Sustainability, Agri, Food and Environmental Research, (ISSN: 0719-3726), 11(X), 2023:
http://dx.doi.org/10.7770/safer-V11N1-art622

Comprehensive evaluation of nutrient and phytochemical profile of bok choy (*Brassica rapa subsp. chinensis*) at different growth stages

Evaluación integral del perfil nutricional y fitoquímico de bok choy *(Brassica rapa subsp. chinensis)* en diferentes etapas de crecimiento

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ABSTRACT

Growing Concern of health-related disorder around the globe has led to need for healthy food alternatives. Green leafy vegetable can be one such alternative. As the green plant grows it goes through different stages, and each stage has its own importance in health and wellbeing. Therefore, the main objective of the study was to evaluate the nutritional and phytochemical profile of Bok choy *(Brassica rapa subsp. chinensis) at* microgreen, baby green and mature green stages. These stages were analysed for there proximate and phytochemical profile. A significant difference ($p<0.05$) was observed in the moisture, protein, and carbohydrate content of different stages, from which microgreens were found to have higher nutrient profile. In the phytochemical assessment, phenolics and flavonoids were found to be higher in microgreens, which can be a possible reason for the higher antioxidant activity. The microgreen stage was found to have higher macronutrient content and phytochemical content which can be a reason for higher antioxidant activity.

Key words: Microgreen, Bok choy, Plant stages, Antioxidant.

RESUMEN

Growing Concern of health-related disorder around the globe has led to need for healthy food alternatives. Green leafy vegetable can be one such alternative. As the green plant grows it goes through different stages, and each stage has its own importance in health and wellbeing. Therefore, the main objective of the study was to evaluate the nutritional and phytochemical profile of Bok choy (Brassica rapa subsp. chinensis) at microgreen, baby green and mature green stages. These stages were analysed for there proximate and phytochemical profile. A significant difference ($p<0.05$) was observed in the moisture, protein, and carbohydrate content of different stages, from which microgreens were found to have higher nutrient profile. In the phytochemical assessment, phenolics and flavonoids were found to be higher in microgreens, which can be a possible reason for the higher antioxidant activity. The microgreen stage was found to have higher macronutrient content and phytochemical content which can be a reason for higher antioxidant activity.

Key words: Microgreen, Bok choy, Plant stages, Antioxidant.

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INTRODUCTION

A plant has different stages of growth and development. All these stages have different time period and may have different physical and chemical characteristics. These stages include germination, sprouts, microgreens, baby greens and mature green plants; these stages differ in their growth period.

Microgreens are young cotyledonary leafy greens which are found in different colours, textures, and flavours, which are harvested and consumed in an immature stage. It is also found to have higher concentrations of functional components including antioxidants, phenolics, micronutrients as compared to their mature greens. Because of these reasons they are considered as functional foods which can help in promoting various health benefits and can help in preventing disease.

Baby greens are the after-microgreen stage of plant. Similar like microgreens they also have higher concentration of functional components. In a research study, comparing microgreens and baby greens, they found that, in general, baby greens (2.68 mg/g FW) contained about 20% more chlorophylls, carotenoids, phenols, anthocyanins (a type of flavonoid) than their corresponding microgreens (2.17 mg/g FW) (Lenzi et al., 2019). Therefore, Baby greens often are richer in bioactive compounds than their microgreens.

Green leafy plants have been studied for many years and are known to have various health benefits due to the presence of innumerable bioactive compounds. Investigation of the nutrient profile of green leafy vegetables shows that these are a rich or primary source of plant metabolites, indicated that higher food intakes of these metabolites such as folate, phylloquinone, and lutein have shown a slower cognitive decline and appeared to have a protective relation of green leafy vegetables to cognitive change. (Morris et al., 2018)

Each green leafy plant has specific health benefit which can include anti-aging, anti-cancer, anti-diabetic, antioxidant, etc properties. Its bioactive compounds concentration might change with its growing stages. Microgreens are proven to have distinct secondary plant distinct metabolite and vast mineral profiles compared with another species and with their mature counterparts. Microgreens were found to have a wide range of plant metabolites which include vitamins, minerals, and bioactive compounds, providing further evidence for their functional benefits on health. But not all metabolites and minerals were higher in microgreens compared with the mature counterparts. For some compounds, it can be present in higher concentration in mature greens as compared to microgreens. They also found that each microgreen species had numerous metabolites that were unique to that species (Johnson et al., 2021).

