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Effect of Cooking on Total Antioxidant Activity, Polyphenols and Flavanoid Content in Commonly Consumed Vegetables

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A B S T R A C T

In India, most of the vegetables are processed before consumption and are cooked by boiling in water or roasting or deep-frying or microwaving. These cooking processes would bring about a number of changes in physical characteristics and chemical composition of vegetables. Thus, the present study helps to know the effect of different commonly used household processing methods on total antioxidant activity, flavanoid content and total polyphenols content of the selected vegetables. A total of six green leafy vegetables and five other vegetables namely red amaranthus, fenugreek, curry leaves, drumstick leaves, shepu, cabbage, ridge gourd, tomato, small brinjal, cluster bean and French bean were selected for the study. Vegetables were sorted, cleaned and edible portion of vegetables were cut into uniform size of one-centimeter cube using vegetable chopper except green leafy vegetables. The green leafy vegetables were also chopped into uniform pieces with knife as used at household level. The total polyphenolic content, flavanoid content and total antioxidant activity were analyzed in raw and vegetable cooked by two different household processing methods namely open boiling and pressure-cooking. It is a concluded that unprocessed or raw foods is healthier, especially vegetables but still cooking vegetables before consumption is a most widely practiced in India. It is important to know the best method of cooking to retain the health beneficial constituents in the vegetables. The present study indicates that pressure cooking in phenolics and flavanoids better to enhance the total antioxidant activity in Green leafy vegetables and other vegetables.

Introduction

Fruits and vegetables account for a small part of our daily caloric intake; however their benefits to health surpass their caloric contribution. The contributory factors are due to the presence of vitamins and provitamins, such as ascorbic acid, tocopherols and carotenoids and, in addition to that, they are also rich in a wide variety of phenolic substances (Loliger, 1991). Phenolic substances are a category of phytonutrients that exert strong antioxidant properties (Ho, 1992). They can be classified into simple phenols, phenolic acids, hydroxycinnamic acid derivatives and flavonoids. The ability of some of the phenolic substances to act as potent antioxidant components has been reported (Velioglu *et al.*, 1998; K€ahkonen *et al.*, € 1999). The principle function of antioxidants is in delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and they may reduce oxidative damage to the human body (Namiki, 1990).

Antioxidants are any substances that delay or inhibit oxidative damage to a target molecule by scavenging the free radicals. At a time one antioxidant molecule can react with single free radicals and are capable to neutralize free radicals by donating one of their own electrons, ending the carbon starting reaction. Antioxidants prevent cell and tissue damage as they act as scavenger. Cell produces defense against excessive free radicals by this preventive mechanisms, physical defenses and oxidant defenses (Jacob, 1995).

Free radicals are generally involved in chain reactions, which is series of reactions leading to regenerate a radical that can begin a new cycle of reactions. The occurrence of such oxidative damage may be a significant causative factor in the development of many chronic diseases, such as cancer and cardiovascular diseases (Lindley, 1998; Papas, 1999). Several epidemiological studies have shown a negative association between intake of fruits and vegetables and certain diseases (Papas, 1999). Cao *et al.,* (1996) found that vegetables, such as kale, beets, pepper, broccoli, spinach, shallots, potato, carrots and cabbage, had high antioxidant activities. Kale, spinach, swamp cabbage, cabbage and shallots are some of the commonly consumed vegetables in Malaysia. Beside antioxidant nutrients such as ascorbic acid, tocopherols, and carotenoids, these vegetables are also a good source of polyphenol components. Vegetables are generally consumed in the cooked form than raw. Cooking may affect the antioxidant content in vegetables, especially components such as tocopherol, carotenoids, ascorbic acid and polyphenols. In recent years, increasing attention has been paid by consumer, food industry and researcher to the role of diet and food in health promotion and diseases prevention. Epidemiological studies have shown that a high intake of plant originated food is strongly associated with a reduced risk of a number of chronic diseases, as well as inflammation, problems caused by free radicals, cutaneous aging (Das gupta and Be, 2007), cataract, muscular degeneration (Turkmen *et al.*, 2005) and diabetes (cai *et al.*, 2004). Number of studies have also shown that diet rich in fruits and vegetables are protective against diseases and population that consume such diets have the higher plasma antioxidants states and exhibit lower risk of chronic degenerative diseases (Azizah *et al.*, 2009; Chipurura *et al.,* 2010; Adefegha and Oboh, 2011).

