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EFFECT OF STEAMING, BOILING AND MICROWAVE COOKING ON THE TOTAL PHENOLICS, FLAVONOIDS AND ANTIOXIDANT PROPERTIES OF DIFFERENT VEGETABLES OF ASSAM, INDIA

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ABSTRACT

All the fourteen vegetables selected for the study are an integral part of the diet in North Eastern India. Vegetables like banana blossom, roselle leaves, black eyed pea and teasle gourd are traditionally known to be good for health. The present study determined the effect of three cooking treatments viz. steaming, conventional boiling and microwave cooking on the phytochemical contents (TPC, TFC) and antioxidant activitites (FRAP, DPPH, MCC) on the fourteen vegetables. Results showed both positive and negative impact on the phytochemical properties of the vegetables depending on the cooking method employed and type of the vegetable. Among the three cooking methods employed, steaming emerged as the most suitable method followed by microwave cooking in most of the cases in terms of retention of phytochemicals and antioxidant activities.

Keywords: Phenolic content, steaming, boiling, microwave cooking, phytochemicals, antioxidant.

INTRODUCTION

Diets naturally rich in bioactive compounds are very popular among the consumers with an increase in awareness for healthy food and living. Fresh fruits and vegetables contain wide range of phytochemicals having antioxidant and other health promoting properties. Phenolic compounds constitute the major portion of the phytochemicals apart from carotenoids and vitamins in fruits and vegetables. The phenolic compounds help in the destruction of free radicals and other toxic compounds in the human body. Although fruits are mostly consumed in raw form, vegetables need to be cooked to enhance their palatibilty and taste. However, cooking brings about a number of physical and chemical changes in the vegetables (Rehman, Islam and Shah, 2003). These changes could be both beneficial and detrimental depending on the extent and type of treatment conditions. Variety of effects like destruction, release and structural transformation of the phytochemicals take place during the cooking process. Cooking treatments like boiling, microwaving (Zhang and Hamauzu, 2004), baking, frying and griddling lead to changes in texture and nutritional properties of the vegetables. Studies had reported that cooking softens the cell walls which lead to increase in the extraction of carotenoids (Rodriguez-Amaya, 1999). However, other studies have reported that cooking can also lead to loss in essential vitamins and antioxidants, mostly water soluble and heat labile compounds. The extent of loss is dependent on the type of cooking treatment (Lin and Chang, 2005) and the phytochemical compositions of the cooked vegetable.

Based on these facts, the present study was carried out to determine the effect of boiling, steaming and microwave cooking on the antioxidant activity of the phytochemicals of cauliflower (*Brassica oleracea* Botrytis), cabbage *(Brassica oleracea captita)*, green pea (*Pisum sativa*), banana blossom (*Musa balbisiana ABB*), beetroot (*Beta vulgaris*), teaslegourd *(Momordica dioica*), black eyed pea (*Vigna unguiculata* subsp. *Unguiculata*), bottlegourd (*Lagenaria siceraria*), tomato (*Solanum lycopersicum)*, carrot *(Daucus carota subsp. Sativus), kharua* brinjal (*Solanum sp.)*, radish (*Raphanus sativus*), knol-khol (*Brassica caulorapa L.) a*nd roselle leaves (*Hibiscus acetosella*) that are widely available and consumed in North eastern India and Assam in particular. Vegetables like banana blossom, roselle leaves, black eyed pea and teasle gourd are traditionally known to have health benefitting properties.

MATERIALS AND METHODS

CHEMICALS

All chemicals used were of analytical grade supplied by Merck and Sigma. All the measurements were done at least in triplicates.

