

Health Promoting Bioactive Phytochemicals Present in Organically and Conventionally Grown Exotic *Brassica* Vegetables

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ABSTRACT

The beneficial effects of *Brassica* vegetables on health improvement have been partly attributed to the presence of various forms of phytochemicals and antioxidants present in it, e.g. ascorbic acid, carotenoids and polyphenol compounds including flavonoids and chlorophyll. Two *Brassica* vegetables viz. broccoli (*Brassica oleracea*) and kale (*Brassica oleracea* L.var *acephala*) which, grown organically and conventionally were analysed for their phytochemicals content. The ascorbic acid content of organically grown broccoli and kale was 89.67 ± 0.33 and 48.67 ± 0.33 mg/100g which was comparatively higher when compared with inorganic counterparts with 84.80 ± 0.40 and 43.33 ± 0.67 mg/100g values respectively. The beta carotene content was found to be higher in case of organic broccoli i.e. 11.17 ± 0.01 ppm as compared to 8.71 ± 0.01 ppm in inorganic broccoli. The chlorophyll content was higher in case of inorganic broccoli (5.87 ± 0.03 mg/L) and kale (8.31 ± 0.01 mg/L). Simple phenol and flavanoids were found to be higher in case of organic kale as compared to inorganic kale. Total phenols, tannins and oxalates were found to be higher in inorganic broccoli and kale. Both broccoli and kale were good sources of phytochemicals. From the findings of the study concluded that they are rich in health-promoting phytochemicals so should be included in our food basket to provide protection against various diseases especially cancer. The cultivation and consumption of broccoli and kale should be promoted to harness their health promoting and health protecting beneficial effects. The phytochemical contents of the leafy vegetables serve as supplements for food and also have the potential to improve the health status of its users as a result of the presence of various compounds vital for good health.

Keywords: Kale, Broccoli, Bio-active, Health benefits, Antioxidants, Phytochemicals.

INTRODUCTION

Vegetables of the *Brassica* group are the most commonly grown and consumed on a global scale. Among plant foods with health benefits,

crops from the family *Brassicaceae* (also known as *Cruciferae*) have been the focus of numerous epidemiological and clinical studies (Piruthiviraj et al., 2016).

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Cruciferous vegetables, in particular those included in to the *Brassica* genus, are good sources of a variety of nutrients and health-promoting phytochemicals (Singh et al., 2006).

Brassica vegetables are a potent modulator of the innate immune response system with potent phytochemicals activity (Chauhan et al., 2012). Several studies have revealed that they exhibit anti-inflammatory, antimycotic, photoprotective, antihyperglycemic, anticarcinogenic and antioxidant activities (Valeria Dal Pra et al., 2015). They are abundant of polyphenolic compounds and contain 15-20 different glucosinolates like compounds (Sikora and Bodziarczyk, 2012). The secondary metabolite glucosinolate, are the characteristic compounds of the crucifer family (Talreja & Moon, 2014).

Broccoli and kale are excellent sources of indole-3-carbinol, a chemical which boosts DNA repair in cells and appears to block the growth of cancer cells. They contain sulforaphane (particularly when chopped or minced), a compound with potent anti-cancer properties (Correa et al., 2014). The beneficial effects of *Brassica* vegetables on health improvement have been partly attributed to their complex mixture of phytochemicals possessing antioxidant. Consumption of *Brassica* vegetables is associated with a lowered risk of cancer, heart disease, hypertension and stroke. This has been attributed to the presence of various forms of phytochemicals and antioxidants present in the foods, e.g. carotenoids and polyphenol compounds including flavonoids and chlorophyll.

Vegetables play an important role in human diets, as they support the normal functioning of the different body systems. They provide our cells with vitamins, minerals, fiber, essential oils and phytonutrients. These vegetables contain low amounts of fat and calories (Banerjee et al., 2012).

