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EFFECT OF COOKING ON TOTAL PHENOL, TOTAL FLAVONOID, DPPH FREE RADICAL SCAVENGING ASSAY AND TOTAL ANTIOXIDANT CAPACITY OF SOME GREEN LEAFY VEGETABLES COMMONLY CONSUMED IN INDIA.

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ABSTRACT Green leafy vegetables (GLVs) are recognized worldwide as rich source of antioxidants. They are usually consumed in India after cooking. Cooking may have an effect on the antioxidant contents of the vegetables. This study was undertaken to evaluate the effect of cooking on the total phenolic content (TPC), total flavonoid content (TFC), 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging assay and total antioxidant capacity (TAC) of some green leafy vegetables commonly consumed in India like Amaranthus viridis L., Raphanus sativus L., Chenopodium album and Spinacia oleracea L. TAC of the raw vegetables used for the study was found to be higher than that of the cooked. However, TPC, TFC and DPPH free radical assay was found to be more in the cooked varieties with Spinacia oleracea L or Spinach having the maximum value of TPC of 144.0 mgGAE/gm, TFC of 150.2 mg QE/gm and 92.42% DPPH free radical assay.

KEYWORDS : Green leafy vegetables, total phenolic content, total flavonoid content, DPPH free radical assay and total antioxidant capacity

INTRODUCTION

Green leafy vegetables (GLVs) are considered as an essential part of the diet to meet the daily nutrient requirements. These are becoming more popular for the masses day by day due to the increased awareness of consumers about natural and organic foods. These possess a high place in the food pyramid and are an essential part of a balanced diet (Randhawa 2015). Green vegetables contain a wide variety of bioactive compounds known as phytochemicals. These phytochemicals impart health benefits beyond basic nutrition (Oomah and Mazza, 2000).

Most of the phytochemicals from plant extracts, fruits and green vegetables have been identified to exhibit antioxidant activities. Natural antioxidants have a diversity of biochemical activities, some of which include the inhibition of reactive oxygen species (ROS) generation, direct or indirect scavenging of free radicals, and alteration of intracellular redox potential (FinkelHolbrook, 2000). In order to prevent or moderate oxidation-related diseases, it is necessary to sequester and eliminate free radicals from the body (Hait-Darshan et.al.2009). Antioxidants may offer some resistance to oxidative stress by scavenging free radicals, inhibiting cell membrane damage, and suppressing lipid peroxidation, thus preventing the onset of chronic disease (Fu et.al 2011). GLVs have different antioxidants such as Vitamin C, Vitamin E, Carotenes, Lycopenes, Polyphenols and other phytochemicals (Prior and Cao, 2000). Much of the total antioxidant activity of fruits and vegetables is related to their phenolic content (Oboh et al., 2007; Oboh and Rocha, 2008). Phenolic compounds can protect the human body from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells. The antiradical activity of flavonoids and phenols is principally based on the structural relationship between different parts of their chemical structure (Rice-Evans, 1996).The present investigation has been undertaken to evaluate the total phenol and flavonoid content, DPPH free radical assay and total antioxidant capacity of raw and cooked forms of some green leafy vegetables like *Amaranthus viridis* L., *Raphanus sativus* L., *Chenopodium album* L .and *Spinacia oleracea* L commonly consumed by people of India.

MATERIALS AND METHODS

Fresh vegetable samples of *Amaranthas viridis, Raphanus sativus, Chenopodium album* and *Spinacia oleracea* were purchased from local vegetable market of Surat in the month of December-January. Vegetables were botanically identified with the help of local flora and authenticated by experts.

Sample preparation:

Fresh green leafy vegetables were rinsed in water, dried on paper towel and the edible portions were separated from the inedible portion. The edible portions were chopped into almost equal small pieces or slices, mixed well and a portion (40 g) of the chopped vegetables was cooked by steaming in 200 ml of distilled water for 10 mins, while the other portion was not cooked. Fresh and cooked samples of the green leafy vegetables were then blended, centrifuged and filtered. The filtrates were stored in the refrigerator for subsequent analysis (Adefegha and Oboh, 2011).

Determination of total phenol content

TPC were determined by the Folin-Ciocalteu method (J. Lachman et al., 1998) with some modifications. The diluted aqueous solution of each extract (0.5 ml) was mixed with Folin Ciocalteu reagent (0.2 N, 2.5 ml). This mixture was allowed to stand at room temperature for 5 min and then sodium carbonate solution (75 g/l in water, 2 ml) was added. After 2 hours of incubation, the absorbance was measured at 760 nm against water blank. A standard calibration curve was plotted using Gallic acid.

Determination of total flavonoid content

TFC were estimated by Aluminium trichloride colorimetric method (Arvouet-Grand et. al. 1994). A diluted methanolic solution (2 ml) of each fruit extract was mixed with a solution (2 ml) of Aluminum trichloride ($AlCl_3$) in methanol (2 %). The absorbance was read at 415 nm after 10 min against a blank sample consisting of a methanol (2 ml) and with $AlCl_3$. A standard calibration curve was plotted using Quercetin.

