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EFFECT OF COOKING METHODS ON BIOACTIVE COMPOUNDS IN VEGETABLES

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Keywords:

Vegetables, Bioactive compounds, Phytochemicals, Open cooking, and pressure cooking, FRAP, AOA, DPPH, Phenols, Flavonoids, Antioxidants

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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 have 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 the 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 91.7 mg RE/100g in the selected vegetables. There was a significant increase ($p< 0.01\%$ and $p<0.05\%$) in antioxidant activity levels in vegetables upon both open and pressure cooking.

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 [1, 2, 3, 4, 5]. The phenolic metabolites, including tocopherols, flavonoids, phenolic acids, alkaloids, chlorophyll derivatives, or carotenoids [6, 7], possess the high antioxidant capacity and have significant health benefits [8].

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The studies have demonstrated that phenols and flavonoids contribute to a greater 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 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 several physical and chemical changes in vegetables [9]. 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 [10].

Studies have reported that cooking softens the cell walls, which lead to an increase in the extraction of carotenoids [11]. However, other studies have reported that cooking can also lead to a loss in essential vitamins, antioxidants, water soluble, and heat-labile compounds. The extent of loss is dependent on the type of cooking treatment [12] and the phytochemical compositions of the cooked vegetable [13]. Cooking processes may affect the antioxidant content of food due to antioxidant release, destruction, or creation of redox-active metabolites [14]. Antioxidant compounds, such as ascorbic acid and some carotenoids, are very sensitive to heat and storage.

Conversely, polyphenols have shown a certain stability when exposed to high temperatures [15]. Previous studies conducted on different vegetables showed that, after cooking, total polyphenol content and antioxidant activity of samples could be higher or lower compared to fresh vegetables. For example, Faller and Fialho, 2009 [15] showed that cooking decreased the antioxidant capacity for most of the vegetables and small differences found between the cooking methods applied.

Wachtel-Galor *et al.*, 2008 [14] 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 contents increased for all steamed vegetables over that of raw vegetables. Effects were variable for boiling and microwaving. Boiling caused a lesser antioxidant loss in cooking than did microwave.

Turkmen *et al*., 2005 [16] 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, [10] pointed out that antioxidant components and antioxidant activity in broccoli samples were lost quickly during cooking.

Based on above the facts, the present study was carried out to investigate the effect of common domestic cooking processes, open cooking, and pressure cooking, on phenolics and 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 of Telangana states, which include beans (*Phaseolus vulgaris*), bitter gourd (*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 ºC) 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 (1000 ml) and a pressure valve for high pressure-cooking and cook till the first whistle. 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 a solvent for extracting the samples from the vegetables.

Analysis of Bioactive Components: Total phenolic content [17], flavonoids [18], and antioxidant activity properties, (DPPH Radical-Scavenging activity, [19] and Ferric reducing antioxidant power FRAP) [20] were estimated in the extracts using standard analysis procedures.

Statistical Analysis: All data were presented as means ± standard deviation. Differences between variables were tested for significance by using the ANOVA test, followed by multiple comparisons.

Total Phenolic Content of Selected Vegetables: Table 1 indicates that in fresh form, 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 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, beans (8.77, 15.09%), bitter guard (202.62, 260.30%) and drumsticks (15.07, 28.10), 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 ranged between 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 total TPC content of selected vegetables within the group and between the treatments (raw (C), open (T1), and pressure cooking (T2)) showed a 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 in both soluble forms and combined with cell-wall complexes. Studies performed on different vegetables after cooking show 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 [21]. 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 [16]. The gain could be to the decomposition of some polyphenols bound to the dietary fiber of vegetables releasing free phenolic compounds that increase their detection [22]. Yamaguchi *et al.*, (2001) [23] has told that heat treatment usually leads to inactivation of polyphenol oxidase and other oxidizing 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 a study carried out by Ismail, [24] 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 (p<0.05).

