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Effect of cooking methods on bioactive compounds in vegetables

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Abstract
Vegetables are very rich in health-promoting phytochemicals like flavonoids and phenolic compounds. The objective of the study was to investigate the effect of commonly used cooking (i.e., open and pressure cooking) methods on phytochemical content (i.e., polyphenols, flavonoids) and antioxidant activity levels as measured by DPPH and FRAP assay. Results showed that cooking methods has a great effect on phytonutrients and caused significant losses of flavonoids and increase in phenolics and antioxidant activity levels when compared with raw vegetables. Bitter gourd scored highest mean value, 1033.8 mg GAE/100g, and drumstick scored the lowest mean value, 222.3 mg GAE/100g, for total phenolic content. The flavonoid content ranged between 14.18 to 91.70 mg RE/100g and both the cooking methods decreased the flavonoid content in all the selected vegetables. Antioxidant activity levels were increased significantly ($p < 0.01\%$ and $P<0.05\%$) in all the vegetables upon both open and pressure cooking methods.

Keywords: Vegetables; bioactive compounds; phytochemicals; open cooking and pressure cooking; FRAP; AOA; DPPH; phenols; flavonoids; antioxidants

Introduction

Vegetables contain dietary antioxidants, such as water-soluble vitamin C and phenolic compounds, as well as lipid soluble vitamin E and carotenoids, contribute to both the first and the second defense lines against oxidative stress. As a result, the consumption of vegetables may protect humans from chronic angiogenic diseases, such as cardiovascular diseases, chronic inflammation, arthritis, and cancer (Middleton *et al*., 2000 [12]; Saleem *et al*., 2002 [17]; Prior, 2003 [13]; Zhang *et al*., 2005 [26]; Chen *et al*., 2005 [4]). The phenolic metabolites, including tocopherols, flavonoids, phenolic acids, alkaloids, chlorophyll derivatives, or carotenoids (Hudson, 1990 [7]; Hall and Cuppett, 1997 [6]), possess high antioxidant capacity and have significant health benefits (McDermott, 2000) [11]).

The previous studies have demonstrated that phenols and flavonoids contribute to a higher extent than vitamin C, carotenoids, and others to the antioxidant capacity of fruits and vegetables. Most of the studies proved that, higher concentrations of vitamin C in fruits contribute only 10–20% of the total antioxidant capacity. However, low concentrations of vitamin E in fruits contribute significantly higher antioxidant capacity than the vitamin C.

The fruits are mostly consumed in raw form, but vegetables need to be cooked to enhance their palatability and taste. However, cooking brings about a number of physical and chemical changes in the vegetables (Rehman, Islam and Shah, 2003) [14]. These changes could be both beneficial and detrimental depending on the extent and type of treatment conditions. The cooking treatments like boiling, microwaving, baking, frying and griddling lead to changes in texture and nutritional properties of the vegetables (Zhang and Hamauzu, 2004) [25]. Studies have reported that cooking softens the cell walls which lead to increase in the extraction of carotenoids (Rodriguez-Amaya, 1999) [15]. However, other studies have reported that cooking can also lead to loss in essential vitamins, antioxidants, water soluble and heat labile compounds. The extent of loss is dependent on the type of cooking treatment (Lin and Chang, 2005 [9]) and the phytochemical compositions of the cooked vegetable (Sangeeta and Charu 2013) [18]. Cooking processes may have an effect on the antioxidant content of food due to antioxidant release, destruction, or creation of redoxactive metabolites (Wachtel-Galor *et al*., 2008) [23]. Antioxidant compounds, such as ascorbic acid and some carotenoids, are very sensitive to heat and storage. Conversely, polyphenols have shown certain stability when exposed to high temperatures (Faller and Fialho, 2009) [5].

