Journal on New Biological Reports
JNBR 4(3) 238 – 242 (2015)

ISSN 2319 – 1104 (Online)

Published by
www.researchtrend.net

Estimation of ascorbic acid, ß carotene, total chlorophyll, phenolics and antioxidant activity of some European vegetables grown in mid hill conditions of western Himalaya

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| **Received**: 23 November 2015 | **Accepted**: 28 December 2015 |

ABSTRACT

Seven European vegetables viz: Chinese Cabbage (*Brassicca* pekinensis), Black Lai (*Brassicca rugossa*), Broccoil (*Brassicca oleracea* var *italica*), Brussel's Sprout (*Brassicca oleracea* var *germifera*), Cauliflower (*Brassicca oleracea* var *botrytis*), Red Cabbage (*Brassicca oleracea* var *rubra*) and Lettuce (*Lectuca sativa*) were grown and estimated for antioxidant phytonutrients. Maximum ascorbic acid content (175.99 mg/100g), ß carotene (52.34 µg/g), antioxidant activity (1.38 IC_{50}) phenol content (13.59 mg/g) and total chlorophyll content (16.92 mg/g) was recorded in Brussels's Sprout. Linear regressions were performed with collected data in order to know which phytonutrients are contributing to antioxidant activity. Results revealed that ascorbic acid, ß carotene, total phenols and total chlorophyll contents were highly correlated with antioxidant activity for European vegetables. It is revealed from the results that European vegetables are rich source of abundant antioxidants and there is significant variation among the different species for antioxidant phytochemicals.

Key Words: European vegetables, antioxidant activity, ascorbic acid, ß carotene, chlorophyll, phenols.

INTRODUCTION

European vegetables are also called temperate and biennial vegetables constitute a group of vegetables which require vernalization (chilling) during a specific stage of their growth for conversion from vegetative to reproductive phase. Nowadays, these vegetables are considered as world healthiest food. They are rich source of vitamins and minerals and having strong anti- oxidant potential. The discovery of phytochemicals in vegetables has generated tremendous attention among scientists. Vegetables act as powerful medicine which can help reduce the risk of chronic diseases (Brown *et al*; 1999; Gosslau and Chen, 2004; Lee *et al*., 2008).
Ascorbic acid or vitamin C is acquired human nutrient. It has a great potential against heart diseases, cancer, blood pressure and high cholesterol (Antonious, G. 2009; Byers and Perry, 1992). ß carotene is a double molecule of vitamin A. It is the precursor of vitamin A. It is a hydrocarbon carotenoid and having powerful antioxidant activities (Perra and Yen, 2007). Chlorophyll is another biomolecule recognized as a health promoting phytochemical. Phenols are the secondary metabolites synthesized by the plants. These are potentially powerful antioxidants that can protect the human organisms from free radicals (Nadeem *et al.*, 2011; Velioglu *et al.*, 1998; Materska and Perucka, 2005; Chu *et al.*, 2002; Kaur and Kapoor, 2002). Antioxidant activity is an important parameter to establish the health functionality of a food product. The majority of the antioxidant activities of vegetables are from ascorbate, carotenoids and phenols (Badami and Channa, 2007).

Tremendous interest in the antioxidant components in vegetables and their possible health promoting effects has stimulated world wide research to identify potential sources. Scientists at John Hopkins University found that lab animals fed with European vegetables had a 90% reduction in their cancer rate after being exposed to aflatoxins, a deadly cancer causing agent. Among European vegetables, some are quite common but towards some, interest is decreasing due to lack of their awareness, limited availability and absence of popularization and documentation. In the present study, some less familiar vegetables were analyzed for ascorbic acid, ß carotene, total chlorophyll, phenolics and antioxidant activity. There is need for quantitative data on the antioxidant contents of European vegetables. Such information will not only increase the understanding of the function of these antioxidant phytochemicals but also be helpful in breeding programme to develop new germplasm with high content of such phytochemicals.