VEGETABLE SAMPLES

Freshly harvested cauliflower (*Brassica oleracea* Botrytis), cabbage *(Brassica oleracea captita)*, green pea

(*Pisum sativa*), banana blossom (*Musa balbisiana ABB*), beetroot (*Beta vulgaris*) teaslegourd *(Momordica dioica*), black eyed pea (*Vigna unguiculata* subsp. *Unguiculata*), bottlegourd (*Lagenaria siceraria*), tomato (*Solanum lycopersicum)*, carrot *(Daucus carota subsp. Sativus), kharua* brinjal (*Solanum sp.)*, radish (*Raphanus sativus*), knol-khol (*Brassica caulorapa L.) a*nd roselle leaves (*Hibiscus acetosella*) were purchased from the local market of Tezpur, Assam. All the vegetables were sorted, washed properly before use and cut into uniform pieces. Each vegetable batch was divided into four equal portions. One portion was retained as raw, and the remaining three were subjected to cooking treatments of boiling, steaming and microwave cooking, respectively.

COOKING TREATMENTS

The vegetables were subjected to three cooking treatments- conventional boiling, steaming and microwave cooking. Prior to choosing the best cooking time for the vegetables, the individual vegetables were cooked for different times and the best cooking time was determined by taking into consideration the surface appearance and tender texture felt both by fingers and teeth. The cooking conditions for each treatment are given in Table 1. Immediately after cooking, the vegetables were cooled in an ice bath to stop the process of cooking and then stored at -20^0 C until analysis for phytochemicals and antioxidant activities.

Table 1- Cooking treatments and cooking time for vegetables

Sl. No.	Vegetable species	Cooking time(min)		
		Steaming	Microwaving (600 W)	Boiling
1.	Cauliflower	8	8	9
2.	Cabbage	7	7	5
3.	Green pea	5	5	6
4.	Banana blossom	5	7	8
5.	Beetroot	7	9	8
6.	Teasle gourd	8	4	10
7.	Black eyed pea	6	5	7
8.	Bottlegourd	5	5	6
9.	Tomato	3	2	3
10.	Carrot	3	3	5
11.	*Kharua* brinjal	4	3	4
12.	Radish	5	4	5
13.	Knol-khol	5	4	5
14.	Roselle leaves	3	3	4

BOILING

Vegetables were added to boiling water in a covered stainless steel container (1:2 sample/water) and cooked. Excess water was drained.

STEAMING

Vegetables were cooked in steam using an autoclave (Equitron Model 7407ST, India) under atmospheric pressure (760 mmHg).

MICROWAVE COOKING

The vegetables were cooked in a microwave oven (Samsung model) at 600W power level with water (1:1 sample/water).

SAMPLE EXTRACTION

For the determination of phytochemicals and antioxidant activities, fresh raw and cooked vegetables were extracted in 80% acetone solvent. One gram of sample was homogenized and then extracted in 10 mL of solvent at 20^o C for 90 min at 200 rpm and then centrifuged (Hettich centrifuge, Germany) at 970 x g (Atala *et al*., 2009). The extracted supernatants were analyzed for total phenolics content, total flavonoid content, ferric reducing antioxidant potential, DPPH radical scavenging activity and metal chelation activity.

PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITIES

DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)

Total phenolic content in fruit extracts were assessed using a modified version of the Folin–Ciocalteu assay (Slinkard and Singleton, 1977). Gallic acid was used as a standard and the aqueous gallic acid solution (500 mg L^{-1}) was diluted with distilled water to give appropriate concentrations for a standard curve. For the analysis, 20µL each of fruit extract, gallic acid standard or blank were taken in separate test tubes and to each 1.58 mL of distilled water was added, followed by 100µL of Folin–Ciocalteu reagent, and content was mixed well. Within 8 min, 300 µL of sodium carbonate was added. The samples were vortexed immediately and the tubes were incubated in the dark for 30 min at 40˚C. The absorbance was then measured at 765 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400). The results were expressed in mg $GAE100^{-1}g$.

DETERMINATION OF TOTAL FLAVONOID CONTENT (TFC)

The flavonoid content was determined by aluminium trichloride method (Chang *et al*., 2002). Briefly, 0.5 mL of the extract was mixed with 1.5 mL of 95% ethanol, 0.1mL of 10% aluminum trichloride ($AlCl_3$), 0.1 mL of 1M potassium acetate, and 2.8 mL of deionised water. After incubation at room temperature for 40 min, the absorbance of reaction mixture was measured at 415 nm against deionised water taken as blank in a UV-Vis spectrophotometer (Cecil, Aquarius 7400). Results were expressed as quercetin equivalent (mgQE $100^{-1}g$) of sample.