Previous studies conducted on different vegetables showed that, after cooking, total polyphenol content and antioxidant activity of samples can be higher or lower compared to fresh vegetables. For example, Faller and Fialho, 2009 [5] showed that cooking decreased antioxidant capacity for most of the vegetables and small differences found between the cooking methods applied. Wachtel-Galor *et al.,* 2008 [23] reported that the antioxidant content was lower in microwaved samples and was followed by boiled and steamed samples and decreased with longer cooking time, regardless of the method in all cooked vegetables. Antioxidant content increased for all steamed vegetables over that of raw vegetables. Effects were variable for boiling and microwaving. Boiling caused lesser antioxidant loss in cooking than did microwaving. Turkmen *et al.*, 2005 [22] reported that after cooking, total antioxidant activity increased or did not change depending on the type of vegetable but not type of cooking. Zhang and Hamauzu, 2004 [25] pointed out that antioxidant components and antioxidant activity in broccoli samples were lost quickly during cooking.

Based on the above facts, the present study was carried out with the objective to investigate the effect of common domestic cooking processes; open cooking and pressure cooking on phenolics, flavonoids and total antioxidant capacity of commonly grown and consumed vegetables in Telangana state, India.

Materials and Methods

Procurement of Samples

The commonly consumed vegetables like beans (Phaseolus vulgaris), bittergourd (Momordica charantia), cluster beans (Cyamopsis tetragonoloba), and drumstick (Moringa oleifera), were purchased from local markets of the AICRP adopted villages in Moinabad Mandal, Rangareddy district, Telangana state, India, during the rainy season.

Processing Treatments

Open Cooking: The edible portion of vegetables was placed into a stainless steel pan with 1000 ml of boiling water (100 ∘C) and covered with a lid. The boiling was continued till vegetables were cooked. The cooked vegetables were cooled rapidly on plenty of ice.

Pressure Cooking: The edible portion of vegetables was placed in a pressure cooker, containing water (1000ml) and cooked till first whistle on a pressure valve. The pressure was slowly released and the cooked samples were cooled rapidly on plenty of ice.

Extraction of the sample

80% methanol acidified with 6N hydrochloric acid (pH 2.0) was used as solvent for extracting the samples from the vegetables.

Analysis of bioactive components

Total phenolic content (Singleton *et al* 1999) [19], flavonoids (Zhishen *et al*., 1999) [27], and antioxidant activity properties, (1-diphenyl-2-picrylhydrazyl (DPPH) radical-Scavenging Activity, (Tadhani *et al*. 2007) [21] and Ferric reducing antioxidant power (FRAP) (Benzie and Strain 1999) [2] were estimated in the sample extracts using standard analysis procedures.

Statistical Analysis

The data was presented as means ± standard deviation. Differences between variables were tested for significance using ANOVA test, followed by multiple comparisons.

Total phenolic content of selected vegetables

Table 1 indicates that bitter gourd scored highest mean value, 1033.8 mg GAE/100g and drumstick scored the lowest mean value, 222.3 mg GAE/100g for total phenolic content in fresh or raw form among the vegetables selected. The ranking of vegetables for the total phenolic content in raw form was bitter gourd> cluster bean>beans>drum stick.

