 1 and Table 2). Since other compounds besides flavonoids are phenolic substances in plants. Ademoyegun & Fariyike (2008).

The content of total phenolics in the extracts of spice is determined using the folin – ciocalteu assay, calculated from regression equation of calibration curve ($y = 0.0111x + 0.0008$, $r^2 = 0.9958$) and is expressed as gallic acid equivalents (GAE). It can be observed that the content of phenolics in the extracts correlates with the total antioxidant activity, ($r^2 = 0.9667$). The fresh spices contained 5.96 – 41.10 mg GAE/g Fw total phenolics and the ranking were Turmeric > Ginger > Basil > Garlic > Onion. The total flavonoids ranged from 0.81 – 27.96 mg catechin equivalent / g Fw and in this decreasing order Turmeric > Ginger > Basil > Onion > Garlic.

In (Table 1), after cooking procedures for 1 hour, the total phenolics content of basil was significantly ($P < 0.05$) reduced and reduction was the same for the 2 hours cooking. Conversely, of Ginger and Turmeric total phenolic content was significantly ($P < 0.05$) increased to various extents for 1 hour. Heat treatment and increment was the same for ginger 2 hours cooking but turmeric reduce its total phenolic content at 2 hours cooking.

For onion and garlic there was not significant difference ($P < 0.05$) for the unheated and heated treatment at 1h and 2 hrs. Data on total phenolic in heat treatment spices are very limited. Khatun *et al.*, (2006) reported that ginger and turmeric contain 20.00 mg GAE/g and 14.5 mg GAE/g uncooked and retained 75% and 172.41% for 1 hour respectively. However, in the present study it was found that raw ginger and turmeric contained 18.01 mg GAE/g Fw and 41.10 mg GAE/g Fw and retention for 1 hour at 184.45 % and 141.87 % respectively. The difference may have been due to the differences in the extraction method, solvent used and cooking method.

To our knowledge, this is the first time that the total flavonoids content of the cooked spices has been reported. The effect of heated spices are significant ($p < 0.05$) for turmeric, onion and garlic. The turmeric show an increase in the total flavonoid 1h at 160.94% and further heated for 2h was observed for 2h. It was reported that heat treatment increased the level of free flavonoid Stewart *et al.* (2000). For Basil and ginger the effect of heat on the total flavonoid content has no significant ($p < 0.05$), which show that the flavonoid content is relatively stable under thermal heat.

Table 1: Effect of different cooking period on total phenolic and retention factors of spices

Spices	Total Phenolic (mg GAE / g FW) ^a				
	Fresh	1 hours		2 hours	
		Amount	% ^b	Amount	%
Turmeric	41.00 ± 1.02b	58.31 ± 1.27a	141.87	43.81 ± 6.12b	106.59
Onion	5.96 ± 0.12a	5.91 ± 0.64a	99.16	5.86 ± 0.82a	98.32
Ginger	18.01 ± 0.25c	33.22 ± 1.86b	184.45	38.04 ± 1.27a	211.22
Garlic	8.29 ± 0.25c	8.74 ± 1.30b	105.56	9.45 ± 0.33a	114.13
Basil	17.45 ± 0.51a	14.93 ± 0.28b	85.56	13.42 ± 1.02c	76.91

a) Data are expressed as means ± SD of triplicate experiments (on Fresh basis); mean values in a row with different letters are significantly different at $P < 0.05$

b) Fresh = 100, GAE = Gallic acid equivalent, FW = Fresh weight

Table 2: Effect of different cooking period on total flavonoid and retention factors of spices

Spices	Total Flavonoid (mg catechin eqv / g FW)				
	Fresh	1 hours		2 hours	
		Amount	% ^b	Amount	%
Turmeric	27.96 ± 0.47c	45.00 ± 7.57a	160.94	33.80 ± 2.88b	120.89
Onion	0.86 ± 0.08a	0.51 ± 0.08b	59.30	0.41 ± 0.09b	47.67
Ginger	4.29 ± 0.37a	3.52 ± 0.38b	81.82	3.35 ± 0.38b	78.19
Garlic	0.81 ± 0.10a	0.48 ± 0.02b	59.26	0.45 ± 0.08b	55.56
Basil	2.77 ± 0.47b	3.41 ± 0.28a	123.11	3.59 ± 0.37a	129.60

a) Data are expressed as means ± SD of triplicate experiments (on Fresh basis), mean values in a row with different letters are significantly different at $P < 0.05$

b) Fresh = 100, eqv = equivalent, FW = Fresh weight

The effect of heating times on antioxidant activity of spices

Total antioxidant activity:

The study reveals that the antioxidant activities of the five spices are in the order: Turmeric > Ginger > Basil > Garlic > Onion (Fig. 1).

The total antioxidant capacity ranged from 79.49 to 29.35 equivalent to ascorbic acid mg/g extract for the unheated spice and for the heated spices range from 101.91 – 20.30 eqv AA mg/g spice extract. There is a strong relationship between the total correlation equation between total antioxidant capacity and total phenolic is 0.9667 in (fig.2). Many reported that high total phenol content increase the antioxidant activity velioglu *et al.* (1998); Holasova *et al.* (2002) and there is a linear correlation between phenolic content and antioxidant activity Gheldof & Enjeseth (2002).

Total antioxidant activity of turmeric, ginger and garlic significantly ($P < 0.05$) increased during the 2h heating compared to the values for the fresh ones however, total antioxidant activity of onion and basil decreased during the 1h and 2h heating time.