Awareness of the important role that fruits and vegetables play in a healthy diet is increasing as is interest in organic foods. Organic foods have become one of the fastest

growing food categories with sales increasing nearly 20 per cent each year since 1990 (Winter & Davis 2006). Organically grown/labeled produce is considered to be healthier and nutritious than conventionally grown produce by the consumers. The nutritional quality of organically and conventionally grown plants has been compared mainly in term of macronutrients, vitamins and minerals. Organically produced vegetables have higher levels of nitrates and lower amounts of some heavy metal (Worthington, 2001).

Organically grown produce is considered to have good amounts of flavonoides and secondary metabolites. There is need to evaluate these content of organically and conventionally grown produce. Therefore, a comparative study for phytochemicals evaluation of organically and conventionally grown vegetables was carried out.

MATERIALS AND METHODS

Procurement of raw materials

Fresh and optimally mature organically grown samples of broccoli (var *Palam Samridhi*) and kale (var *DSK-1*) was procured from the Department of Organic Agriculture, CSKHPKV, Palampur whereas, same varieties of broccoli and kale was grown conventionally using inorganic inputs. The optimally mature samples were selected and after sorting and proper washing, the samples were evaluated for various phytochemicals content. Data for analysis of different parameters is reported as average of triplicate determinations.

Estimation of Phytochemicals

Determination of Ascorbic acid (Ranganna, 2007)

The content of ascorbic acid was determined titrimetrically using 2,6-dichlorophenolindophenol. For determination 10 ± 0.001 g of sample was accurately weighted, grinded in porcelain mortar, than quantitatively transfer in 100 mL tubes, blended with 3 per cent metaphosphoric acid and filtered. Then titrated 5 ml aliquot with standard dye to a pink colour end point which persisted for at least 15 seconds. Milligram of ascorbic acid per 100 g of sample was calculated as:

$$\text{Milligram of ascorbic acid/100g} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up} \times 100}{\text{Aliquot of extract} \times \text{Weight of sample taken for estimation}}$$

Determination of Flavonoid (Boham and Kocipia 1994)

0.5ml of the plant sample was repeatedly extracted at room temperature with 100 ml of 80% aqueous methanol. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was then transferred into a china dish and evaporated to dryness over a hot plate and weighed.

Determination of Oxalates (Abaza et al., 1968)

Two grams of sample was taken in a 250 ml volumetric flask. To this 190 ml of distilled water and 10 ml of 6 N HCl was added and digested for 1 hour on boiling water bath. It was cooled, volume was made up to the mark (250 ml) with distilled water and the supernatant was filtered. To 50 ml of the filtrate 20 ml of 6 N HCl was added and evaporated to half of its volume and then

filtered. Precipitates were washed several times with hot distilled water to make the volume 125 ml. To the filtrate 3-4 drops of methyl red indicator followed by concentrated ammonia was added till the solution turned faint yellow. It was heated to 90 to 100°C, cooled and filtered, then the filtrate was boiled and 10 ml of 5 per cent calcium chloride was added with constant stirring. It was then allowed to stand overnight. It was filtered through Whatman filter paper No. 41 and precipitate was washed several times with hot water to make it free from calcium ions. The precipitate was transferred to original beaker, distilled water and sulphuric acid solution (1:4) was added to it till the precipitates were completely dissolved. The contents were warmed at 70°C and titrated with N/20 KMnO₄ to the near end point. The calculation was done using following formula:

$$\text{Oxalates (g/100g)} = \frac{\text{N/20 KMnO}_4 \text{ used (ml)} \times 0.002225 \times \text{Volume made}}{\text{Aliquot taken} \times \text{Weight of sample}} \times 100$$

Extraction for phenolic content

Finely ground sample weighing 200 mg was transferred to a beaker of 20 ml capacity. To this 10 ml of 70 per cent aqueous acetone was added and beaker was kept in water shaking bath at 37°C for 2 hours. After that sample was centrifuged for 20 minutes at 3000 rpm Supernatant was collected in a test tube for estimation for total and simple phenols.