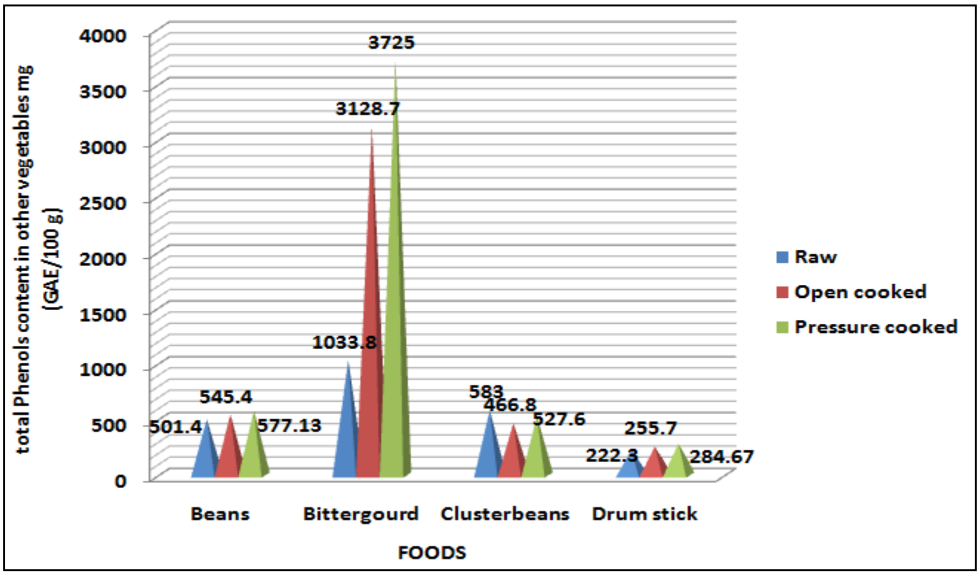
The results showed increase in total phenolic content in open and pressure cooking treatments in all the vegetables studied with 8.77% and 15.09% in beans, 202.62% and 260.30% in bitter guard, and 15.07%, 28.10% in drumsticks, whereas decrease in phenolic content was observed in cluster beans (-19.97, -9.55%). The increase in total phenolic content was ranged from 8.77% to 202.6% in open cooking while in pressure cooking it was from 28.10% to 260.30% in selected vegetables.

Table 1: Total phenolic content of selected vegetables (mg GAE/100 g)

S. No	Foods	Raw (Control)	Open cooked (T1)	% Change	Pressure cooked (T2)	% Change	F-value
1	Beans	501.4(±0.019)	545.4(±1.98)	8.77	577.13(±2.02)	15.09	1874.5**
2	Bitter gourd	1033.8(±2.27)	3128.7(±1.9)	202.62	3725(±2.4)	260.30	1569440**
3	Cluster beans	583.0(±2.42)	466.8(±3)	-19.97	527.6(±2.3)	-9.55	1972.7**
4	Drumstick	222.3±0.02	255.7(±1.7)	15.07	284.67(±2.03)	28.10	936.6**

The TPC content of selected vegetables within the group and between the treatments (raw (C), open (T_1) and pressure cooking (T_2)) showed significant increase at 1% level.

In India, open cooking and pressure cooking methods are popularly used means for preparation of recipes with vegetables. This study indicated that the different food processing methods did not affect the phenolic compounds in the vegetables. Phenolic compounds in vegetables are present both in soluble forms and combined with cell-wall complexes. Studies performed on different vegetables after cooking showed that the total polyphenol content and antioxidant capacity could be either higher or lower in comparison to fresh food. The cooking treatment usually causes significant changes in the total phenolic content in the vegetables (Saikia and Mahanta, 2013) [16]. The dissimilarity may have been due to the differences in the extraction and cooking methods. The loss could be due to phenolic breakdown during cooking (Turkmen, Sari, and Velioglu, 2005) [22]. The gain could be to decomposition of some polyphenols bound to dietary fiber of vegetables releasing free phenolic compounds that increase their detection (Stewart, Bozonnet *et al.*, 2000) [20]. Yamaguchi *et al*., (2001) [24] 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 and deactivation of these enzymes avoid the loss of phenolics and, therefore, lead to the increase in total phenolic content. In another study carried out by Ismail, Marjan and Foong, 2004 [8], spinach was found to have the highest TPC, followed by swamp cabbage, kale, shallots and cabbage after cooking like boiling, steaming, microwaving and this increase was not significant.