Bok Choy (Brassica rapa subsp. chinensis), also known as Pak choi, is a Chinese cabbage belonging to Brassicaceae or Cruciferae family, which has been used in various dishes in East and South-east Asia. Chinensis is a variety of Chinese cabbage which doesn't form head and only have leaves with bulbs. Bok choy is known to have high bioactive components specially glucosinolates. Effect of glucosinolates have been studied on various animal model which have suggested that these compound helps in the prevention of various disease such as dyslipidaemia, cancer, hypertension, diabetes, neurological disorders and helps in maintaining muscle and bone health.

The main Objective of the study was analysed and compare the nutritional and phytochemical profile of the three stages of Bok choy plant, i.e., microgreen, baby green and the mature plant. This can help in understanding the inclusion of different stages of plant in the diet for maximizing the potential benefit of greens.

MATERIALS AND METHODS

Procurement of Samples: Bok choy microgreens were grown at household level at room temperature (27-30^{o} C & RH: 76%), where the potting mix soil and seeds were bought from local store and was used to grow the microgreens. The microgreens were harvested between 7th -10th day. The bok choy baby greens and mature greens were procured from the local market. All the three samples were washed and cleaned and were then dried in hot air oven for 3-4 hours at around 60^{o} C. Dried leaves were then used for different evaluation.

Proximate analysis: All the three Bok choy plant samples were evaluated for their proximate analysis as mentioned in the AOAC 2016 international guidelines. They were analysed for Macronutrients, i.e., carrbohydrates, proteins and fat, along with the percentage moisture and ash content.

Phytochemical and anatioxidant activity analysis: The phytochemicals were extracted from the dried plant samples using methanol in the ratio of 1:10. And following test were formored for the same.

Total Phenolic compounds: The plant methanolic extract was analysed for total phenolic content by the Folin-Ciocalteu method (F-C). The assay is based on the transfer of electrons from the phenolic compounds present in the sample in alkaline medium to form a blue coloured phosphomolybdic complexes which is determined spectroscopically at approximately 765 nm. In this method, Gallic acid is used as the comparison standard whose values are compared as mg of gallic acid is equivalent to one kg or one litre of extract of sample. (Sánchez-Rangel *et al.,* 2013*)*

Flavonoid content: Flavonoids are generally estimated calorimetrically by Aluminium chloride method. The basic principle of this method is that Aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids. It is generally estimated at 510 nm. (Kalita et al, 2013)

Alkaloid content: Alkaloids are naturally occurring nitrogen containing organic compounds. These compounds are known to have various medicinal properties such as analgesic, treatment of arrhythmias, etc. These compounds can be estimated using acid-base titration method. In this method, the HCl dissolves the alkaloids from methanolic extract. And this mixture is then neutralized using NaOH. *(*Debnath *et al.,* 2015)

Antioxidant Activity: The antioxidant activity of the plant samples was assessed by their scavenging ability to DPPH (2, 2-diphenyl l-picrylhydrazyl) stable radical. The main principle of DPPH method is that the reduction of DPPH in the presence of hydrogen donating antioxidant. The extract reduces the colour of DPPH by donating the hydrogen atom. The absorbance is read at 517 nm calorimetrically. (Kalita et al, 2013)

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RESULTS AND DISCUSSION

Proximate analysis: It is a system of analysis in which the components such as carbohydrates, protein, fat, moisture and ash of the food material are analyzed. The proximate analysis was carried out for the different stages of plant, i.e., microgreens, baby greens and mature greens, to assess the difference between the nutrition content of the different plant samples.

Table 1 depicts the mean results of proximate analysis of different stages of plants. The results were taken in triplicates and the mean is then taken to find the difference between the plant samples.

From the table 1, it was observed that the moisture content of baby greens (96.64 ±1.03)% was higher as compared to microgreens (93.71 ± 0.41)% and mature greens (94.15 ± 1.21)%. The results of mean ash content of the different stages of plant shows that the ash content of baby greens (1.10 ± 0.14)% is higher than mature (1.01 ± 0.18)% and microgreens (0.89 ± 0.09)%.