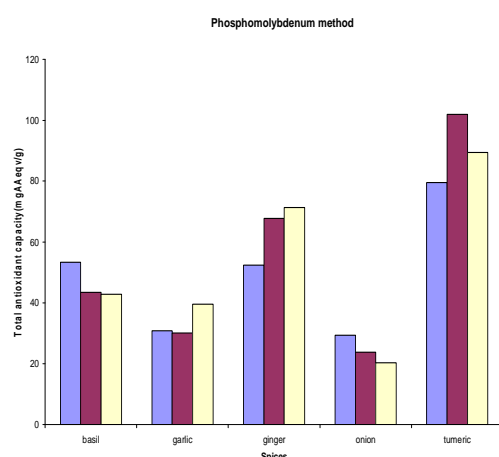


Fig. 1: Total Antioxidant Activity: Phosphomolybdenum Method

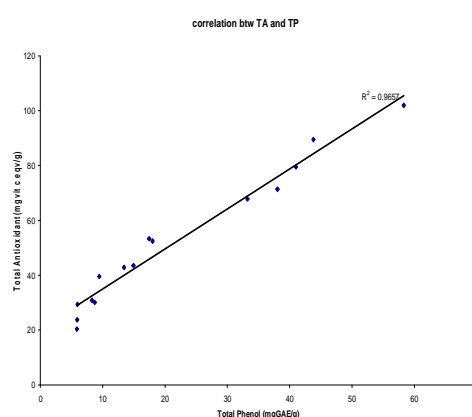


Fig 2: Correlation between Total Antioxidant and Total Phenolic

EFFECT OF HEATING TIME ON THE DPPH RADICAL SCAVENGING ACTIVITY

The DPPH radical scavenging method decreased in the order Turmeric > Ginger > Basil > Garlic > Onion in (Fig. 3).

Among the five spices, Turmeric showed highest scavenging activity with a inhibition of 89.58% whereas onion had lowest activity with 9.39%. Shobana and Naldn (2000) reported the relative antioxidant activities of some spices: the order of the activities was clove, ginger, pepper and onion, which is related to this study.

Changes in the DPPH radical scavenging activity of spices for different heating times are shown in (fig. 3). After heating, a significant change in the activity occurring in many spices. An increase in radical – scavenging activity was found in Turmeric and Ginger. The main active component of spices such as *Curcumin* (were found in ginger and turmeric is usually fat – soluble. But the present study was carried out in absolute methanol. So, the active components of these spices might not – dissolve completely in this solution before heating. After heating, the solubilities of the components probably increased because of decomposition of the cell wall and by radical scavenging activity total antioxidant activating and total phenolic of two spices might be observed after heating. Sohobana and Naidu (2000) reported that the bound antioxidants might be released due to heat treatment, resulting in the higher antioxidant activity compared that in that of fresh spices extract. Dewanto *et al.* (2002) reported that thermal processing disrupt the cell membranes and cell walls to release lycopene from the insoluble portion of tomato, which might cause the antioxidant activity of tomato to increase. Takamuru *et al.*, (2002) found a decrease of radical – scavenging activity of curry paste and cooked curry, possibly due to decomposition or evaporation of the active compounds. Since the spices were heated with butter at high temperature.

In this study, a decrease in the radical – scavenging activity was observed in garlic and basil. After heating, coagulation of these spices was observed therefore, the extraction ability might be decreased by coagulation after heating, resulting in a reduction in the radical – scavenging activities of these spices. Onion showed no significant change due to heating.

The DPPH radical – scavenging activity of all the spices was highly correlated ($R^2 = 0.8$) with total phenol content fig 4). Form these results the active components of spices are considered to be mostly polyphenol compounds. (Several polyphenols did not show any DPPH radical – scavenging activity) is reported by Parejo *et al.*, (2001); Oktay *et al.*, (2003) found a high correlation between phenolic context and DPPH radical – scavenging activity.

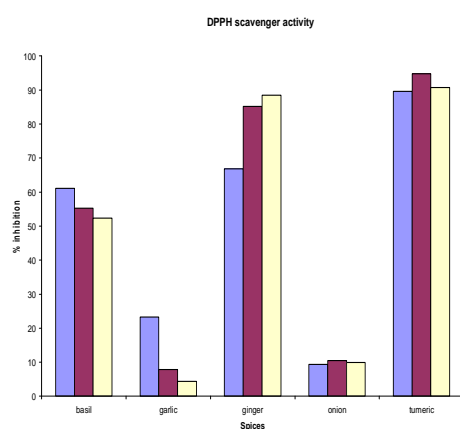


Fig. 3 Antioxidant activity: DPPH Scavenging Assay

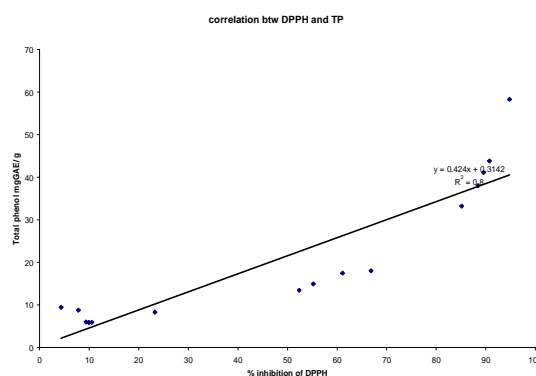


Fig. 4: Correlation between Total Phenolic and Free Radical Scavenging Activity

From the results of this study, it is clear that that spices have strong antioxidant activities in a methanol extract solution. All the tested activities of spices remained after heating, suggesting that the bioactive components are relatively stable during thermal heating at boiling point of water. But we have results which show apparent change in antioxidant properties of these spices. In conclusion, the results illustrated that the health benefits from plant sources remained in the products after thermal process that it, heat do not denatured the antioxidant activities in all the selected spices studied. Spices are expected to be a valuable food constituent for promoting good health in our daily lives.

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