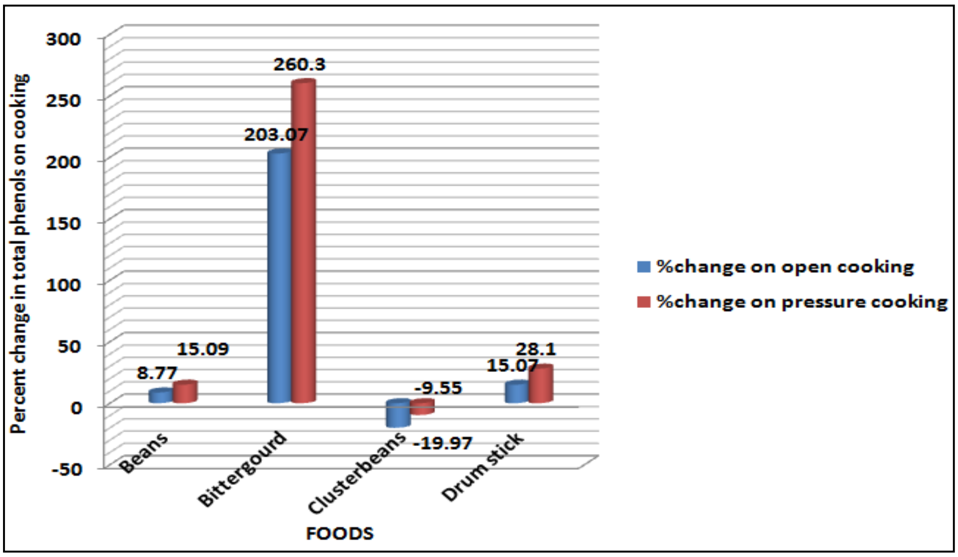


Fig 1: Total phenolic content of selected vegetables mg (GAE/100 g)

Total flavonoid content of selected vegetables

In the selected vegetables, 14.18 - 91.7 mg RE/100g of flavonoid content was observed in raw form. The sequence of selected vegetables (fresh form) in the flavonoid content was Bitter gourd > Beans> Cluster beans> Drumstick. Table 2 revealed that both the cooking methods decreased the flavonoid content in all the vegetables (beans, bitter gourd, cluster beans and drumstick).

Cooking had both positive and negative effects on the flavonoid content depending on the type of vegetables. Usually, thermal treatments have destructive effect on the flavonoid as they are highly unstable compounds (Ismail, Marjan and Foong, 2004) [8]. According to Adefegha and Oboh, 2011 [1], total flavonoids of cooked vegetables were higher in total flavonoid content compared to the total flavonoid content of raw vegetables, indicating a possible release of some flavonoids during cooking of the green leafy vegetables. In another study by Saikia and Mahanta, 2013 [16] beetroot showed an increased TFC in steamed and boiled samples.

In open cooking (T_1) the loss of flavonoids ranged from -26.2% to -53.88% whereas, in pressure cooking (T_2) it was ranged from -8.43% to -36.11%. However, the reduction of flavonoid content was found to be higher in open cooking method ($T_{1)}$ compared to pressure cooking method (T_2).

The total flavonoid content of selected vegetables was compared with in the group and between the treatments, i.e. raw (C), open (T_1) and pressure cooking (T_2). The results showed that the reduction in flavonoids content was at 1% significance difference level in bitter gourd and cluster beans whereas in beans and drumsticks it was at 5% significant difference level.

Table 2: Total flavonoids content of selected vegetables (mg RE/100g)

S. No	Foods	Raw (Control)	Open cooked (T1)	% Change	Pressure cooked (T2)	% Change	F-value
1	Beans	17.17±1.36	10.89±2.36	-36.56	13.84±1.29	-19.35	9.75*
2	Bittergourd	91.7±2.18	41.3±1.52	-53.88	58.6±2.23	-36.11	474.0**
3	Clusterbeans	16.75±1.19	9.33±1.26	-44.29	12.58±1.96	-24.89	18.1**
4	Drum stick	14.18±2.02	10.46±1.86	-26.20	12.99±2.27	-8.43	2.54*