Recent reports suggest that vegetables act as a good source of natural antioxidants due to the high levels of carotenoids, tocopherols and ascorbic acid. In addition to carotenoids, tocopherols and ascorbic acid, most of the anti-oxidative effect related to plant food intake is mainly due to the presence of phenolic compounds which have been associated with flavor and color characteristics of vegetables. The majority of the antioxidant of a fruit or vegetables may be from polyphenolic compounds rather than from vitamin C, E or β-carotene (Wang *et al.*, 1996; Kahkonen *et al.*, 1999)

In India most of the vegetables are processed before consumption and are cooked by boiling in water or roasting or deep frying or microwaving. These cooking processes would bring about a number of changes in physical characteristics and chemical composition of vegetables (Rahman *et al.*, 2003; Zhang and Hamauzu, 2004). Many studies have showed that the nutritional value of fresh fruits and vegetables are higher than those of processed one. Thus the present study helps to know the effect of different commonly used household processing methods on total antioxidant activity, Flavanoid content and total polyphenols content of the selected vegetables.

Materials and Methods

Selection and procurement of vegetables

A total of six green leafy vegetables and five other vegetables namely red amaranthus, Fenugreek, Curry leaves, Drumstick leaves, shepu, Cabbage, Ridge gourd, Tomato, Small Brinjal, Cluster bean and French bean were selected for the study. All selected vegetables were purchased fresh from local market, cleaned and edible portions were used for the analysis. Bioactive compounds were extracted on the same day.

Household processing of vegetables

Vegetables were sorted, cleaned and edible portion of vegetables were cut into uniform size of one centimeter cube using vegetable chopper except green leafy vegetables. The green leafy vegetables were also chopped into uniform pieces with knife as used at household level. The total polyphenolic content, flavanoid and total antioxidant activity were analyzed in raw and vegetable cooked by two different household processing methods namely open boiling and pressure cooking.

Open boiling

The selected vegetables were boiled in a stainless steel vessel till cooked. The vegetable to water ratio was 1:10. The samples were drained on stainless steel sieve until cold and then used for extraction and analysis of total polyphenolic content, flavanoid and total antioxidant activity.

Pressure cooking

Vegetable sample were cooked in domestic pressure cooker of one litre capacity, care was taken to avoid water entering into the sample container by placing the weighed sample in a container covered with an aluminum foil. The samples were drained on stainless steel sieve until cold and then used for extraction and analysis.

Extraction of bioactive compounds

Raw as well as cooked samples, 2-5g were weighed, grounded manually using pestle and mortar by adding 10ml acidified 80 percent methanol. The ground aliquot was collected in 50 ml propylene centrifuge tube with cap. 10ml of acidified methanol was again added to pestle and mortar, washing thoroughly to ensure no residue is left in pestle and mortar, and then transferred to the centrifuge tube.

Propylene centrifuge tube containing sample extract was kept for 30 minutes on mechanical shaker at room temperature. After 30 minutes, the extract was centrifuged at 10,000 rpm for 10 minutes and supernatant was collected in 100 ml conical flask. 10 ml of acidified 80 per cent methanol was added again into the same centrifuge tube containing remaining residues and kept shaking for 30 minutes, centrifuged for 10 minutes and then the supernatant was collected in the same conical flask. The process was repeated three times to ensure that all bioactive compounds were extracted completely. The conical flask containing all three aliquots was filtered with whatman no.1 filter paper into 50 ml volumetric flask and volume was made up to 50 ml with 80 per cent acidified methanol. Then the filtered extract was transferred into 60 ml sterile propylene bottles and stored at -20^0 c and used for estimation of total polyphenolic content, flavanoid and total antioxidant activity.

Estimation of Total Polyphenolic Content (TPC)

The total polyphenolic content of the extract was determined using the method reported by Singleton *et al.*, (1999)

Calculation of Total Polyphenolic Content (TPC)

$$\text{TPC(mg GAE/100gm)} = \frac{\text{Std. Conc}}{\text{Std .O.D}} \times \frac{\text{Sample O.D}}{\text{Aliquot taken}} \times \frac{\text{Volume make up}}{\text{weight of sample}} \times \frac{100}{1000}$$

Total polyphenolic content was expressed as milligram of Gallic acid equivalent per 100g of sample (mg GAE/100g)

Estimation of Total Antioxidant Activity (TAA)

The total antioxidant activity (TAA) of the extract was determined using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method reported by Brand-William *et al.*, (1995.)