DETERMINATION OF FERRIC REDUCING ANTIOXIDANT POTENTIAL (FRAP)

FRAP activity of the samples was measured by the method of Benzie and Strain (1996). Briefly, a 40 µL an aliquot of sample extract not properly diluted fruit extract was mixed with 3.0mL of FRAP solution. The reaction mixture was incubated at 37˚C for 4 min and the absorbance was determined at 593 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400) against a blank that was prepared using distilled water. FRAP solution was pre warmed at 37˚C and prepared freshly by mixing 2.5 mL of a 10 mM 2,4,6-TPTZ [2,4,6-tri(2-pyridyl)-1,3,5-triazine] solution in 40mM hydrochloric acid with 2.5 mL of 20mM ferric chloride and 25 mL of 0.3M acetate buffer (pH 3.6). A calibration curve was prepared, using an aqueous solution of ferrous sulfate (1-10 mM). FRAP values were expressed as µM of ferrous equivalent Fe (II) 100^{-1}g of sample.

DETERMINATION OF DPPH RADICAL SCAVENGING ACTIVITY

Radical scavenging activity of the fruit extracts was measured by determining the inhibition rate of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical (Brand-Williams, Cuvelier and Berset, 1995). Precisely, 100 µL of extracts were added to 1.4 mL of 10^{-4} M DPPH radical methanolic solution. The absorbance at 517 nm was measured at 30 min against blank (100 µL methanol in 1.4 mL of DPPH radical solution) using a UV-Vis Spectrophotometer (Cecil Aquarius 7400). The results were expressed in terms of radical scavenging activity using the following equation:
Radical scavenging acitivity (%) = $[(A_o-A_s)/ A_o] \times 100$. Where, A_o is absorbance of control blank, and A_s is absorbance of sample extract.

DETERMINATION OF METAL CHELATING CAPACITY (MCC)

Metal chelating capacity was determined based on the given method (Dinis *et al.,* 1994). For the estimation, 1.0 mL of 0.125 mM $FeSO_4$ (ferrous sulphate), and 1.0 mL of 0.3125 mM Ferrozine were mixed with 0.2 mL sample. The mixture was allowed to equilibrate for 10 min at room temperature and the absorbance at 562 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400) was recorded. The control contained all the reaction reagents except the extract. Decreased absorbance of the reaction mixture indicated increased activity.
Chelation activity [%] = $[(A_o-A_s)/ A_o] \times 100$
Where, A_o is absorbance of control blank, and A_s is absorbance of sample extract

STATISTICAL ANALYSIS

All experiments were carried out in triplicates and presented as mean ± standard deviation of mean (SEM). Using SPSS version 20, TPC, TFC, FRAP, DPPH and MCC data were statistically analyzed by one way ANOVA followed by Duncan's multiple range tests at $P \leq 0.05$ significance level.

RESULTS AND DISCUSSION

TOTAL PHENOLIC CONTENT (TPC)

The cooking treatments caused significant changes in the total phenolic content in the selected vegetables (Table 2). But cooking processes were not always detrimental to the phytochemical properties. It depended in some cases on the method used and species considered for cooking (Boari *et al.,* 2013). Steaming of banana blossom and cauliflower floret caused an increase in TPC value but had a negative effect on microwave and boiling treatments. Similarly, in beetroot and teasle gourd, steaming and boiling had a positive effect on the phenolics content compared to raw samples. In black eyed pea, while steaming drastically reduced TPC, boiling was found to increase it. Likewise, cabbage showed an increased TPC on steaming and microwave cooking. Moreover, the remaining vegetables viz. tomato, *kharua* brinjal, knol-khol and carrot exhibited increased TPC during all the three methods of cooking. However, not all the samples followed an increasing effect on phenolics upon cooking. Green pea, bottle gourd, radish and roselle leaves showed negative effect of thermal treatments on their TPC values. Most importantly, among the studied vegetables, banana blossom (5481.48 mg GAE/100^{-1}g), beetroot (1063.89 mg GAE/100^{-1}g), teasle gourd (1166.67 mg GAE/100^{-1}g), black eyed pea (2059.52 mgGAE/100^{-1}g), *kharua* brinjal (1516.13 mg GAE/100^{-1}g) and roselle leaves (3118.11 mg GAE/100^{-1}g) were found to be rich in TPC and could be exploited for their phenolic content in the food industries and should be included in the diet as a good source of phenolic compounds.