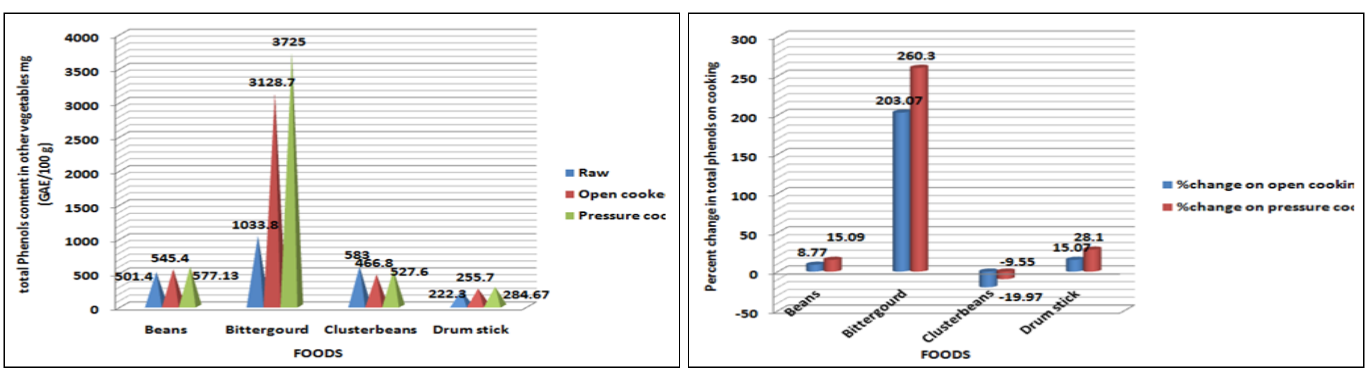

FIG. 1: TOTAL PHENOLIC CONTENT OF SELECTED VEGETABLE mg (GAE/100 g)

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 a destructive effect on the flavonoid as they are highly unstable compounds [24]. According to Adefegha and Oboh, 2011 [25], 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, this indicates some flavonoids release during cooking. In the study of Saikia and Mahanta, 2013, [21] beetroots showed an increased TFC in steamed and boiled samples. According to Adefegha and Oboh, 2011 [25] 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.

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 the pressure cooking method (T_2).

The total flavonoid content of selected vegetables was compared within 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 the reduction in flavonoid content was at the significant difference level of 5%.

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	Bitter gourd	91.7 ± 2.18	41.3 ± 1.52	-53.88	58.6 ± 2.23	-36.11	474.0**
3	Cluster beans	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*

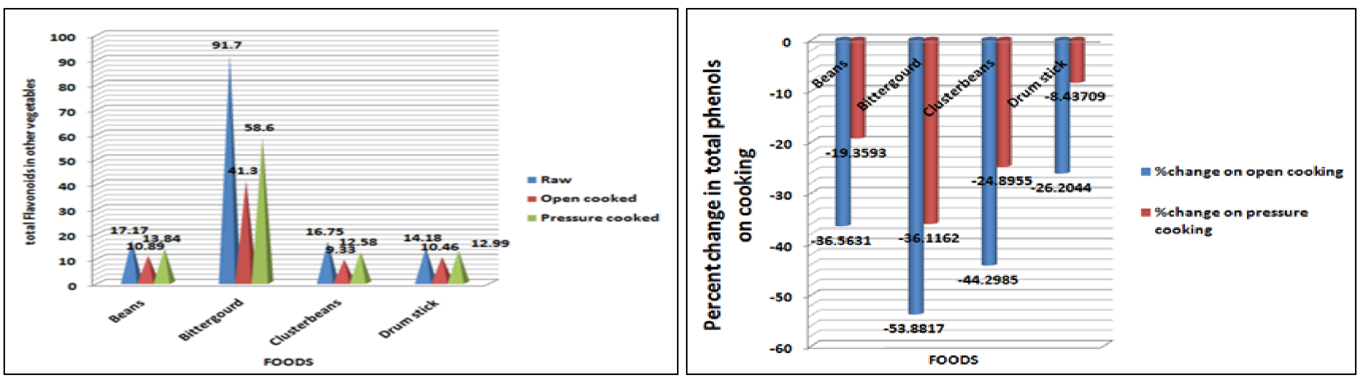

FIG. 2: TOTAL FLAVONOIDS CONTENT OF SELECTED VEGETABLES (mg RE/100g)

The Total Antioxidant Activity Level of Selected Vegetables:

Frap Method: 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**

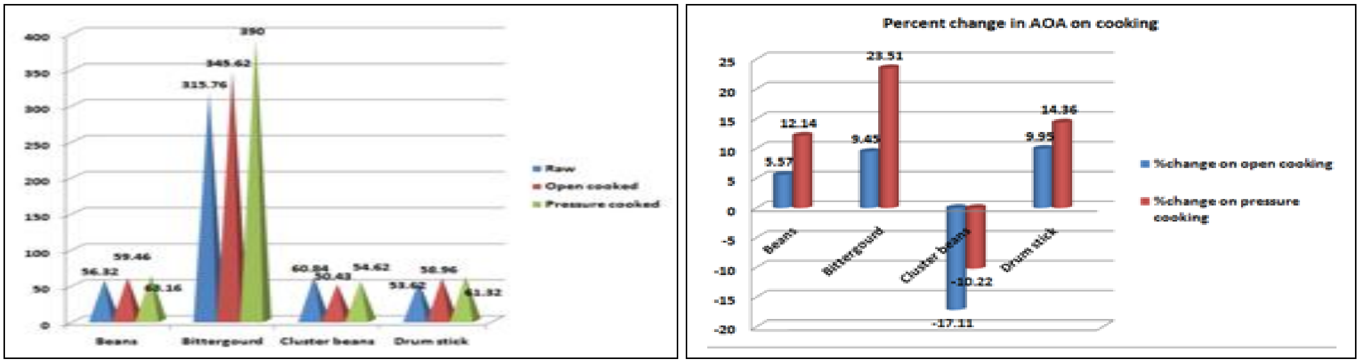

FIG. 3: THE TOTAL AOA LEVEL OF SELECTED VEGETABLES BY FRAP METHOD (mg TE/100g)

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 the percent difference between the control and treatment extracts are 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 a significant increase in AOA by DPPH method in both the cooking 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*

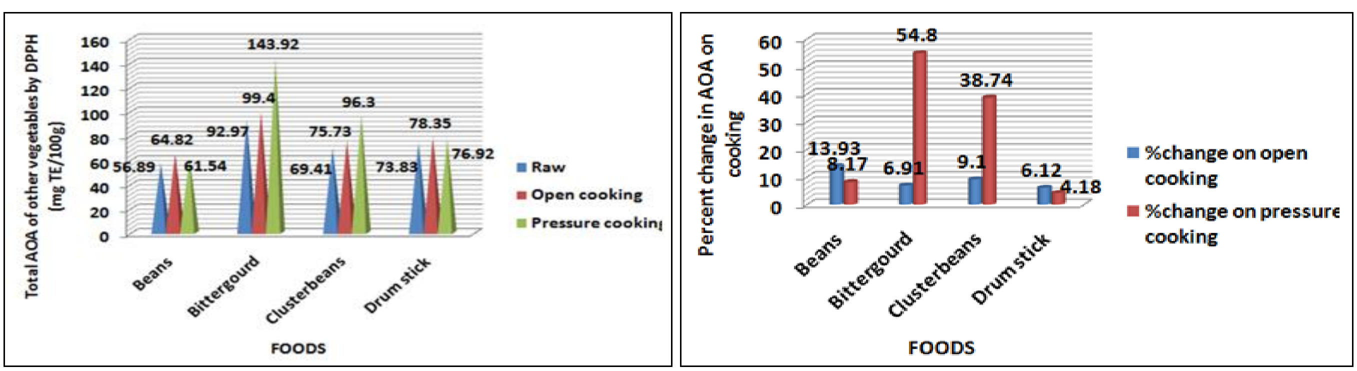

FIG 4: THE TOTAL AOA OF SELECTED VEGETABLES BY DPPH METHOD (mg TE/100g)

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 significant increase in total AOA content was observed at 1% level in beans, bitter gourd, and cluster beans except for drumstick, it showed 5% significant difference level. Cooking procedures led to an increase in antioxidant activity in vegetables. This effect is perhaps due to the 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 the antioxidant activity of red radish, broccoli, and white cabbage. Wachtel-Galor *et al.,* 2008 [14] showed that the effects of cooking on antioxidant capacity of different vegetables might be different. According to Saikia and Mahanta, 2013, beetroot has shown 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 [16] Beetroot has shown an increased and positive effect on FRAP and TPC for all the three cooking treatments like steamed, microwaved and boiled [21] In the earlier study by Zhang and Hamauzu, 2004 [10] said that there were 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 the improvement of antioxidant properties of naturally occurring compounds or formation novel compounds such as Maillard reaction products having antioxidant activity [26]. Ismail, Marjan, and Foong, 2004 [24] 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 the destructive effect of the flavonoid and phenolic compounds as they are highly unstable compounds [24]. In some cases, an increasing trend in phenolic and flavonoid 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 high reactivity with Folin Ciocalteau reagent and thus leads to an increased value of total phenolic content [17]. 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. 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 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 antioxidant activity, total phenolics, and flavonoid content. As the flavonoids are highly unstable compounds, application of cooking/heat resulted in a 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 plants are consumed after cooking. The study highlighted that the antioxidant activity of the vegetables did not significantly affect by the commonly used cooking methods and proved safe for phytonutrients.

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CONFLICT OF INTEREST: Nil

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