MATERIALS AND METHODS

Vegetables viz: Chinese Cabbage (*Brassicca* pekinensis), Black Lai (*Brassicca rugossa*), Broccoil (*Brassicca oleracea* var *italica*), Brussel's Sprout (*Brassicca oleracea* var *germifera*), Cauliflower (*Brassicca oleracea* var *botrytis*), Red Cabbage (*Brassicca oleracea* var *rubra*) and Lettuce (*Lectuca sativa*) were planted in randomized block design with three replications at Defence Institute of Bio Energy Research, Pithoragarh, (Uttarakhand) at an altitude of 5500 feet above the sea level. This place is situated in western Himalaya, which extends from $29^{0}29'$ N to $30^{0}49'$ N latitude and $85^{0}05'$ E to $81^{0}31'$ E longitude. The annual rainfall is approximately 1250mm, out of which 70-75% is received during the rainy season. The temperature of the place ranged from a maximum of 35^{0}C in summer to a lower of -2^{0}C during winter. The plants were spaced 60 cm apart between and within rows. Net plot size was 5.40 $m^{2.}$ Recommended cultural practices were adopted for the proper growth and stand of the crop. Three replicates each comprising of a homogenous mass from 5 randomly selected plant parts were screened for ascorbic acid (mg/100g), β carotene (µg/g), phenols (mg/g), total chlorophyll (mg/g) and anti oxidant activity (IC_{50}). The chemical analysis of fresh plant parts included determination of ascorbic acid by 2, 6 di chlorophenol indophenols titration method (AOAC, 1990), antioxidant activity using DPPH method (Hatano *et al*, 1989), β carotene estimation through spectrophotometer and results expressed as µg/g (AOAC, 1980), phenol estimation with Folin – Ciocalteau reagent (Singleton *et al.*, 1999) and chlorophyll content was estimated through the method developed by (Rangana, S. 1976).

Vitamin C as % RDA was based on 60 mg/100g. Provitamin A as RE/100g was calculated: 1 RE = 1 µg Retinol = 6 µg ß carotene = 3.33 IU vitamin A. Statistical analysis was carried out by (Gomez and Gomez, 1984). Data were found significant and presented in table 1.

Ascorbic acid assay

Ascorbic acid was estimated by volumetric method. 5 g of the fresh sample was extracted with 4% oxalic acid and volume made to 100 ml and centrifuged. 5 ml of this supernatant was pipette out, added with 10 ml of 4% oxalic acid and titration was done against the dye. Ascorbic acid reduces the 2, 6-dichlorophenol dye to a colorless leuco- base and gets oxidized to dehydroascorbic acid. Ascorbic acid was measured in mg/100g.

β carotene assay

5 g of dried sample was taken in 150 ml glass stopper Erlenmeyer flask and 40 ml water saturated butanol (WSB) was added. The contents of the flasks were mixed vigorously for 1 minute and kept overnight (16-18 hrs) at room temperature under dark condition for complete extraction of β carotene. The contents were shaken and filtered through the Whatman no.1 filter paper into a 100 ml volumetric flask. The optical density of clear filtrate was measured at 440 nm using ECIL, Double Beam UV- VIS Spectrophotometer 5704SS. Pure WSB was used as blank. WSB was prepared by mixing n-butanol with distilled water in 8:2 ratios. The β carotene contents were calculated by calibration curve from the known amount of β carotene and expressed as parts per million (ppm). Standard solution of β carotene (Sigma) was prepared in WSB having concentration of 5µg/ml. Calibration curve was made by known amounts of pure β carotene from 0.25 µg/ml to 1.5 µg/ml. The results were expressed as µg/g.

Anti oxidant assay

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) method was used for estimating free radical scavenging activity of the methanol extracts of samples. 2 ml of methanol extract (4 mg/ml) taken in test tube and final volume of 3 ml was made with methanol. The absorbance of the mixture was measured after 40 min at 517 nm against methanol as blank. Ascorbic acid was used as standard. The free radical scavenging activities (%) of tested samples were evaluated by comparing with a control (2 ml DPPH and 1 ml of methanol). Each sample was then measured in triplicate and averaged. The free radical scavenging activity (FRSA) was calculated using the formula: FRSA= [(Ac-At)/Ac-As] x 100, where Ac=Absorbance of control, As=Absorbance of standard and At= Absorbance of test.