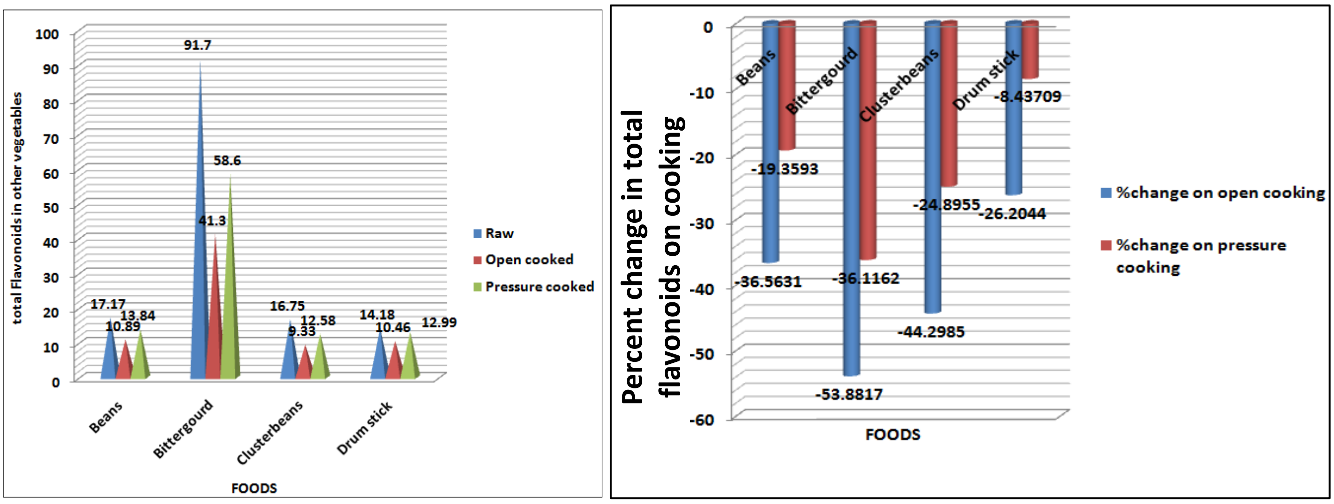


Fig 2: Total flavonoids content of selected vegetables (mg RE/100g)

Total antioxidant activity level of selected vegetables frap method

The sequence of selected vegetables (fresh form) in the flavonoid content was Bitter gourd > Cluster beans > Beans > Drumstick. The mean FRAP value was reduced in cluster beans by -17.11% in open cooking (T_1) and -10.2% in pressure cooking when compared to raw (C). The remaining other vegetables, i.e. beans, bitter gourd and drumstick, the FRAP value was increased at a range of 5.57% to 9.45% in open cooking (T_1), and 12.14% to 23.51% in pressure cooking (Table 3).

Table 3: The total AOA of selected vegetables by FRAP method (mg TE/100g)

S. No	Foods	Raw (Control)	Open cooked (T1)	% Change	Pressure cooked (T2)	% Change	F-value
1	Beans	56.32(±3.01)	59.46(±2.13)	5.57	63.16(±2.6)	12.14	5.17*
2	Bitter gourd	315.76 (±1.46)	345.62(±1.8)	9.45	390(±2.06)	23.51	1302NS
3	Cluster beans	60.84(±1.3)	50.43(±1.8)	-17.11	54.62(±2.86)	-10.22	18.79**
4	Drum stick	53.62(±1.8)	58.96(±1.11)	9.95	61.32(±2.01)	14.36	16.3**