Table 2 - Total phenolics content (mg GAE/100^{-1}g DW) in acetone extracts of raw and cooked vegetables

Samples	Raw	Steamed	Microwaved	Boiled
Banana blossom	5481.48 ± 0.29^{c}	6070.00 ±0.21^{d}	5100.00 ± 0.28^{b}	2320.51 ± 0.21^{a}
Cauliflower floret	583.33 ± 0.12 ^{c}	684.68 ± 0.29 ^{d}	209.09 ± 0.17 ^{a}	446.33 ± 0.29^{b}
Beetroot	1063.89 ± 0.19^{b}	2003.03± 0.11 ^{d}	866.67 ± 0.15^{a}	1434.27 ± 0.22^{c}
Green pea	184.06 ± 0.11^{d}	110.10± 0.07^{b}	144.76 ± 0.15^{c}	105.21 ± 0.16^{a}
Teasle gourd	1166.67± 0.18^{b}	1230.77±0.15^{c}	1146.67 ± 0.17^{a}	1912.12 ± 0.19^{d}
Black eyed pea	2059.52± 0.22^{c}	1420.37± 0.27^{a}	1878.79± 0.18^{b}	2381.82± 0.15^{d}
Cabbage	266.64±0.23b	567.14±0.29d	272.07±0.11c	250.00±0.09a
Bottlegourd	406.25±0.29d	319.15±0.27a	393.94±0.19c	386.36±0.29b
Radish	837.50±0.16d	647.73±0.23c	493.51±0.22b	337.35±0.17a
Tomato	443.66±0.11a	633.33±0.19d	577.59±0.07c	485.50±0.17b

Kharua brinjal	1516.13±0.12a	2449.44±0.11c	2617.65±0.34d	1623.29±0.11b
Knol-khol	199.47±0.22a	386.36±0.23c	564.36±0.13d	340.52±0.23b
Carrot	206.52±0.31a	326.61±0.23c	508.47±0.11d	253.33±0.27b
Roselle leaves	3118.11±0.17d	2178.57±0.25c	1723.14±0.33b	1487.80±0.29a

**- Means with the same letter within row are not significantly different at P≤0.05 by DMRT. Superscript of DMRT describes significant difference between the treatments

TOTAL FLAVONOID CONTENT (TFC)

Cooking had both positive and negative effects on TFC depending on the type of vegetables (Table 3). Banana blossom, cauliflower, green pea, black eyed pea, bottlegourd and roselle leaves exhibited a lowering trend while, knol-khol, cabbage, tomato and carrot showed increased TFC values upon cooking. Apart from that, beetroot showed an increased TFC in steamed and boiled samples. Radish also showed maximum increase in steamed sample although microwaved and boiling treatment led to destruction of flavonoids. Lastly, in *kharua* brinjal, boiling caused lowering of TFC but steaming and microwave cooking had a positive effect.

