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Effect of cooking on total antioxidant activity in selected vegetables

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Abstract
Introduction: The recent focus in research has been on functionality of foods to improve quality of diet and enhance health. Common chronic diseases such as inflammation, ulcers, diabetes, heart diseases, osteoporosis, are caused due to oxidative damage. The damage caused by oxidative stress or free radicals can be reduced by the consumption of antioxidants. Vegetables are rich source of antioxidants. Most vegetables are consumed after cooking. The cooking processes brings about a number of changes in physical and chemical composition of vegetables. Effect of cooking treatment on antioxidant properties of vegetables has seldom been reported.
Methodology: In the current study 5 locally available tropical vegetables such as spinach, drumstick, beetroot, mushroom and cluster beans were selected and their nutrient profile in raw form and total antioxidant, phenolic and flavonoid contents were assessed in raw as well as three commonly used cooking methods such as sautéing, boiling and pressure cooking. The antioxidant activity was analysed by DPPH (Diphenylpicrylhydrazyl) radical scavenging activity method.
Results: The selected vegetables were found to be rich in phytonutrients, antioxidant activity and macronutrients. The effect of cooking had increased the antioxidant activity and decreased total phenolic and flavonoid content than the raw form, however the difference was not significant. The correlation of antioxidant activity and total phenol content found that sautéing and pressure cooking has strong positive effect and boiling had negative effect. Moderate heat treatment and open cooking therefore would be useful in improving health properties of vegetables. This study has brought out that cooking does not significantly decrease the phytonutrient content of the selected vegetables.

Keywords: antioxidant activity, phytonutrients, phenol content, flavonoids

1. Introduction
Vegetables provide a varied, flavoured, colourful, tasty, low calorie, and protective, micro-nutrient rich diet. In addition to providing the body with many essential nutrients, vegetables are foods rich in antioxidants, which help combat the damaging effects of free radicals. The components of vegetables that function as antioxidants are beta-carotene, lutein, zeaxanthin, luteolin, quercetin, vitamin C, tocotrienols (vitamin E), organosulphurs, glucosinolates and some minerals (Lekshmi S. L and Dr. I. Sreelathakumary. 2013) [11]. The occurrence of oxidative damage may be a significant causative factor in the development of many chronic diseases such as inflammation, ulcers, diabetes, stroke, other heart diseases, osteoporosis, rheumatoid arthritis, and Alzheimer's disease. The approximate death due to chronic diseases in India in 2004 was 10·3 million (WHO) [19]. The important function of antioxidants is in retarding the oxidation of other molecules by hindering the initiation or propagation of oxidizing chain reactions by free radicals and they may decrease the oxidative damage to the human body (Amin., Zamaliah., and Chin. 2004) [2]. Vegetables are considered to be the major contributors of reactive oxygen species scavenging antioxidants. Most vegetables are consumed after cooking. The cooking processes brings about a number of changes in physical and chemical composition of vegetables (Rehman., Islam., and Shah. 2003) [15]. Epidemiological studies have suggested association between the consumption of plant foods and the prevention of chronic diseases.

Methodology
Selection of Sample
The selected vegetables were purchased fresh for analysis. The vegetables were washed with tap water after removing manually inedible parts with a knife. The cleaned vegetables were

diced in equal size and divided into four parts for raw, boiling, pressure cooking and sautéing. The individual doneness of the cooked vegetables was determined by taking into consideration the surface appearance and texture felt by fingers. Immediately after cooking, the vegetables were cooled to stop the process of cooking and the extract prepared for further assays. The raw and cooked vegetable extracts were simultaneously analysed for phenol, flavonoid and total antioxidant activity.

Sample Preparation

The selected vegetables were dried, weighed and ground into paste with pestle and motor. Then the sample was extracted up to 5ml with distilled water. The extract was then separated from the residue by centrifugation. After the process of centrifuging the extract was used for further analysis. The aqueous extract was freshly prepared for each analysis.