Calculation of % DPPH inhibition

$$\% \text{ Inhibition} = \frac{(Ac-Ae)}{Ac} \times 100$$

(Where, Ac is absorbance of control and Ae is absorbance of sample extract)

Calculation of Total Antioxidant Activity (TAA)

$$\text{TAA(mg TE/100gm)} = \frac{\text{Std. Conc}}{\text{Std \% Inhi.}} \times \frac{\text{Sample O.D}}{\text{Aliquot taken}} \times \frac{\text{Volume make up}}{\text{weight of sample}} \times \frac{100}{1000}$$

Estimation of Flavanoids (TF)

The flavanoid content (FC) of the exctract was determined using Rutin method reported by Zhishen *et al.*, (1999)

Calculation of Flavanoids

$$\text{Flavanoids (mg RE/100gm)} = \frac{\text{Std. Conc.}}{\text{Std. O.D}} \times \frac{\text{Sample O.D}}{\text{Aliquot taken}} \times \frac{\text{Volume made up}}{\text{weight of sample}} \times \frac{100}{1000}$$

Results and Discussion

Total Antioxidant Activity (TAA)

Among the green leafy vegetables analyzed in raw farm, curry leaves had higher content of total antioxidant of 2.84mg TE/gm, followed by cabbage (2.56 mg TE/gm), red amaranthus (2.32 mg TE/gm), Shepu (1.76 mg TE/gm) Fenugreek (1.61 mg TE/gm) and drumstick leaves (0.64 mg TE/gm) per 100gm of sample analyzed.

Pressure cooking was found to increase the total antioxidant in fenugreek (3.03 mg TE/gm), drumstick (0.67 mg TE/gm) and cabbage leaves (2.95 mg TE/gm) but found to be decrease in red amaranthus (1.43 mg TE/gm), curry leaves (0.73 mg TE/gm) and shepu (0.78 µg TE/gm). However, the reduction in total antioxidant was slightly lower in pressure-cooking than open cooking.

The difference TAO from raw to cooked and between the green leafy vegetables was found to be statistically significantly. Sreeramulu *et al.*, (2013) and Jimenez-monreal *et al.*, (2009) have reported similar findings in a study on antioxidant in raw and cooked green leafy vegetables.

Kenny and Obairna (2009) indicated that loss of antioxidants activity was relating to the contact area between vegetables and as well as processing time, longer the cooking time, more is loss. Further during open cooking, leaching of antioxidants with the boiling media results in the decrease of TAA compared to pressure cooking.

Table.1 Anti-oxidant scavenging activity (mg TE $100g^{-1}$) in leafy vegitables measured by DPPH and FRAP method

Leafy vegetables	Anti-oxidant scavenging activity					
	DPPH Method (mg TE $100g^{-1}$) ± SE			FRAP Method (mg TE $100g^{-1}$) ± SE		
	Raw	Open cooking (With husk)	Pressure cooking (Without husk)	Raw	Open cooking (With husk)	Pressure cooking (Without husk)
Red amaranthus (*Amaranthus cruentus*)	2.32±0.07[d]	1.76±0.01[e]	1.43±0.06[f]	39.28±3.20[g]	38.18±0.92[g]	36.64±0.35[g]
Fenugreek (*Trigonella foenum-graicum*)	1.61±0.13[ef]	2.50±0.12[cd]	3.03±0.04[a]	28.61±2.03[h]	25.06±1.2[h]	9.91±0.18[ij]
Curry leaves (*Murraya koenijii*)	2.84±0.01[ab]	0.70±0.01[g]	0.73±0.02[g]	152.21±0.38[a]	122.73±1.89[d]	128.02±2.18[c]
Drum stick leaves (*Moringa oleifera*)	0.64±0.01[g]	0.64±0.04[g]	0.67±0.04[g]	150.65±2.29[a]	121.25±1.51[d]	135.19±0.28[b]
Shepu (*Anethum graveolens*)	1.76±0.10[e]	0.57±0.01[g]	0.78±0.03[g]	50.39±1.33[ef]	52.66±1.64[ef]	54.84±1.93[e]
Cabbage (*Brassica oleracea var-capitata*)	2.56±0.04[c]	2.79±0.03[b]	2.95±0.11[ab]	5.61±0.42[j]	14.33±0.72[i]	12.17±1.28[i]
LSD @ 0.05	0.18			4.40		
CV %	6.52			4.56		