Table 3 - Total flavonoid content (mgQE/100^{-1}g DW) of acetone extracts of raw and cooked vegetables

Samples	Raw	Steamed	Microwaved	Boiled
Banana blossom	359.26± 0.10^{d}	180.83± 0.13^{b}	273.96± 0.13^{c}	67.31± 0.15^{a}
Cauliflower floret	482.46 ± 0.11^{d}	102.48 ± 0.09^{b}	87.12 ± 0.13^{a}	142.66 ± 0.18^{c}
Beetroot	200.69±0.19^{b}	358.33± 0.17^{d}	137.91± 0.10^{a}	303.99± 0.16^{c}
Green pea	32.97 ± 0.11^{d}	26.77± 0.11^{c}	19.05± 0.11^{a}	23.18± 0.11^{b}
Teasle gourd	87.03 ± 0.06^{c}	74.36 ± 0.11^{b}	41.67 ± 0.03^{a}	87.88 ± 0.15^{c}
Black eyed pea	495.83± 0.20^{d}	169.44± 0.19^{a}	293.18± 0.19^{c}	246.59± 0.10^{b}
Cabbage	35.14±0.19^{a}	85.45±0.25^{d}	69.88±0.19^{c}	46.41±0.17^{b}
Bottlegourd	125.00±0.12^{d}	58.51±0.22^{b}	45.45±0.32^{a}	73.86±0.13^{c}
Radish	45.31±0.17^{b}	60.61±0.11^{c}	19.48±0.14^{a}	22.59±0.05^{a}
Tomato	112.68±0.13^{a}	216.67±0.24^{c}	213.36±0.19^{c}	182.97±0.21^{b}
Kharua brinjal	446.24±0.08^{b}	529.49±0.34^{c}	527.57±0.11^{c}	375.00±0.09^{a}
Knol-khol	15.29±0.25^{a}	40.72±0.11^{c}	45.79±0.22^{d}	35.56±0.21^{b}
Carrot	40.76±0.23^{a}	81.65±0.22^{c}	133.47±0.13^{d}	59.17±0.29^{b}
Roselle leaves	269.75±0.20^{c}	190.63±0.34^{b}	116.75±0.12^{a}	109.50±0.23^{a}

**-Means with the same letter within row are not significantly different at P≤0.05 by DMRT. Superscript of DMRT describes significant difference between the treatments

Application of heat during cooking involves changes in the structural integrity and cellular matrix of the vegetables and this causes both positive and negative effects on the phytochemical properties. It was observed that cooking caused a significant change in the phenolic and flavonoid content in the selected vegetables. Usually, thermal treatments have destructive effect on the flavonoid and phenolic compounds as they are highly unstable compounds (Ismail, Marjan and Foong, 2004). The black eyed pea and roselle leaves which has anthocyanin, a class of flavonoid as its major pigment showed decreased TPC and TFC upon cooking as these are heat labile. But again, the pattern of change in phenolics depends on the severity of the heat treatments, exposure to the air, light, leaching of soluble phenolics (Pellegrini *et al.*, 2009), the bioactive structures of the studied vegetables, the cutting, chopping and cooking method, bioavailability and heat stability of the present phenolics (Sultana, Anwas and Iqbal, 2008). Moreover, heat treatment usually leads to inactivation of the polyphenol oxidase and other oxidising enzymes which in turn slows down the phenolic destruction by oxidation on exposure to the surrounding environment (Yamaguchi *et al*., 2003). In some cases, an increasing trend in phenolic and flavanoid content was observed upon thermal treatment. These could be due to breakdown of the cellular matrix which helped in the binding of the total phenolics with pectin or cellulose networks and making them more extractable into the solvents. Moreover, in some instances, application of heat could cleave the phenolic-sugar glycosidic bonds resulting in the formation of phenolic aglycons, which has high reactivity with Folin Ciocalteau reagent and thus leads to an increased value of total phenolic content (Singleton, Orthofer and Lamuela-Raventos, 1999). Also cooking could lead to decomposition of some polyphenols bound to dietary fibre of vegetables releasing free phenolic compounds that increase their detection (Stewart *et al.*, 2000).

Apart from that, the phenolics can be hydrophilic or lipophilic depending on their solubility pattern. The overall difference in the results of the total phenolics and flavonoids of the selected vegetables could be due to the presence of different phenolic groups in the vegetables and their susceptibility to change or destruction during the three cooking treatments (Bernhardt and Schlich, 2006). Cooking treatments altered the TPC and TFC of the vegetables although the direction of change and extent of change was not uniform across all vegetables and across all treatments.