Phenol assay

Total phenol estimation was carried out with the Folin –Ciocalteau reagent (FCR).Stock solution was prepared by dissolving 50 mg of catechol in 50 ml of distilled water. The absorption value for the catechol solution with concentration ranging from 10-50 µg was measured with the help of Spectrophotometer. One ml from the alcohol extract was evaporated to dryness and volume made up to 5 ml. 0.5 ml of extract was pipette out and to this 1 ml of 1N FCR was added followed by 2 ml of sodium carbonate solution and mixed properly. The contents were then placed in boiling water bath for exactly 1 minute. It was cooled and 10 ml of distill was added. Absorbance value was measured at 650 nm. The amount of phenols present was calculated by using standard graph.

Chlorophyll assay

1 g finely cut and well mixed sample of leaf or fruit tissues was made to a fine pulp with the addition of 20 ml of 80% acetone. Centrifuged (5000 rpm for 5 min) and the supernatant were transferred to a 100 ml volumetric flask. The residue was grind with 20 ml of 80% acetone, centrifuged and transferred the supernatant to the same volumetric flask. This process was repeated until the residue is colorless. The clear washings were collected in the volumetric flask and volume was made to 100 ml with 80% acetone. Thus chlorophyll was extracted in 80% acetone and absorption at 663 nm and 645 nm were read in a spectrophotometer. Using the absorption coefficients, the amount of chlorophyll was calculated.

RESULTS AND DISCUSSION

The results of antioxidant phytonutrients are given in Table 1.

Ascorbic acid ranged from 41.22 mg/100g to 175.99 mg/100g depicting 4 folds variation. Brussels Sprout exhibited maximum ascorbic acid content (175.99 mg/100g) followed by Black Lai (158.68 mg/100g) and Cauliflower (138.00 mg/100g). All the vegetables except Lettuce were found to be excellent source of ascorbic acid and fulfilled more than 100% RDA values for vitamin C.

The range of ß carotene (µg/g) ranged from 20.98-52.34. The maximum content was expressed by Brussel's Sprout (52.34 µg/g) followed by Black Lai (44.69 µg/g). The provitamin A ranged from 3.72 RE/100g to 8.72 RE/100g and vitamin A ranged from 11.64 IU to 28.78 IU.

Antioxidant activity (IC_{50}) from 1.38- 6.77. IC_{50} value is the inhibition concentration at which DPPH molecules were reduced by 50%. A low IC_{50} value is the sign of strong anti oxidant activity. Brussel's Sprout exhibited maximum antioxidant activity (1.38 IC_{50}) followed by Black Lai (1.72 IC_{50}).

Total phenol ranged from 2.05 mg/g to 13.59 mg/g. Maximum phenol content was exhibited by Brussels Sprout (13.59 mg/g) followed by Black Lai (12.61mg/g).

Chlorophylls are the essential components for photosynthesis. Total chlorophyll content ranged from 2.23 mg/g to 16.92mg/g. Brussels Sprout exhibited 16.92 mg/g total chlorophyll followed by Black Lai (14.90 mg/g).

Correlations

Linear regressions were performed with collected data in order to know which phytonutrients are contributing to antioxidant activity. Comparison of the ascorbic acid content of European vegetables with their corresponding antioxidant activity revealed a strong correlation (R^2=0.9595) **(Fig 1)**. ß carotene contents were highly correlated with

Table 1 Antioxidant phytonutrients in vegetables

Vegetables	Ascorbic acid (mg/100g)	Vitamin C (% RDA)	ß carotene (µg/g)	Provitamin A (RE/100g)	Vitamin A IU	Antioxidant activity (IC_{50})	Total Phenols (mg/g)	Total Chlorophyll (mg/g)
Chinese Cabbage	65.78	109.63	20.98	3.49	11.64	5.85	2.76	4.31
Lettuce	41.22	68.42	22.32	3.72	12.27	6.77	2.05	2.23
Black Lai	158.68	263.40	44.69	7.44	24.57	1.72	12.61	14.90
Broccoli	104.75	173.89	34.12	5.68	18.77	4.37	1.34	8.47
Cauliflower	138.00	229.08	43.32	7.22	23.83	2.06	11.04	10.18
Brussel's Sprout	175.99	192.14	52.34	8.72	28.78	1.38	13.59	16.92
Red Cabbage	99.93	129.36	41.44	6.90	22.79	3.53	6.14	9.16
CD @ 5%	20.57		4.737			0.798	0.155	0.475
CD @ 1%	28.83		6.639			1.118	0.217	0.666