Research tools

The tools used in the study were

- The carbohydrate content was estimated using Anthrone method of Hedge and Hofreiter (1962).
- The protein content was estimated using Lowry's method of Lowry., Rosebrough., Farr and Randall (1951).
- The amount of crude fibre was estimated using the method of Maynard (1970).
- The moisture content was estimated using the method of Maynard (1970).
- The total antioxidant activity was estimated using DPPH (Diphenylpicrylhydrazyl) radical scavenging activity – method of Zhang and Hamauzu (2004).
- The total phenol content was estimated using Folin-Ciocalteu assay *in vitro* method of Lister and Wilson (2001).
- The total flavonoid content was estimated using Aluminium trichloride method of Woisky and Salatino (1998).

Nutrient Analysis

The raw vegetables extract were analysed for carbohydrate, protein, fibre and moisture content.

i. Carbohydrate Content

Carbohydrate content of the vegetables were determined using Anthrone method with anthrone reagent and glucose solution. Carbohydrate are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxy methyl furfural. This compound with anthrone forms a green coloured product with an absorption maximum at 630nm.

ii. Protein Content

Protein content of enzyme extract was determined by Lowry's method using Folin-Ciocalteau reagent. The blue colour developed by the reduction of the components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartrate was estimated colorimetrically.

iii. Crude Fibre Content

The crude fibre content in substrate was estimated by the method by Maynard. During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occur. The residue obtained after final filtration was weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fibre content. Total dietary fibre was calculated as the sum of insoluble and soluble dietary fibre. The extract was made into dry ash under 600 degree centigrade and results were noted.

iv. Moisture Content

The basic principle of this technique is that water has a lower boiling point than the other major component within foods. One gram of selected vegetable was subjected to drying at 60^0 C in hot air oven for 24 hours. Moisture content was calculated using fresh weight and dry weight.

Total Antioxidant Activity

Antioxidant assay of selected vegetables of raw and cooked were estimated with its free radical scavenging method by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. In this screening method the observation for the samples discolouration from purple to yellow and pale pink were considered as strong and weak positive respectively. In the quantitative assay, DPPH is a stable free radical with purple colour absorbed at 517nm and compared with known synthetic standard 0.16% of Butylated hydroxy toluene (BHT). If free radicals have been scavenged, DPPH will degenerate to yellow colour. This assay uses this character to show free radical scavenging activity.

Inhibition of DPPH (%) = $[(A_o – A_s) / A_o] * 100$

Where, A_o is absorbance of control and A_s is absorbance of sample

Total Phenolic Content

The phenol content of selected vegetables of raw and cooked were estimated using Folin-Cicolteau reagent according to Lister and Wilson with slight modifications. Reduction of phosphomolybdic-phosphotungstic acid (Folin) reagent to a blue-coloured complex in an alkaline solution occurs in the presence of phenolic compounds. The absorbance is measured at 765 nm after a 15-min heating at 45 °C; a mixture of water and reagents was used as a blank. The results were expressed in mgGAE/ 100g.

Total Flavonid Content

The flavonoid content of selected vegetables in raw and cooked form were determined by Aluminium chloride colorimetric method that was modified from the procedure reported by Woisky and Salatino. The principle involved in Aluminium chloride (AlCl3) colorimetric method is that AlCl3 forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids. 10mg of quercetin was dissolved in 100ml methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100µg/ml using methanol. 10% aluminium chloride and 1M potassium acetate were prepared using distilled water. Absorbance was taken at 415 nm against the suitable blank. The results were expressed in mgQE/100g.

Results and Discussions

Nutrient analysis

The macronutrient content of the vegetables in raw form was estimated. The values was compared with the ICMR nutritive value.