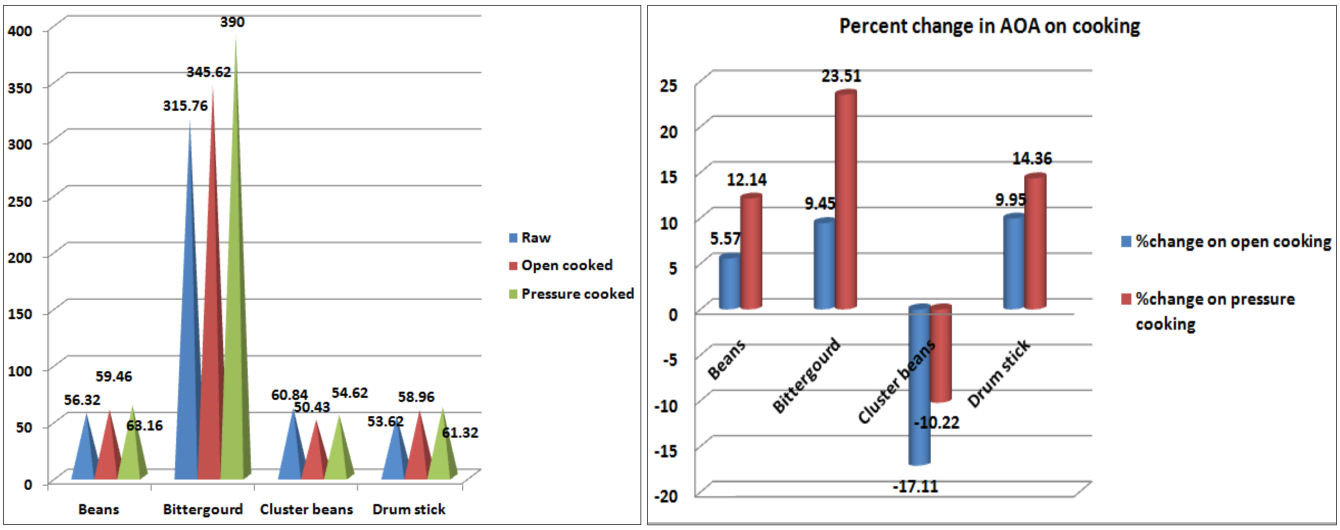


Fig 3: The total AOA level of selected vegetables by FRAP method (mg TE/100g)

The total AOA content of selected vegetables in FRAP method was compared with the group of selected vegetables and between the treatments, i.e. raw (C), open (T_1) and pressure cooking (T_2) and found AOA in FRAP method was decreased in cluster beans and increased in drumsticks and beans at 1% level and 5% level respectively whereas bitter gourd showed no significant difference in total AOA upon cooking.

DPPH Method

The mean values for DPPHRSA in selected vegetables and percent difference between the control and treatment extracts is shown in Table 4 and Fig 4. The mean value of DPPHRSA in selected vegetables in raw form ranged from 69.41 to 92.97 mg TE/100g. The ranking of selected vegetables in AOA by DPPH method was bitter gourd> drumstick> cluster beans> beans. All the selected vegetables showed significant increase in AOA by DPPH method in both the cooked methods (open and pressure cooking) than in raw form. The range of increase in total AOA of selected vegetables in open cooking was ranged between 6.91% and 13.93%, while in pressure cooking it was ranged from 4.18% to 54.8% when compared to raw.

Table 4: The total AOA of selected vegetables by DPPH method (mg TE/100g)

S. No	Foods	Raw (Control)	Open cooked (T1)	% Change	Pressure Cooked (T2)	% Change	F-value
1	Beans	56.89 (±1.56)	64.82(±1.49)	13.93	61.54 (±1.46)	8.17	21.09**
2	Bitter gourd	92.97 (±2.37)	99.4(±1.63)	6.91	143.92 (±1.4)	54.80	673.5**
3	Cluster beans	69.41 (±2.41)	75.73(±2.41)	9.10	96.3 (±2.05)	38.74	112**
4	Drum stick	73.83 (±2.57)	78.35(±0.8)	6.12	76.92(±1.53)	4.18	5.01*