Determination of total phenol (Makkar et al., 1993)

For this, 0.1 ml of sample extract was taken and volume was made up to 1 ml. Then 2.5 ml of sodium carbonate solution and 0.5 ml of Folin Ciocalteu reagent was added. Contents were kept for 40 minutes for developing purplish blue colour. Finally, absorbance at 725 nm in spectrophotometer was recorded

and total phenols were calculated in g/100g using standard curve (Figure 1).

Estimation of simple phenols (Makkar et al., 1993)

Sample extract (1 ml) was taken to which 1 ml of PVP was added. Contents were kept in ice cubes for 15 minutes. Then 0.20 ml of aliquot was taken in a test tube and 2.50 ml of sodium carbonate solution was added followed by Folin Ciocalteu reagent and incubated for 30-40 minutes for developing purplish blue colour. The absorbance was recorded at 725 nm and simple phenols using standard curve were calculated as g/100g (fig. 1). Total tannins were calculated as; Total Tannins = Total phenols- Simple phenols

Determination of Beta-carotene (AACC 1976)

Water saturated n-butanol was used to extract pigments. Weighed sample (3 g) was taken in stoppered conical flask to which water saturated n-butanol (40 ml) was added. The content were mixed and kept for 2 hours, mixing occasionally before filtering. Absorbance was measured at 440 nm.

Total pigments (ppm as β -carotene) = O.D X 30.1

Determination of total chlorophyll (Ranganna 2007)

Dry sample was ground and mixed thoroughly. Took 2 g of the sample to which was added a small amount of calcium carbonate. The sample was extracted with acetone in a blender using purified sand. Decanted the supernatant liquid. Extraction was repeated with acetone till the residue was colourless. Filtered the extract to 100 ml volumetric flask, washed the filter paper and made up to mark with acetone. Took 50 ml of ether in separating funnel. Pipetted 50 ml of acetone into this. Added water from the sides of the separating. Drained off the water layer. Washed the ether layer 5 times with 10 ml portion of distilled water or until the ether layer was free of acetone. Transferred the ether extract to a 100 ml volumetric flask, diluted to volume with ether and mixed. Transferred the solution to 100 ml amber coloured reagent bottle and added 3g of anhydrous Na_2SO_4 . Waited till solution became clear. An aliquot (5 ml) of this solution was pipetted into another dry bottle and diluted with ether (20 ml) such that OD of colour at 660 nm was between 0.2 and 0.8 and preferably near about 0.6.

Total chlorophyll (mg/L) = $(7.12 \times \text{OD at } 660 \text{ nm}) + (168.8 \times \text{OD at } 642.5 \text{ nm})$

RESULTS AND DISCUSSION**Phytochemical content in organic and inorganic broccoli and kale**

Data of Table1 pertains to phytochemicals content of organically and inorganically grown broccoli and kale.

Ascorbic acid content

Ascorbic acid is considered as one of the most important water soluble vitamins with different important biological functions. Sufficient amount of vitamin C was determined in broccoli and Kale (Figure 2). The ascorbic acid content of organic broccoli was 89.67 ± 0.33 mg/100g which was comparatively higher than that of inorganic broccoli where the value for ascorbic acid content was 84.80 ± 0.40 mg/100g. The ascorbic acid content of organically grown kale was 48.67 ± 0.33 mg/100g which was also comparatively more than that of inorganic kale with 43.33 ± 0.67 mg/100g ascorbic acid. A significant difference was found in both organic broccoli and kale when compared with inorganic produce. The results are in conformity with those reported by Kandil and Gad (2009), Dogra and Awasthi (2010), Sikora and Bodziarczyk (2012) Duma et al., 2014, and Bhandari and Kwak (2015).