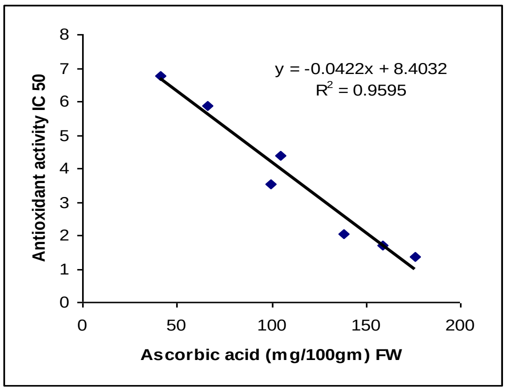

Fig. 1. Correlation between ascorbic acid and AA.

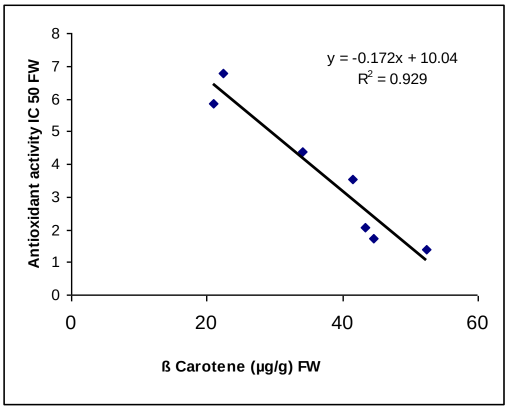

Fig. 2. Correlation between ß carotene and AA.

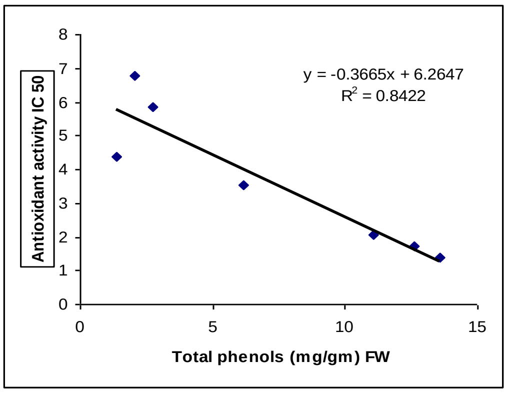

Fig. 3. Correlation between Total phenol and AA.

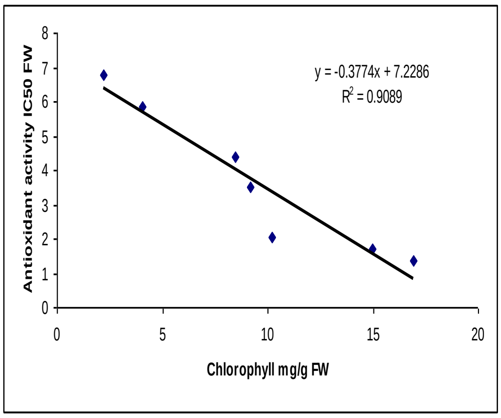

Fig. 4. Correlation between chlorophyll and AA.

antioxidant activity. The correlation coefficient R^2 was 0.9290 (Fig 2). Comparison of the total phenol content of European vegetables with their corresponding antioxidant activity revealed a strong correlation (R^2=0.8422) (Fig 3). The correlation coefficient (R^2 =0.9089) revealed that there was strong correlation between chlorophyll content with antioxidant activity (Fig 4).

The study on phyto-nutrients of some uncommon vegetables was carried out by Khanzadi Fatima Khattak (2011), Dasgupta and De (2007), Iqbal *et al*; 2006, Odhav *et al*; 2007, Xin et al; 2004. In the present study variations were observed in the phytochemicals studied with the earlier reports. The reasons behind this may be due to environmental factors, genetic factors, the differences in the methods of estimation and the harvesting stage of the samples. It is revealed from the data that European vegetables are having strong antioxidant potential and these vegetables should be commercialized.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge State Agricultural Universities for providing germplasm for studies.

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