CHANGES IN ANTIOXIDANT CAPACITY OF COOKED VEGETABLES

FERRIC REDUCING ANTIOXIDANT POTENTIAL (FRAP)

The vegetables showed varied results for ferric reducing antioxidant potential upon cooking compared to the raw uncooked vegetables. The FRAP values upon cooking showed an increased and positive effect on beetroot, green pea, black eyed pea, radish, tomato, *kharua* brinjal and knol-khol for all the three cooking treatments. Banana blossom showed high FRAP value in microwaved and boiled samples but low value in steamed blossom. Cabbage showed increased value during steam cooking. Steamed carrot retained the FRAP value found in raw but exhibited an increase in microwaved and boiled samples. Likewise, cauliflower floret showed no significant change in FRAP on steaming. However, in the remaining vegetables viz. teasle gourd, bottlegourd and roselle leaves a decrease in FRAP value was observed.

Table 4 - Ferric reducing antioxidant potential (µM Fe (II)/100^{-1}g) of acetone extracts of raw and cooked vegetables

Samples	Raw	Steamed	Microwaved	Boiled
Banana blossom	16319.44± 0.33^{b}	14956.27± 0.13^{a}	39570.47± 0.19^{c}	55825.62± 0.29^{d}
Cauliflower floret	3944.32± 0.13^{c}	3941.32± 0.23^{c}	2725.99± 0.19^{a}	2756.46± 0.11^{b}
Beetroot	4480.72± 0.13^{a}	7215.18± 0.15^{c}	7892.03± 0.17^{d}	7154.27± 0.27^{b}
Green pea	417.67± 0.04^{a}	603.86± 0.13^{b}	649.15± 0.11^{c}	1036.63± 0.10^{d}
Teasle gourd	3665.12± 0.27^{d}	2864.58± 0.15^{b}	3067.13± 0.19^{c}	2372.69± 0.25^{a}
Black eyed pea	7155.26± 0.31^{a}	21192.96 ± 0.12^{c}	16319.44± 0.29^{b}	22656.25± 0.33^{d}
Cabbage	1512.93± 0.28^{c}	2235.45±0.12^{d}	425.75±0.14^{a}	1103.12±0.28^{b}
Bottlegourd	3356.72±0.17^{d}	1548.19±0.19^{a}	3018.75±0.29^{c}	2913.75±0.19^{b}
Radish	1862.44±0.11^{a}	3176.25±0.39^{d}	2182.50±0.29^{c}	2066.48±0.11^{b}
Tomato	2151.80±0.17^{a}	4652.78±0.23^{c}	3621.89±0.11^{b}	5308.98±0.27^{d}
Kharua brinjal	8923.32±0.26^{a}	19505.24±0.37^{c}	22165.83±0.32^{d}	14049.88±0.41^{b}
Knol-khol	331.76±0.11^{a}	1561.88±0.33^{c}	2984.71±0.42^{d}	1209.76±0.26^{b}
Carrot	1148.72±0.21^{a}	1131.71±0.35^{a}	2995.17±0.17^{c}	1755.60±0.37^{b}
Roselle leaves	4482.64±0.27^{d}	3281.25±0.23^{c}	2434.03±0.39^{b}	1906.25±0.21^{a}

**-Means with the same letter within row are not significantly different at P≤0.05 by DMRT. Superscript of DMRT describes significant difference between the treatments

DPPH RADICAL SCAVENGING ACTIVITY

The DPPH radical scavenging effect of the selected vegetables was affected significantly during the cooking treatments. Banana blossom retained the DPPH activity in boiled sample but the activity increased on steaming (92.89%). Similarly, black eyed pea and knol khol showed increased activities during microwave cooking but steaming had a destructive effect. In *kharua* brinjal, maximum increase in DPPH activity was observed in steamed samples (85.50%) compared to raw (47.79%).