Total phenols, Simple phenols and tannins content

According Samec et al. (2014), Brassica vegetables are rich in polyphenolic compounds that have recently receive considerable attention as potential protective factors against cancer and heart diseases, particularly because of their antioxidative properties. Phenolic compounds perform different biological activities, but the most important are the antioxidant activity, the capillary protective effect and the inhibitory effect elicited in various stages of tumor (Cartea et al., 2011). The total phenols, simple phenols and tannins were reported as mg GAE/100g and the corresponding values were 1.03 ± 0.00 , 0.23 ± 0.00 , 0.08 ± 0.00 and 1.33 ± 0.01 , 0.29 ± 0.01 , 0.13 ± 0.01 for organic and inorganic broccoli, respectively while total phenols and tannins content results of kale had also similar trend with broccoli 2.46 ± 0.01 , 0.20 ± 0.00 and $2.79 .31 \pm 0.01$, 0.31 ± 0.00 for organic and inorganic kale. Simple phenol content of organic kale was comparatively

higher than that of inorganic kale and the corresponding values was 0.61 ± 0.00 mg GAE/100g. Statistically ($p < 0.05$) non significant result was observed in both produce. Further scrutiny of total phenols, simple phenols and tannins content were higher in kale as compared with broccoli. The similar results are also find by Duma et al. (2014), Bhandari and Kwak (2015), Desire Ndayazi et al., (2017) and Shah et al. (2017) in *Brassicaceae* family vegetables.

Flavonoids content

Phenolic compounds, especially flavonoids, perform different biological activities, but the most important are the antioxidant activity, the capillary protective effect and the inhibitory effect elicited in various stages of tumor (Cartea et al., 2011). The flavonoid contents of organic and inorganic broccoli were 10.78 ± 0.01 and 12.41 ± 0.01 mg/100g, respectively. The organic kale was comparatively higher than that of inorganic kale and the corresponding values were 10.11 ± 0.00 and 8.42 ± 0.01 mg/100g, respectively. Further scrutiny of data revealed that flavonoids content was higher in broccoli as compared with kale and organic produce of broccoli and kale had significant. The results of present study conformity with the study of Podsedek 2007, Sikora and Bodziarczyk (2012) and Bhandari and Kwak (2015)

Oxalates content

The value of oxalates in organic and conventionally grown broccoli was 1.07 ± 0.01 and 1.55 ± 0.00 respectively. Inorganic kale samples contained oxalates 0.37 ± 0.01 mg/100g whereas, the organic kale contained oxalates 0.23 ± 0.01 mg/100g difference in organic and inorganic was statistically non significant. Further scrutiny of data revealed that oxalate content was higher in broccoli as compared with kale. The similar results are also find by Sikora and Bodziarczyk (2012),

Bhandari and Kwak (2015) Desire Ndayazi et al. (2017) and Shah et al. (2017) in *Brassicaceae* family vegetables.

β -carotene content

Carotenoids present in dark green leafy vegetables might be involved in the prevention of several diseases related to oxidative stress (Girard-Lalancette et al., 2009). An intake of this bioactive compound has been implicated in a reduced risk of certain cancers and de- generative diseases, immune dysfunction and aged-related macular degeneration. The beta carotene content of organic and inorganic broccoli was 11.17 ± 0.01 and 8.71 ± 0.01 ppm, respectively. Beta carotene content of organic kale was 11.87 ± 0.04 and that of inorganic kale was 7.07 ± 0.01 ppm. Significantly higher amount of Carotenoids content present in organic produce (Figure 3). The results of Bhandari and Kwak (2015) and Shah et al., (2017) studies are also similar to present study.

Chlorophyll content

Chlorophyll is often referred to as the green blood of plants due to the identical molecular structure with hemoglobin with only difference in centre atom (iron or magnesium). This similarity makes chlorophyll so important to our health, it improve digestive, immune and detoxification systems of human body (Kopsell et al., 2005). The chlorophyll content of inorganic broccoli was slightly higher and was to the tune of 5.87 ± 0.03 mg/l when compared with organic broccoli where corresponding value was 4.90 ± 0.01 mg/l. The chlorophyll content of inorganic kale was 8.31 ± 0.01 mg/l which was comparatively higher than that of organic kale (5.18 ± 0.01 mg/l) (Figure 4). The obtained results confirm the results mentioned in the study of Banerjee et al., 2012, Duma et al., 2014 and Bhandari and Kwak (2015).