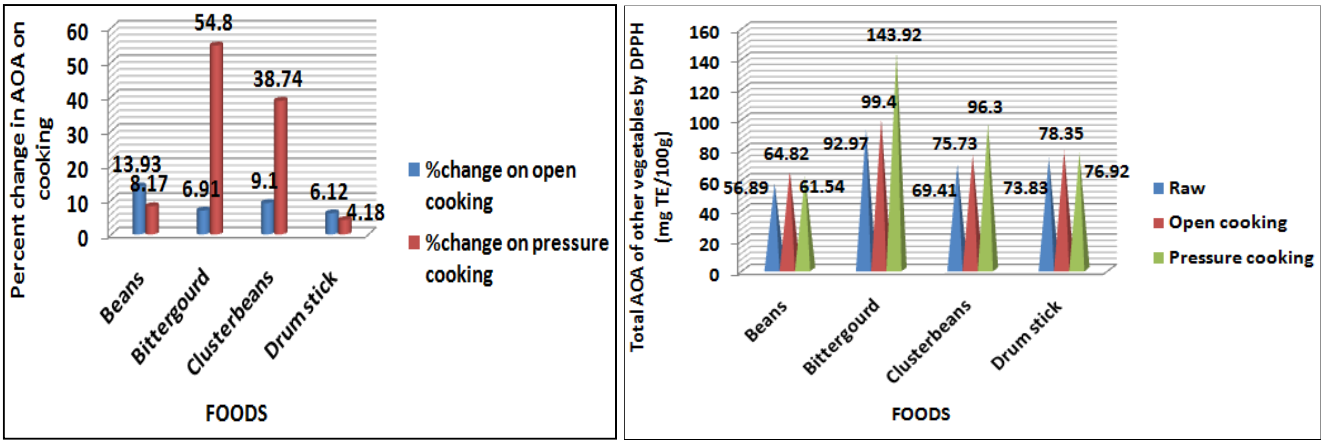


Fig 4: The total AOA of selected vegetables by DPPH method (mg TE/100g)

The total AOA content of selected vegetables in DPPH method was compared with the group of vegetables and between the treatments, i.e. raw (C), open (T_1) and pressure cooking (T_2). The results showed that a significance increase in total AOA content was observed at 1% level in Beans, Bitter gourd and Cluster beans except Drumstick, it showed 5% significant difference level.

Cooking procedures led to an increase in antioxidant activity in vegetables. This effect is 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 intracellular proteins, changes in plant cell wall structure, matrix modifications, and more efficient release of antioxidants during homogenization. Conversely, boiling reduces the antioxidant activity of beet, turnip, red cabbage, white cabbage, and broccoli. Microwave cooking of beet, black radish, red cabbage, broccoli, and white cabbage resulted in a significant reduction (P <0.01) of antioxidant activity when compared to the respective raw state. Stir-frying increased antioxidant activity of red radish, broccoli, and white cabbage. Wachtel-Galor *et al.*, 2008 [23] showed that the effects of cooking on antioxidant capacity of different vegetables may be different. According to Saikia and Mahanta, 2013 [16] beetroot has showed an increase in radical scavenging activity of 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) [22] Beetroot has shown 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 [25] 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 *et al*., 2001) [10]. Ismail, Marjan and Foong, 2004 [8] reported that antioxidant activities of the 1 min boiled vegetables were similar to the fresh ones.

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 of the flavonoid and phenolic compounds as they are highly unstable compounds (Ismail, Marjan and Foong, 2004) [8]. In some cases, an increasing trend in phenolic and flavanoid content was observed upon thermal treatment. These could be due to the 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, the application of heat could cleave the phenolic-sugar glycosidic bonds resulting in the formation of phenolic aglycons, which has a high reactivity with Folin Ciocalteau reagent and thus leads to an increased value of total phenolic content (Singleton *et al.*, 1999 [19]). 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) [2]. 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.

Conclusion

The selected vegetables were found to be rich in total phenols, flavonoids and antioxidant activity levels. It was found that cooking had an impact on the antioxidant activity, total phenolics and flavonoid content. As the flavonoids are highly unstable compounds, application of cooking/heat resulted in decrease in flavonoid content. Cooking had a positive impact on phenolic and antioxidant levels in selected vegetables. Five servings of vegetables per day are recommended by the

ICMR and mostly the vegetables are consumed after cooking. The study highlighted that the antioxidant activity of the vegetables do not significantly affected by the commonly used cooking methods and proved safe for phytonutrients.

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