The remaining vegetables viz. cauliflower florets, beetroot, teasle gourd, green pea, bottle gourd and carrot showed increase in radical scavenging activity on cooking while a decrease in activity was observed in cabbage, radish, roselle leaves and tomato. There was decrease in activity in tomato on cooking compared to raw; however, there was no significant difference between the treatments. The DPPH activity was maximum in banana blossom (above 90% activity) and black eyed pea (above 75% activity) followed by kharua brinjal (above 45% activity) on cooking by the three treatments.

Table 5 - DPPH activity (%) of acetone extracts of raw and cooked vegetables

Samples	Raw	Steamed	Microwaved	Boiled
Banana blossom	91.19± 0.31^{b}	92.89± 0.21^{c}	90.27± 0.18^{a}	91.45± 0.26^{b}
Cauliflower floret	7.30± 0.17^{a}	19.53± 0.18^{c}	11.77± 0.19^{b}	11.00± 0.12^{b}
Beetroot	24.96± 0.23^{a}	51.38± 0.30^{d}	35.21± 0.26^{b}	41.48± 0.27^{c}
Green pea	7.35± 0.07^{a}	10.30± 0.16^{c}	10.23± 0.11^{c}	8.51± 0.17^{b}
Teasle gourd	0.33± 0.03^{a}	19.10± 0.28^{b}	35.52± 0.37^{c}	48.65± 0.30^{d}
Black eyed pea	91.06± 0.11^{c}	75.82± 0.23^{a}	80.43± 0.27^{b}	91.34± 0.25^{c}
Cabbage	30.62± 0.18^{d}	6.16± 0.14^{c}	3.59± 0.12^{b}	2.37± 0.13^{a}
Bottlegourd	3.77±0.09^{a}	25.58±0.15^{d}	18.17±0.13^{b}	22.03±0.16^{c}
Radish	21.72±0.07^{d}	2.86±0.05^{b}	0.52±0.03^{a}	3.96±0.06^{c}
Tomato	16.18±0.10^{a}	44.31±0.15^{b}	44.78±0.13^{b}	43.49±0.19^{b}
Kharua brinjal	47.79±0.18^{a}	85.50±0.21^{c}	61.22±0.21^{b}	44.58±0.39^{a}
Knol-khol	4.79±0.10^{c}	1.72±0.03^{b}	6.93±0.20^{d}	0.86±0.05^{a}
Carrot	3.87±0.15^{a}	11.06±0.13^{d}	7.30±0.09^{c}	6.23±0.22^{b}
Roselle leaves	64.15±0.21^{d}	48.79±0.22^{c}	34.26±0.09^{a}	41.31±0.09^{b}

** Means with the same letter within row are not significantly different at P≤0.05 by DMRT. Superscript of DMRT describes significant difference between the treatments

METAL CHELATING CAPACITY (MCC)

The MCC values of the raw and cooked vegetable samples are given in Table 6. Cooking caused an increase in MCC in banana blossom in all the three cooked forms. Green pea and radish retained MCC in boiled samples, while an increase in steamed and microwaved samples was observed. Likewise, black eyed pea and carrot showed an increased activity in microwaved samples but showed no change in steamed and boiled vegetables except in carrot where a decrease was observed on boiling. Similarly, steaming had a positive effect on bottle gourd and knol khol. However, a decrease in activity was observed in tomato, *kharua* brinjal and roselle during steaming and boiling. Teasle gourd showed reduction in MCC on cooking. Therefore, depending on the type of vegetables and cooking method, the MCC values varied. In majority of the vegetables, MCC activity was retained or was enhanced.