Table 1: Phytochemical content of broccoli and kale

Parameter	Organic	Inorganic	CD(p<0.05)
a) Broccoli			
Ascorbic acid (mg/100g)	89.67±0.33	84.80±0.40	0.37
Total Phenol (mg GAE /100g)	1.03±0.00	1.32±0.01	0.06
Simple Phenol (mg GAE /100g)	0.23±0.00	0.28±0.01	0.05
Tannins (mg GAE /100g)	0.08±0.00	0.12±0.01	0.02
Flavonoids (mg/100g)	10.78±0.01	12.41±0.01	0.02
Oxalates (mg/100g)	1.07±0.01	1.55±0.00	0.05
Beta-Carotene (ppm)	11.17±0.01	8.71±0.01	0.02
Chlorophyll (mg/L)	4.90±0.01	5.87±0.03	0.12
b) Kale			
Ascorbic acid (mg/100g)	48.67±0.33	43.33±0.67	0.13
Total Phenol (mg GAE /100g)	2.46±0.01	2.78±0.01	0.03
Simple Phenol (mg GAE /100g)	0.61±0.00	0.55±0.00	0.01
Tannins (mg GAE /100g)	0.20±0.00	0.31±0.00	0.01
Flavonoids (mg/100g)	10.11±0.00	8.42±0.01	0.03
Oxalates (mg/100g)	0.23±0.00	0.37±0.01	0.04
Beta-Carotene (ppm)	11.87±0.04	7.07±0.01	0.13
Chlorophyll (mg / L)	5.18±0.01	8.31±0.01	0.03

The values are expressed on dry weight basis except for β- carotene and chlorophyll

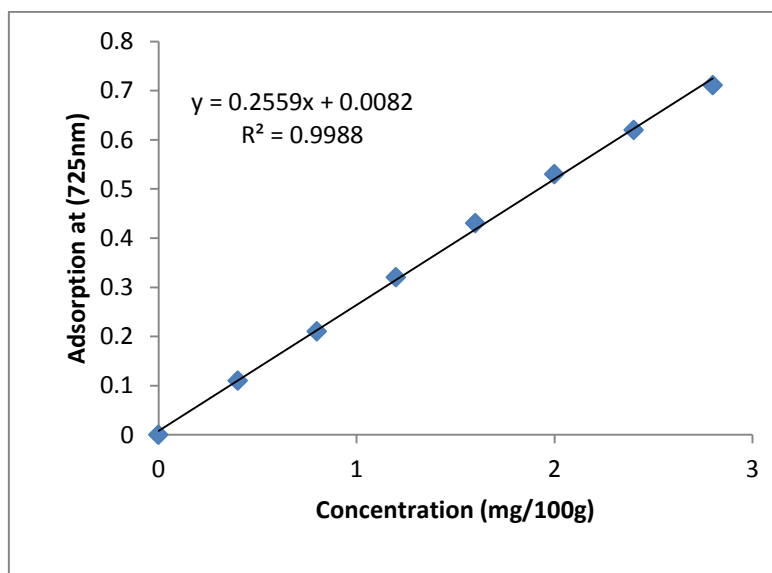


Fig. 1: Standard curve gallic acid equivalent (GAE)

