

Phytochemical profiling of basil (*Ocimum basilicum*) microgreens

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Abstract

Micro greens (seedlings of edible vegetables and herbs) have gained popularity as a new culinary trend over the past few years. Although small in size, micro greens can provide surprisingly intense flavors, vivid colors, and crisp textures and can be served as an edible garnish or new salad ingredient. The consumption of micro greens has nowadays increased due to higher concentration of bioactive components such as vitamins, minerals and antioxidants than mature greens, which are important for human health. The present study was conducted to determine the physicochemical status of microgreens sold in Delhi market, India. The most popular micro green was found to be *Ocimum basilicum* (basil) based on the survey of retail outlets. Twenty five samples of basil microgreens were analyzed and found to be good source of ascorbic acid (71.0 mg/100g), total phenolic content (8.6 mg/gm), titratable acidity (0.024 [H⁺] mol/L), pH (5.82) and water content (94.3%).

Keywords: micro greens; bioactive components; polyphenols; basil; functional foods

Introduction

Microgreens contain higher concentrations of functional components such as antioxidants, phenolics, vitamins and minerals than found in mature greens or seeds (Janovska, 2010). Thus, they are considered as —functional foods which contain health promoting or disease preventing properties that are additional to their normal nutritional values. Demand for these products is growing rapidly due to the recent attention of consumers towards functional foods and they are highly prized for rich source of bioactive components (Xiao Z. L., 2015). Micro greens are loaded with nutritional benefits that can support our everyday intake of vitamins and minerals, helping us to live a healthy lifestyle. Because microgreens are harvested right after germination, they hold high levels of Vitamin C, Vitamin E and other essential nutrients that can help to keep us healthy. However, growing, harvesting, and postharvest handling conditions have a considerable impact on the synthesis and degradation of phytonutrients, including vitamins and carotenoids, of micro greens.

Variety of high quality micro greens is grown commercially and sometimes they are grown by individuals at lower scale for home use. Mixed cultivation of microgreens is also done by the growers (Poorva and Aggarwal, 2013). The time from seeding to harvest varies greatly from crop to crop (Pinto *et al.*, 2015). The growers are selecting the crops having a similar growth rate during seeding a mixture of crops, so the entire crop can be harvested at once. Micro greens are also produced in garden bed, in window sill as well as in containers, depending upon the requirement. They are grown in a standard, sterile, loose soil and many mixes with peat, vermiculite, perlite and bark (Kou *et al.*, 2013). Micro greens consumption has been steadily increasing in recent years due to consumer awareness of their unique color, rich flavor and concentrated bioactive compounds. However, industrial production and marketing is limited due to their short shelf life associated with rapid deterioration in product quality (Bergquist, 2006).

During the germination of seeds, macromolecules such as lipids and proteins are broken down to form nutrients that are more easily digested and absorbed. Hence, they have a good concentration of bioactive components. So, the purpose of this study is to assess bioactive components of micro greens.

Methodology

Sample collection and Preparation

Twenty five samples of basil microgreens were analyzed during the course of this entire study. The chemical analysis of approximately 25 samples was done during a 3 months study period. These samples were randomly collected aseptically in a sterilized container maintained in cold conditions, delivered to laboratory immediately and analyzed.

Chemical composition analysis

Ascorbic Acid Content

Standardization of dye was done by titrating standard ascorbic acid and metaphosphoric acid with the dye solution giving pink color as end point. Dye factor is calculated. Sample is prepared by blending 10 gm of microgreens sample with 3% Metaphosphoric acid (HPO₃) and making up the volume to 100 ml with HPO₃. The sample is filtered and from this, 2ml of aliquot is taken which is further titrated with standard dye giving pink color as an indicator which persists for 15 seconds. In order to eliminate the interference due to Sulphur dioxide (SO₂), 10 ml of filtrate is taken and to this, 1 ml of 40% formaldehyde and 0.1 ml of HCl is added, kept for 10 min and titrated as before. After this, calculation was done and result was obtained in mg/100g.

Titratable Content

Sample was prepared by homogenising 100 gm microgreens in 10ml water using Bag Mixer (Interscience). From this, 25 gm was transferred to conical flask with 50 ml of hot water

and boiled for 30 minutes in a reflux motion. The content was then transferred to volumetric flask and volume was made up to 100 ml. Standardization of NaOH was done with Oxalic acid. The sample was titrated against standardised NaOH after adding 2-3 drops of phenolphthalein indicator till pink color persists for 30 seconds. After this, the result was obtained in H^+ mol/100mL. pH was measured with the help of analytical pH meter. pH meter was first calibrated with buffer of pH 4.0, 7.0 and 9.2 and then testing of sample was done.