Table 1: Nutrient Analysis of Raw Vegetables

Samples	Carbohydrates g/100g		Protein g/100g		Moisture content g/100g		Crude Fibre g/100g	
	Sample	Reference ICMR value	Sample	Reference ICMR value	Sample	Reference ICMR value	Sample	Reference ICMR value
Spinach	2.5	2.9	2	2.0	93.76	92.1	0.8	0.6
Drumstick	3.5	3.7	3	2.5	88.12	86.9	5.2	4.8
Beetroot	9.2	8.8	1.9	1.7	87.89	87.7	1	0.9
Mushroom	3	4.3	3.5	3.1	90.95	88.5	0.4	0.4
Cluster Beans	11	10.8	3.5	3.2	88.26	81	3.2	3.2

*Nutritive value of Indian Foods by C.Gopalan, B.V. Rama Sastri and B.S. Balasubramanian. National Institute of Nutrition, ICMR, Hydreabad.

From the table, carbohydrate content of the vegetables ranged between 3 – 4 g/ 100g. The highest carbohydrate content was found in cluster beans (11g/100g) and the lowest carbohydrate content was found in spinach (2.5g/100g). The carbohydrate content was compared with the ICMR nutritive value and the values of the samples had very minimal difference. Protein content of the vegetables ranged between 2-3.5g/100g. The highest protein content was found in cluster beans and mushroom (3.5g/100g) and the lowest protein content was found in beetroot (1.9g/100g). The protein content of the samples were compared with the ICMR nutritive value and the values were more or less equal. The moisture content of the vegetables was slightly differed when compared with the ICMR nutritive value except beetroot. The moisture content ranged from 88 – 90 g/100g. Spinach (93.76g/100g) has the highest moisture content and decreased as mushroom, cluster beans, drumstick and beetroot. The highest fibre content was found in drumstick (5.2 g/100g) and the lowest was found in mushroom (0.4g/100g).

Antioxidant activity

The antioxidant activity of the vegetables in raw and after cooking were found. The percentage of inhibition in total antioxidant activity is given in table 2.

Table 2: Percentage of Inhibition in Total Antioxidant Activity

% of Inhibition					
Samples	**Spinach**	**Drumstick**	**Beetroot**	**Mushroom**	**Cluster beans**
Raw	96.1	96.1	87.4	89.0	96.1
Sauteing	100.0	102.5	101.8	108.0	100.0
Boiling	98.4	99.2	109.0	108.0	99.2
Pressure cooking	100.0	100.0	110.8	110.6	100.0

It is revealed from the table, that the antioxidant activity of the raw spinach, drumstick and cluster beans are around 96% while those of beetroot and mushroom are between 87-89%. On comparing the 3 methods of cooking the antioxidant activity on pressure cooking followed by boiling. There was no drop on the antioxidant level with cooking in any of the chosen vegetables. However, the percentage of inhibition in total antioxidant activity by different cooking methods had no significant difference ($p<0.05$). According to Hunter and Fletcher (2002) [6] cooking by boiling and microwaving caused no significant losses of water and lipid soluble antioxidant activities evaluated by FRAP has an increase in frozen spinach. However, our results showed that total antioxidant activity of spinach was higher than reported by Turkmen, Sari and Velioglu (2005) [18]. The study of Kirisattayakul, Tong-Un, Wattanathorn, Muchimapura, Wannanon and Jittiwat (2012)[9] had concluded that antioxidant activities of drumstick extract via DPPH and FRAP assays were 96.49 ± 0.87 µg/ml and 171.54 ± 1.21 µML- ascorbic acid equivalent / mg extract similar to the results obtained in this study.

According to Saikia and Mahanta (2013) [16] beetroot has showed increase in radical scavenging activity on cooking and metal chelating capacity (MCC) was not detected. It was reported that the antioxidant activity of the vegetables increased by cooking. This suggests that the pro-oxidant activity was due to peroxidases which were inactivated at high temperatures [Turkmen, Sari and Velioglu (2005) [18]]. Beetroot has showed an increased and positive effect on FRAP and TPC for all the three cooking treatments like steamed, microwaved and boiled [Saikia and Mahanta (2013) [16]. In the earlier study by Zhang and Hamauzu (2004) [21] said that there was no significant differences in the content of antioxidant components and antioxidant activity between conventional and microwave cooking. Another study indicated that enhanced effect was due to improvement of antioxidant properties of naturally occurring compounds or formation novel compounds such as maillard reaction products having antioxidant activity (Manzocco, Calligaris, Masrrocola, Nicoli and Leici, 2001) [11]. Ismail, Marjan and Foong (2004) [7] reported that antioxidant activities of the 1 min boiled vegetables were similar to the fresh ones.