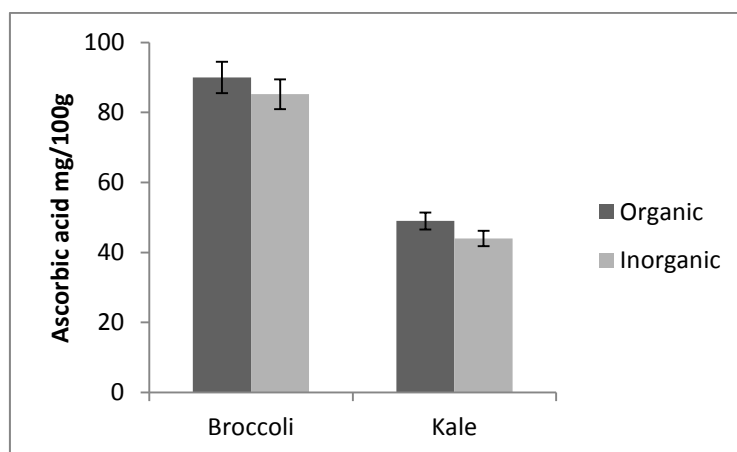


Fig. 2: Ascorbic acid content

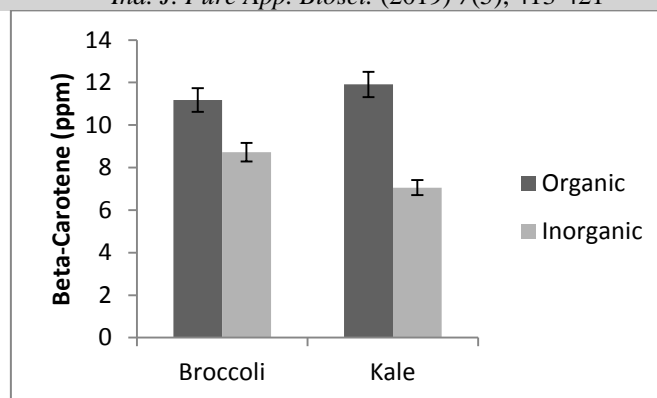


Fig. 3: Beta carotene content

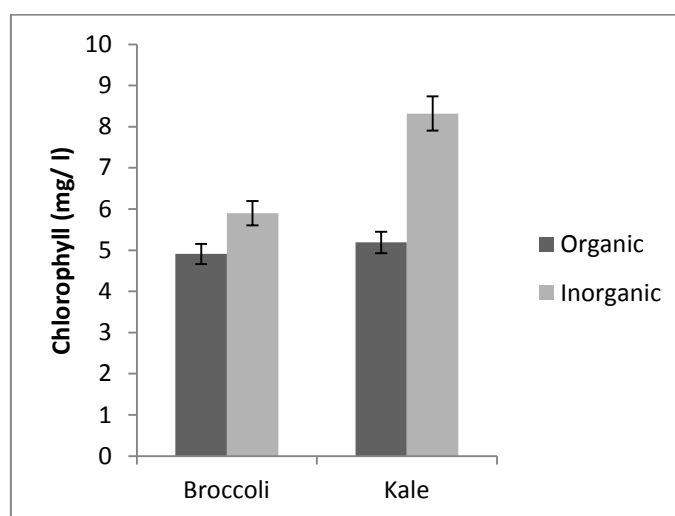


Fig. 4: Chlorophyll content

CONCLUSIONS

All the results of study suggested that phytonutrients in *Brassica* are affected in different ways depending on the nature of the compounds. The ascorbic acid content of organically grown broccoli and kale was higher when compared to conventionally grown broccoli and kale. Beta carotene content was found to be higher in case of organic broccoli i.e. 11.17 ± 0.01 ppm as compared to 8.71 ± 0.01 ppm in inorganic broccoli. The chlorophyll content was higher in case of inorganic broccoli and kale. Simple phenol and flavanoids were found to be higher in case of organic kale as compared to inorganic kale. Total phenols, tannins and oxalates were found to be higher in inorganic broccoli and kale. Broccoli and kale are rich in bioactive compounds so should be included in our food basket to provide protection against various diseases. The cultivation and consumption of broccoli and kale should be promoted to

harness their health promoting and health protecting beneficial effects.

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