Determination of DPPH free radical scavenging assay

The DPPH antioxidant assay was determined as described by Shimada et al., (1992). Briefly, 0.5 ml of DPPH radical solution was mixed with an extract of 2 ml. An equal volume of ethanol was added in the control test. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance at 517 nm was measured with a UV spectrophotometer. The percentage of scavenging of DPPH was then calculated in the following way:

DPPH scavenging effect (%) = [1□ (Test sample absorbance/blank sample absorbance)] ×100(%)

Determination of total antioxidant capacity (TAC)

The total antioxidant capacity of the methanol extract was evaluated by the phosphomolybdenum method according to the procedure described by Prieto et al. (1999). 0.2 ml extract was combined with 2 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against blank after incubation it at 95ºc for 90 minutes and cooling to room temperature. Reagent (2 ml) in the place of extract was used as the blank.

The results of the all these study were presented as the mean of three determinations along with standard deviation. Statistical analysis was done using MS Excel software (CORREL Statistical function).

RESULTS AND DISCUSSION

Present study established that all the selected green leafy vegetables are rich in total phenol and total flavonoid content. TAC and DPPH free radical scavenging was also found to be significant in all the varieties.

For determination of TPC, Gallic acid was used as a reference compound. The total phenols were expressed as mg/g Gallic acid equivalent (mg GAE/gm) using the standard curve equation: y = 0.0095x + 0.37, R^2= 0.9912, Where y is absorbance at 760 nm and x in total phenolic content of the vegetables. (Figure1).

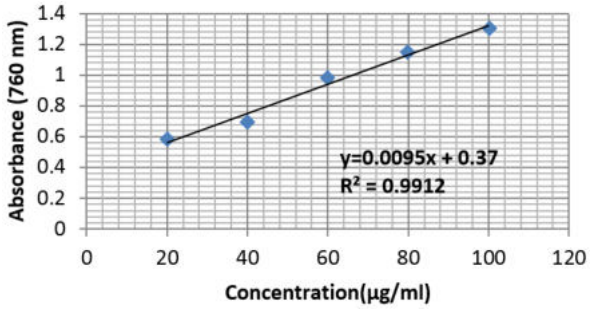

Figure 1. Standard graph of Gallic acid

Phenols are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals (Olajire and Azeez,2011).

Flavonoids are another class of phenolic substance. They are known to possess high antioxidant activity which is due to their ability to reduce free radical formation and to scavenge free radicals (Pietta, 2000).

The amount of total flavonoid was determined with the Quercetin reagent used as standard and the total flavonoids were expressed as mg/g Quercetin equivalent (mg QE/gm) using the standard curve equation: y = 0.005250x + 0.123, R^2= 0.998, Where y is absorbance at 415 nm and x is total flavonoid content (Figure 2).

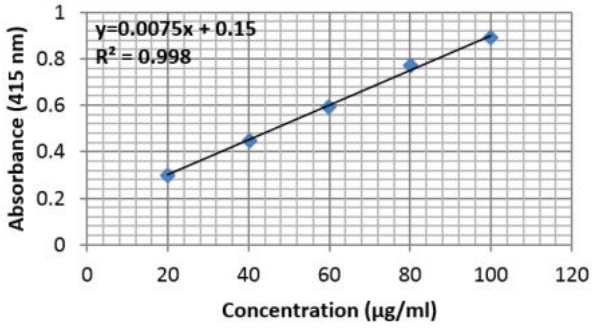

Figure 2. Standard graph of Quercetin

In the present study TPC and TFC of cooked vegetables were found to be higher than that of raw. The result is in agreement with previous work reported by Shaimaa et al. (2016) who reported that TPC and TFC of sweet and chilli pepper increased after boiling, with the antioxidant activity exhibiting a positive relationship with TPC and TFC. Bhave and Dasgupta [2018] also reported significant increase of total phenolic content (TPC) of *Plectranthus amboinicus* after cooking. This indicates that most of the phenolic compounds trapped in fibre of green leafy vegetables are actually more available in the cooked compared to the raw. The percent gain in the total phenol content during cooking may be due to the breakdown of tough cell walls and release of phenolic compounds trapped in the fibre of green leafy vegetables for easier absorption in the small intestine (Oboh and Rocha,2007; Oboh and Rocha,2008).

In the current investigation TPC and TFC of *Spinacia oleracea* (Spinach) was found to be maximum with TPC at 144 ± 1.73 mg GAE/gm and TFC at 150 ± 1.44 mg QE/gm (Fig. 1,2 and table 1). *Chenopodium album* Linn., an under exploited vegetable was also found to be promising with respect to TPC (111 ± 1.40 mg GAE/gm) TFC (144± 1.39 mg QE/gm) and was rightly regarded as a prospective wild vegetable and is worth exploration and utilization (Poonia and Upadhayay,2015).