This study showed that cooking by different methods enhanced the antioxidant activity in mushroom, drumstick and beetroot, and no changes caused in spinach, cluster beans.

Total Phenolic content

The basic mechanism of the Folin- Ciocalteau assay of TPC is an oxidantion / reduction and as such, is considered another antioxidant assay by some researchers [Chun, Kim, Smith, Schroeder, Han and Lee, (2005) [3]; Prior, Wu and Schaich, (2005) [14]. The total phenolic content of raw and cooked vegetables was determined by folin – ciocalteu reagent method. The phenol content is expressed in mg GAE/100g.

Table 3: Effect of Cooking Methods on Total Phenolic Content

Samples	Raw (mg GAE / 100g)	Sauteing (mg GAE / 100g)	Boiling (mg GAE / 100g)	Pressure cooking (mg GAE / 100g)
Spinach	433 ± 1	354.7 ± 1	104.9 ± 1	84.09 ± 0.1
Drumstick	255.8 ± 1	72.42 ± 0.1	17.36 ± 0.1	36.92 ± 0.1
Beetroot	196.07 ± 1	150 ± 1	141.6 ± 1	57.52 ± 0.1
Mushroom	46.7 ± 0.26	36.01 ± 0.1	35.75 ± 0.1	25.82 ± 01
Cluster Beans	160.68 ± 1	72.80 ± 0.1	80.94 ± 0.1	26.56 ± 0.1

In this table, the phenol content was the highest in spinach followed by beetroot, cluster beans, drumstick and mushroom. The phenol content of raw vegetables ranged from 46.7 – 433 mg GAE / 100g. After cooking procedures, the total phenolic content reduced and the reduction were in all three cooking methods. Turkmen, Sari and Velioglu (2005) [18] observed a little increase in total phenolic content of spinach in cooking methods like boiling, steaming, microwaving and this increase was not significant ($p<0.05$). In the study carried out by Ismail, Marjan and Foong (2004) [7] spinach was found to have the highest TPC, followed by swamp cabbage, kale, shallots and cabbage. According to Kirisattayal, Tong-Un, Wattanathorn, Muchimapura, Wannanon and Jittiwat (2013) [9] has showed that the TPC of drumstick extract was 68.16 ± 0.71 mg/L GAE.

The cooking treatments caused significant changes in the total phenolic content in the vegetables but cooking process were not always detrimental to the phytochemical properties [Saikia and Mahanta (2013) [16]. The difference may have been due to the differences in the extraction and cooking methods. This study indicated that cooking caused loss of phenol but not significantly. The loss could be due to phenolic breakdown during cooking (Turkmen, Sari, and Velioglu, 2004) [18].

The relationship in the antioxidant activity and total phenolic content in sample extracts was determined and it was found there was a very positive strong relationship in raw, sautéing and pressure cooking. Boiling was found to have negative correlation in antioxidant activity and total phenolic content. Gan, Amira and Asmah (2013) [5], suggest that phenol do not act as the major antioxidant components in the aqueous extracts. There might be possibility of the other active constituents, which are nonphenolic in nature that can be extracted in water extracts. According to Chye, Wong and Lee (2008)[4] and Perez – Jimenez and Saura – Calixto (2006) [13], the presence of non-antioxidant compounds especially amino acids and uronic acids in the test solutions may produce higher antioxidant capacity to that produced by the polyphenols alone. Jasna *et al*., (2011) [8] has found that correlation coefficients exhibited a positive relationship between the antiradical activities of beetroot pomace and the contents of total phenolic, flavonoid anthocyanin and betaxanthins.