Moisture Content

10 g of the sample was weighed and transferred to pre-dried moisture dish and weighed again. Moisture dish was placed in hot air oven (temp 130-133°C for 90 mins). Dish was cooled in a desiccator and weighed. The process was repeated until the loss in weight between two successive weighing was less than 1 mg. The result was calculated and obtained in % by weight.

Total phenolic Content

The Total Phenolic Content (TPC) was determined by using the Folin-Ciocalteu assay. An aliquot (1 ml) of extracts and

standard solution of Gallic acid (100, 200, 300, 400, and 500mg/ml) was added to 100 ml of volumetric flask, containing 100 ml of distilled water. Reagent blank using distilled water was prepared. 10% of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes, 4 ml of 7.5% Na_2CO_3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 60 minutes at room temperature, the absorbance against the reagent blank was determined at 705 nm with an UV-Visible spectrophotometer. Total Phenolic Content was expressed as mg Gallic acid Equivalents (GAE). The amount of total phenol was determined with the Folin-Ciocalteu method. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g Gallic acid equivalent using the standard curve equation:

$$y = 0.014x - 0.0121, R^2 = 0.9862 \quad (1)$$

Where y is absorbance at 705 nm and x is total phenolic content in the extracts of basil microgreens expressed in mg/gm as shown in (Figure 1).

$$Y = 0.1083 \text{ and } X = 8.6 \text{ mg/gm} \quad (2)$$

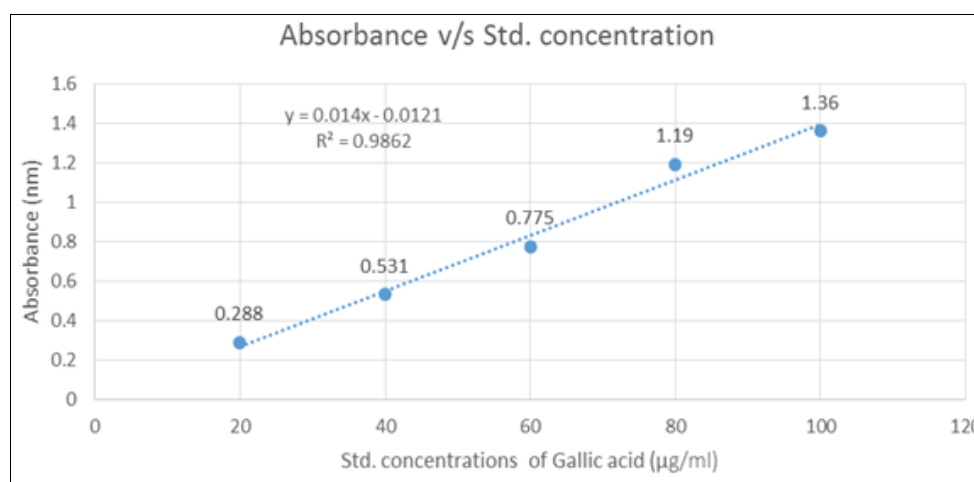


Fig 1: Showing straight line obtained by plotting standard concentration of Gallic acid against Absorbance

Results and Discussion

The ascorbic acid content of the basil micro greens sample came out to be 68.4 mg/100g which is slightly lower than the value of 71.0 mg/100g reported by Xiao *et al.*, 2012. The Titratable acidity is generally used as a quality parameter and relates to the concentration of free organic acid present in a food and describes its flavor (Xiao, 2015; Francis *et al.*, 2012).

The Titratable acidity was calculated and came up to be 0.233 H^+ mol/100mL. Micro greens sample had a pH of 5.82 which is quite closer to the value of 5.77 reported by Xiao, 2015. The moisture content of the basil micro greens sample was 90.7% which is lower than the value of 94.3% by weight as stated by Xiao, 2015. The total phenolic content was 8.6 mg/gm which is slightly higher than the normal value of 7.0 mg/gm reported by Xiao, 2015 (Table 1). ANOVA one-way was performed to find out any significant difference between the observed and reference values for all the chemical parameters. It was found that the observed values of microgreens for ascorbic acid, titratable acidity, moisture content and total phenolic content were

significantly different from the reference values. pH values were not significantly different.