Table 1 Total phenol and flavonoid content of cooked and raw green leafy vegetables

GLVs Species	Cooked		Raw	
	TPC mg GAE/gm	TFCmg QE/gm	TPC mg GAE/gm	TFCmg QE/gm
Amaranthus viridis	103.33 ± 1.33	133.11 ± 1.28	77.2 ± 1.12	124.66± 1.2
Raphanus sativus	100.0 ± 1.3	142.54 ± 1.37	39.33 ± 0.95	102.0± 0.98
Chenopodium album	111.66 ± 1.40	144.74 ± 1.39	79.01 ± 1.17	125.48± 1.3
Spinacia oleracea	144.0 ± 1.73	150.2 ± 1.44	83.74 ± 1.2	94.73± 0.91

Each value represents the mean of three replicates ± Standard Deviation

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [Soares et. al., 1997]. A freshly prepared DPPH solution is of deep purple colour with absorption maximum at 517 nm and in the presence of antioxidant this colour disappears due to quenching of DPPH free radicals and convert them into a colourless product i.e. 2,2-diphenyl-1-hydrazine. Antioxidant mechanism performed by providing hydrogen atoms or electron [Ferreira, et., al., 2007].The results of the DPPH radical scavenging ability of the current investigation was found to be significantly high in the cooked vegetables with cooked *Spinacia oleracea* showing maximum activity at 92.42± 0.44% followed by *Chenopodium album* at 85.0± 0.32% (Table 2). The assay was positively correlated with TPC (0.891) and TFC (0.879). This observation is in agreement with an earlier report by Hossain et al. (2017) where an increment in radical scavenging activity was found to be correlated with the increase of the TPC of the green leafy vegetables.

Table 2- DPPH radical scavenging and total antioxidant activity of cooked and raw green leafy vegetables

DPPH %scavenging activity			TAC (mg AAE / gm)	
GLVs SPECIES	Cooked (%)	Raw (%)	Cooked	Raw
Amaranthus viridis	77.2+ 1.2	23.28±0.39	33.80 + 0.7	102.75 + 0.91
Raphanus sativus	78.55+ 0.3	22.78±0.39	63.33 +1.4	109.0 + 1.24
Chenopodium album	85.0+ 0.32	19.17±0.33	57. 66+ 0.8	120.33 + 0.52
Spinacia oleracea	92.42+ 0.44	22.5±0.14	59.99+0.1. 2	81.93 + 1.87

Each value represents the mean of three replicates ± Standard Deviation

TAC assay by Phosphomolybdenum is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH (Prieto et al., 1999).The Phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid. The value was expressed as mg/g Ascorbic equivalent using the standard curve equation: y = 0.0056x + 0.03, R^2= 0.9887 (Figure 3).

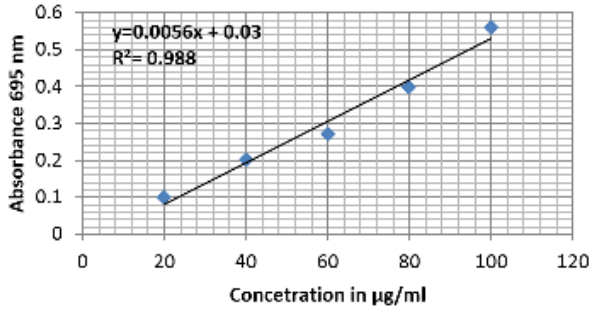

Figure 3. Standard curve of Ascorbic Acid for total antioxidant activity

In the present study the raw extracts was found to show maximum total antioxidant activity with *Chenopodium album* showing maximum

activity at 120.33 ± 0.52 mg AAE / gm. TAC assay is that, by definition, gives an estimation of the antioxidant components of a sample in a global way (Rubio et. al.2016). Antioxidant activities are not limited to the phenolic only, other secondary compounds such as ascorbic acid, β-carotene, α-carotene and different xanthophylls might be playing a significant role for the antioxidant activity (Kondo et al 2005). So in the present study it might be possible that while phenol and flavonoid content increased during cooking but possible that other bioactive compounds responsible for the antioxidant activity were not activated or released (Sultana et.al 2008) hence there is reduction of TAC in the cooked vegetables.

CONCLUSION

Cooking improves the digestibility of the vegetables and also can prevent food borne illness caused by harmful microorganisms. Hence it is always safe to consume cooked vegetables (Adefegha and Oboh,2011). The present study showed that all the four edible green leafy vegetables are having moderate to strong antioxidant activity. Phenol and flavonoid content was found to increase during cooking but total antioxidant capacity was found to be less in cooked vegetables as some antioxidants might have been lost during the process. However more detailed study with respect to temperature, cooking time, and technique used are required to asce\rtain the antioxidant status of the cooked vegetables.

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