Cooking could lead to decomposition of some polyphenols bound to dietary fibre of vegetables releasing free phenolic compounds that increase their detection [Stewart, Bozonnet, Mullen, Jenkins, Lean and Crozier (2000) [17]. Yamaguchi *et al*., (2001) [20] has told that heat treatment usually leads to inactivation of polyphenol oxidase and other oxidising enzymes which in turn slows down the phenolic destruction by oxidation on exposure to the surrounding environment.

Total Flavonoid content

The total flavonoid content of raw and cooked vegetables was estimated by aluminium trichloride method. The flavonoid content is expressed as mg QE/ 100g.

Table 4: Effect of Cooking Methods on Total Flavonoid Content

Samples	Raw (mgQE/ 100g)	Sauteing (mgQE/ 100g)	Boiling (mgQE/ 100g)	Pressure cooking (mgQE/ 100g)
Spinach	324 ± 1	119.8 ± 1	21.89 ± 0.1	37.96 ± 0.1
Drumstick	42.43 ± 0.1	29.98 ± 0.1	8.14 ± 0.01	0
Beetroot	187.5 ± 1	116.71 ± 1	44.25 ± 0.1	44.92 ± 0.1
Mushroom	83.41 ± 0.1	28.13 ± 0.1	74.87 ± 0.1	41.97 ± 0.1
Cluster Beans	50.21 ± 0.1	14.22 ± 0.1	42.14 ± 0.1	42.83 ± 0.1

From the table, it was seen that spinach has the highest flavonoid content. Cooking had both positive and negative effects on flavonoid content depending on the type of vegetables. The flavonoid content of raw vegetables ranged from 42.43 – 324 mg QE / 100g and in decreased order spinach, beetroot, mushroom, cluster beans and drumstick. The cooking process has reduced the flavonoid content but not significantly ($p < 0.05$). Pressure cooking has drastic impact in the flavonoid content of the vegetables especially in drumstick the flavonoid content became 0. So it is recommended in the incorporation of vegetables in Indian recipes such as sambar, should be cooked in open kadai rather than pressure cooking in order to retain the nutrient content of the vegetables. In the study of Saikia and Mahanta (2013) [16], beetroot showed an increased TFC in steamed and boiled samples. Usually, thermal treatments have destructive effect on the flavonoid as they are highly unstable compounds (Ismail, Marjan and Foong 2004) [7]. According to Adefegha and Oboh (2011) [1], total flavonoids of cooked vegetables were higher than total flavonoids of raw vegetables, indicating a possible release of some flavonoids during the cooking of the green leafy vegetables, this indicates some flavonoids release during cooking.

Conclusion

The selected vegetables was found to be rich in phytonutrients, antioxidant activity and the macronutrients. The commonly used cooking methods in India were sautéing, boiling and pressure cooking. The effect of cooking had increased the antioxidant activity and decreased in total phenolic and flavonoid content than the raw form, however the decrease was not significant. As flavonoids are highly unstable compounds, any method of cooking is bound to decrease its content. The correlation of antioxidant activity and total phenol content found that sautéing and pressure cooking has strong positive effect and boiling has negative effect. Moreover, moderate heat treatment and open cooking would be a useful tool in improving health properties of vegetables. The vegetables was similar to the value of nutrients to the ICMR nutritive value. The difference of the values may be due to the difference in extraction method, environment factors such as climate, growth, ripening stage, temperature, duration of storage (postharvest storage) and thermal treatment had influenced. This study has brought out that cooking does not significantly decrease the phytonutrients. The USDA national nutrition data base and National Institutes of Health both recommend a minimum of five servings of vegetables per day and mostly vegetables are consumed after cooking. The study brings to light the antioxidant activity of the chosen vegetables do not significantly change due to the chosen methods of cooking

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