Table 6 - MCC (%) values of acetone extracts of raw and cooked vegetables

Samples	Raw	Steamed	Microwaved	Boiled
Banana blossom	6.08± 0.12^{a}	7.35± 0.13^{b}	8.20± 0.08^{c}	9.23± 0.10^{d}
Cauliflower floret	5.79± 0.15^{b}	5.89± 0.16^{b}	5.17± 0.12^{a}	4.97± 0.14^{a}
Beetroot	n.d	n.d	n.d	n.d
Green pea	6.26± 0.12^{a}	8.36± 0.15^{c}	7.28± 0.13^{b}	6.60± 0.17^{a}
Teasle gourd	14.42± 0.07^{c}	7.34± 0.13^{b}	7.43± 0.15^{b}	3.51± 0.10^{a}
Black eyed pea	2.72±0.10^{a}	2.11± 0.09^{a}	4.61± 0.10^{b}	3.18± 0.14^{a}
Cabbage	7.39± 0.19^{b}	6.28± 0.10^{a}	7.55± 0.17^{b}	5.98± 0.15^{a}
Bottlegourd	3.89±0.07^{b}	5.73±0.11^{c}	2.28±0.10^{a}	7.06±0.10^{d}
Radish	3.18±0.05^{a}	8.28±0.10^{c}	6.55±0.09^{b}	3.69±0.10^{a}
Tomato	4.66±0.08^{c}	2.63±0.04^{a}	4.86±0.10^{c}	3.26±0.16^{b}
Kharua brinjal	4.46±0.07^{b}	3.14±0.04^{a}	5.14±0.10^{c}	3.37±0.09^{a}
Knol-khol	4.08±0.09^{c}	5.75±0.04^{d}	1.92±0.07^{a}	2.76±0.11^{b}
Carrot	4.29±0.09^{b}	4.60±0.10^{b}	5.37±0.06^{c}	2.22±0.10^{a}
Roselle leaves	6.11±0.20^{b}	4.88±.06^{a}	7.43±0.02^{c}	5.03±0.10^{a}

* * Means with the same letter within row are not significantly different at P≤0.05 by DMRT. Superscript of DMRT describes significant difference between the treatments, **** n.d- not detected

The possible reason for the above results could be that there are many hundreds of different phytochemicals present in food, and each has different characteristics of reacting to the changes in their cellular matrix caused by heat treatments or cooking. This could lead to an increase or decrease in the antioxidant activities of the vegetables. The phenolic content and antioxidant activity have a strong relationship between them (Velioglu *et al.,* 1998) and thus phenolic content is a significant factor in most of the cases for increase or decrease in antioxidant activity. In beetroot, tomato, *kharua* brinjal, knol khol, and carrot an increase in both TPC and antioxidant activity was observed in most of the cases with some exceptions. The increase in antioxidant activity in tomato could be due to the increased bioavailibilty and accessibility of its lycopene content. In some cases, increase in antioxidant activity was observed due to transformation of phytochemicals into more active compounds like deglycosylation of some flavonoids. Other factors like polymerization of polyphenols during cooking may result in higher antioxidant activities (Nicoli, Anese and Parpinel, 1999). During steaming increase in antioxidants activity for all the selected vegetables with the exception of one or two vegetables was observed. This effect was perhaps due to production of redox-active secondary plant metabolites or breakdown products, but is highly likely to be related to release of antioxidants from intercellular proteins, changes in plant cell wall structure and matrix modification (Rechkemmer, 2007). Apart from these, inactivation of oxidative enzymes which are responsible for increase in oxidation of phenolic compounds could lead to an increased activity. Moreover, enhanced antioxidant activity could also be witnessed due to the production of novel compounds due to Maillard reaction (Morales and Babel, 2002).

CONCLUSIONS

The results presented here clearly show that cooking can make the phenolics and antioxidants of cooked vegetables quite different from that of uncooked form. This is probably due to variety of effects like destruction, release and transformation of the phytochemicals. Cooking enhanced the antioxidant activity of the selected vegetables in most of the cases. Overall, steaming was the most preferred method for cooking. But, in case of cooked banana blossom and black eyed pea, a decrease in flavonoid content was observed. Among the vegetables, banana blossom, beetroot, teasle gourd, black eyed pea, *kharua* brinjal and roselle leaves were found to be rich in TPC and antioxidant properties. Therefore, from the above discussed results, it could be inferred that cooking had both positive and negative impact on the phytochemicals and antioxidant activities on the vegetables. In most cases cooking increased the release of phenolics into the extraction medium and among the three cooking methods employed steaming emerged as the most suitable method followed by microwave cooking in most cases.

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