Feizi *et al.*, 2016 also showed in their study that water is the preferred solvent in the solvation shell of chrysin in water-rich mixtures. Polyphenols present in fruits and vegetables as secondary metabolites are considered as non-essential for sustenance of life but also contribute potentially to the maintenance of human health (Patil *et al.*, 2009). Composition of the digested food matrix, the synergisms and antagonisms of the different components are influenced by the bioavailability (Fernández-García *et al.*, 2009), but also by physicochemical properties, such as temperature, pH and texture of the matrix (Neilson *et al.*, 2011).

Independent sample t-test was performed to find out if the values of chemical parameters (i.e. ascorbic acid, pH, moisture content and total phenolic content) tested for Basil microgreens are superior from those of basil leaves. On assuming equal variances, ascorbic acid and pH values were not significantly different for basil microgreens and basil leaves but moisture content and total phenolic content values were found to be significant. The comparison of basil microgreens with basil leaves at 5% level of significance

($p < 0.05$) showed that the ascorbic acid content and phenolic content of basil microgreens was much higher than those of basil leaves and thus have more nutritional value. The mean difference between basil microgreens and basil leaves for ascorbic acid content and total phenolic content was also large. The pH values were comparable for basil leaves and basil microgreens although no significant difference was observed in case of moisture content. (Table 2).

The chemical analysis result showed microgreens are rich in ascorbic acid and polyphenol content which is attributed to their anti-oxidant property. The observed chemical analysis results showed slight deviation from the reference values. The parameters like ascorbic acid are also prone to oxidation in environmental conditions. Preharvest and post-harvest factors such as seed source, growth location, growth environments and storage time affects the nutritional profile of produce (Goldman *et al.*, 1999).

Table 1: Comparison of chemical analysis results with reference values.

Chemical analysis parameter	Observed Value	Reference Value	Sources
Ascorbic acid content (mg/100g)	69.42 ± 0.54*	71.0	(Xiao, Lester, Luo, & Wang, 2012)
Titrate acidity ([H] ⁺ +mol/100mL)	0.231 ± 0.01*	0.024	(Xiao Z. L., 2015)
pH	5.83 ± 0.05**	5.77	(Xiao Z. L., 2015)
Moisture content (% by weight)	91.43 ± 0.50*	94.3	(Xiao Z. L., 2015)
Total phenolic content (mg/gm)	8.47 ± 0.09*	7.0	(Xiao Z. L., 2015)

The values expressed as mean ± SE. Values with * superscript differ significantly at 5% level of significance ($p \leq 0.05$). **No significant difference ($p \geq 0.05$) between observed and reference values.

Table 2: Comparison of Basil Micro Greens with Basil Leaves.

Chemical parameter	Basil Micro greens	Basil leaves	Sources
Ascorbic acid content (mg/100g)	69.42 ± 0.54*	27.04	(Dumbrava & Delia, 2012)
pH	5.82 ± 0.05*	6.00	(Agunbiade, M.O., Ojezele, & Alao, 2015)
Moisture content (% by weight)	91.43 ± 0.50**	91.20	(Danso-Boateng, 2013)
Total phenolic content (mg/gm)	8.46 ± 0.08*	0.30	(Agunbiade, M.O., Ojezele, & Alao, 2015)

The values expressed as mean ± SE. Values with * superscript differ significantly at 5% level of significance ($p \leq 0.05$).

**No significant difference ($p \geq 0.05$) between Basil microgreens and Basil leaves.

Conclusion

Microgreens is a less explored and unknown foodstuff in India. A review of the published literature yielded no studies on the cultivation, nutritional composition and shelf life study of microgreens in Indian scenario. This pilot study helped to understand the concept better and to project the status of microgreens in India esp. Delhi.

Microgreens provide high concentration of antioxidant, vitamins and minerals which are linked with the promotion of good human health and they also overcome the detriment of sprouts as they are more tolerant to pathogenic microorganisms. The comparison of the basil microgreens with reference values of basil leaves bioactive components also showed that the content is much more in microgreens. Hence, they can be considered as super food, having antioxidant property, which is need of the hour.

Conflict of Interest

The authors declare that they have no conflict of interest.

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