Table.2 Total flavonoids (mg RE $100g^{-1}$) and Total phenols (mg GAE $100g^{-1}$) content in leafy vegetables

Leafy vegetables	Total flavonoids (mg RE $100g^{-1}$) ± SE			Total phenols (mg GAE $100g^{-1}$) ± SE		
	Raw	Open cooking (With husk)	Pressure cooking (Without husk)	Raw	Open cooking (With husk)	Pressure cooking (Without husk)
Red amaranthus (*Amaranthus cruentus*)	559.85±10.03[e]	338.15±25.70[g]	618.20±7.78[d]	129.47±3.84$_i$	94.34±3.00[j]	169.26±0.87[gh]
Fenugreek (*Trigonella foenum-graicum*)	173.01±1.0[h]	165.90±4.87[h]	137.75±0.50[h]	87.44±0.66[j]	87.01±2.76[j]	35.88±0.23[k]
Curry leaves (*Murraya koenijii*)	562.53±24.35[e]	1020.33±25.66[b]	1118.29±18.21[a]	245.02±12.50[f]	492.09±9.17[b]	586.78±24.98[a]
Drum stick leaves (*Moringa oleifera*)	989.75±39.19[b]	683.39±28.05[c]	1039.29±4.18[b]	432.54±5.51[c]	352.96±1.31[e]	386.32±2.79[d]
Shepu (*Anethum graveolens*)	315.75±11.22[g]	452.00±10.10[f]	526.32±5.00[e]	135.94±6.60[i]	184.40±4.72[g]	158.88±0.37[h]
Cabbage (*Brassica oleracea var-capitata*)	48.27±0.59[i]	64.59±5.63[i]	73.49±0.70[i]	26.23±0.57[k]	31.80±1.16[k]	26.97±4.26[k]
LSD @ 0.05	**48.07**			**21.62**		
CV %	**5.88**			**4.41**		

Table.3 Anti-oxidant scavenging activity (mg TE $100g^{-1}$) in other vegetables measured by DPPH and FRAP method

Other vegetables	Anti-oxidant scavenging activity					
	DPPH Method (mg TE $100g^{-1}$) ± SE			FRAP Method (mg TE $100g^{-1}$) ± SE		
	Raw	Open cooking (With husk)	Pressure cooking (Without husk)	Raw	Open cooking (With husk)	Pressure cooking (Without husk)
Ridge gourd (*Lufa acutangula*)	2.66 ± 0.24^{bc}	3.15 ± 0.05^{ab}	$2.59\pm0.06_{bc}$	7.22 ± 1.03^{ef}	6.71 ± 0.71^{ef}	4.05 ± 0.83^{f}
Tomato (*Solanum lycopersicum*)	2.71 ± 0.09^{bc}	2.42 ± 0.09^{cd}	2.43 ± 0.02^{cd}	8.30 ± 0.78^{de}	10.76 ± 0.98^{d}	7.75 ± 0.70^{de}
Small brinjal (*Solanum melongena*)	2.78 ± 0.07^{bc}	2.17 ± 0.06^{cd}	1.85 ± 0.08^{d}	15.00 ± 0.76^{c}	24.75 ± 1.44^{b}	29.43 ± 2.16^{a}
Cluster bean (*Cyamopsis tetragonoloba*)	3.73 ± 0.02^{a}	2.56 ± 0.05^{bc}	2.68 ± 0.04^{bc}	16.16 ± 1.12^{c}	16.66 ± 0.35^{c}	16.63 ± 1.03^{c}
French bean (*Phaseolus vulgaris*)	3.71 ± 0.35^{a}	2.74 ± 0.06^{bc}	1.80 ± 0.65^{d}	7.94 ± 1.24^{de}	9.93 ± 0.21^{de}	6.94 ± 0.03^{ef}
LSD @ 0.05	**0.59**			**3.11**		
CV %	**3.76**			**4.85**		

Table.4 Total flavonoids (mg RE $100g^{-1}$) and Total phenols (mg GAE $100g^{-1}$) content in other vegetables

Other vegetables	Total flavonoids (mg RE $100g^{-1}$) ± SE			Total phenols (mg GAE $100g^{-1}$) ± SE		
	Raw	Open cooking (With husk)	Pressure cooking (Without husk)	Raw	Open cooking (With husk)	Pressure cooking (Without husk)
Ridge gourd (*Lufa acutangula*)	80.74 ± 1.98^{ef}	90.40 ± 9.73^{e}	55.19 ± 2.97^{h}	20.81 ± 1.64^{gh}	24.11 ± 1.23^{fg}	18.22 ± 1.51^{h}
Tomato (*Solanum lycopersicum*)	65.49 ± 2.36^{gh}	70.23 ± 3.61^{fg}	77.44 ± 4.42^{fg}	19.58 ± 0.70^{gh}	30.40 ± 0.32^{e}	23.11 ± 1.80^{fgh}
Small brinjal (*Solanum melongena*)	146.63 ± 0.39^{c}	243.74 ± 1.02^{b}	264.98 ± 7.88^{a}	56.22 ± 1.21^{c}	101.13 ± 3.88^{a}	103.82 ± 1.48^{a}
Cluster bean (*Cyamopsis tetragonoloba*)	118.39 ± 1.54^{d}	115.31 ± 1.79^{d}	141.79 ± 3.33^{c}	61.78 ± 0.60^{b}	59.55 ± 0.17^{bc}	61.57 ± 0.22^{b}
French bean (*Phaseolus vulgaris*)	81.72 ± 3.15^{ef}	92.33 ± 1.76^{e}	75.91 ± 1.86^{fg}	26.07 ± 2.06^{ef}	37.94 ± 2.08^{d}	29.19 ± 1.77^{e}
LSD @ 0.05	**9.70**			**4.78**		
CV %	**4.07**			**3.39**		

Total antioxidants may increase or decrease due to cooking, because of maillard reaction. In some instances maillard reaction may reduce the total antioxidants after processing due to pro-oxidant activity of maillard reaction.

Total polyphenols and flavanoids

The total phenolic content was found to increase after cooking compared to raw form in most of the green leafy vegetables and other vegetables under study. Among the raw green leafy vegetables, drumstick leaves had highest phenolic content of 432.54 mg GAE /100g) followed by curry leaves (245.02 mg GAE /100g), shepu (135.94 mg GAE /100g) red amaranthus (129.47 mg GAE /100g) fenugreek (87.44 mg GAE /100g) and cabbage (26.23 mg GAE /100g) respectively. Pressure cooking was found to increase the phenolic content in red amaranths (169.26 mg GAE /100g), drumstick leaves (386.32 mg GAE /100g), curry leaves (586.78 mg GAE /100g) whereas open cooking had increased phenolic content in fenugreek (87.01 mg GAE /100g), shepu(184.40 mg GAE /100g) and cabbage(31.80 mg GAE /100g) among the other vegetables, pressure cooking had increased phenolic content in small brinjal (103.82 mg GAE /100g) and cluster bean (61.57 mg GAE /100g), whereas open cooking increased phenolic content in ridge gourd (24.11 mg GAE /100g), tomato (30.40 mg GAE /100g) and French bean (37.94 mg GAE /100g) in general cooking improved the phenolic content compared to raw, however, open cooking was better than pressure cooking to increase phenolic content.

The results are in tune with the findings reported on effect of cooking on phenolic content green leafy vegetables by sreeramulu 2013, other vegetables by Sasipriya (2012) and Sengul *et al.,* (2014). The increase in polyphenols may be due to the release of phenolic compounds, which is the free or bound form in vegetables because of cooking.

Among the green vegetables cooked the total flavanoids was found to increase from raw form except in fenugreek leaves. The increase in flavanoids was more in pressure cooked samples Compared to open cooking.

The raw farm drumstick leaves had highest flevioneds (989-75 mg RE/100g) fold by curry leaves (562-53 mg RE/100g), red amaranths (560 mg RE/100g), shepu (315.75 mg RE/100g) fenugreek & Cabbage (48.75 mg RE/100g).

Almost similar trend was observed for the other vegetables small brinjal had highest flavoniod content (146.63mg RE) fold by cluster bean (118.39 mg RE), French bean (81.72mg re), Ridge guard (80.74mg RE) & Tomato (65.49mg RE) in raw form.

It is a common notion that unprocessed or raw foods is healthier, especially vegetables but still cooking vegetables before consumption is a most widely practiced in India. It is important to know the best method of cooking to retain the health beneficial constituents in the vegetables. The present study indicates that pressure cooking in phenolics and flavanoids better to enhance the total antioxidant activity in Green leafy vegetables and other vegetables.

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