Table 1: Proximate analysis of different stages of plants

Parameters	Micro Greens	Baby Greens	Mature greens	p-value
Moisture (%)	93.71 ± 0.41[a]	96.64 ± 1.03[b]	94.15 ± 1.21[a]	0.01*
Ash (%)	0.89 ± 0.09	1.10 ± 0.14	1.01 ± 0.18	0.27[NS]
Protein (g/100gm)	26.02 ± 1.8[a]	19.31 ± 0.56[b]	24.21 ± 1.62[a]	0.003*
Fat (g/100g)	1.67 ± 0.58	1.00 ± 0.00	1.33 ± 0.58	0.29[NS]
Carbohydrates (g/100g)	23.02 ± 2.97[a]	18.97 ± 0.72[b]	13.62 ±0.72[c]	0.001*

Data expressed as Mean ±Standard deviation of Triplicates

*: indicates p-value is <0.05 by ANOVA

NS: indicates no significance difference between means

a,b and c: the same superscript in row indicates the same to each other and different superscripts in row indicates difference with each other, are significantly different (p<0.05) by DMRT.

The table also demonstrates that the microgreens were found to have slightly high protein content of (26.02 ± 1.8)g when compared to mature greens (24.21 ± 1.62)g and baby greens (19.31 ± 0.56)g. The mean fat content of microgreens (1.67 ± 0.58)g were found to be, whereas mature greens (1.33 ± 0.58)g and baby green (1.00 ± 0.00)g have lower fat content. The above table shows that the microgreens have higher carbohydrates content of (23.02 ± 2.97)g, but the baby greens (18.97 ± 0.72)g and mature greens (13.62 ±0.72)g have lower carbohydrate content.

A significant difference was observed (p<0.05) between the microgreens, baby greens and mature greens in moisture content, protein content and carbohydrate content. It is evident that microgreens have higher moisture, protein, fat and carbohydrate content than baby greens and mature greens. Whereas baby greens were found to have higher ash content, than mature greens and microgreens.

In a similar study by Zou et al., 2021 on Choy sum (Brassica rapa subsp. chinensis var. parachinensis) nutritional metabolites at different growth stages, it was observed that all the essential amino acid decreased as the plant grows from microgreen (15.8%) to 7.8% and 10.4% in seedlings and mature plant, respectively, which can suggest that there is a decline in the protein content as the plant grows. In another study, Waterland et al., 2017 has studied the mineral content of microgreens, baby greens and mature green of kale, whose result suggested that on dry weight basis microgreens have higher mineral content and on fresh weight basis baby greens have higher mineral content, which suggest that the ash content on the basis of dry weight and fresh weight is higher in microgreens and baby greens respectively. These results were in par with the present study, whose ash content was estimated on the basis of fresh weight.

Phytochemical Analysis: Green leafy vegetables are high in bioactive compounds including phenolic compounds, flavonoids and alkaloids. These compounds have essential antioxidant properties capable of producing beneficial effects in the body.

Table 2 summarizes the mean bioactive component content of microgreens, baby greens and mature greens. The table 2 illustrates the mean total phenolic content of different stages of plant. The results clearly indicates that the microgreens have higher phenolic content of (82.18 ± 1.08)mg, than baby (72.85 ± 0.38)mg and mature greens (68.92 ± 0.62)mg. It was also found that as the plant grows the phenolic content decreases from microgreens to mature greens. The mean content of flavonoids of different plant stages were presented from the table 2, states that the flavonoid content of microgreen (19.42 ± 4.29)mg was found to be higher, followed by mature greens (6.62 ± 5.00)mg and baby greens (2.93 ± 2.74)mg. The table 2 depicts the alkaloid content of plant stages. Mature greens (10.69 ± 0.81)mg have higher mean alkaloid content, followed by microgreens (10.37 ± 1.14)mg and baby greens (9.40 ± 0.43)mg.

Table 2: Bioactive component of different stages of plants

Parameters	Micro Greens	Baby Greens	Mature greens	P- value
Phenolics (mg/100g)	82.18 ± 1.08^{a}	72.85 ± 0.38^{b}	68.92 ± 0.62^{c}	<0.01*
Flavonoids (mg/100g)	19.42 ± 4.29^{b}	2.93 ± 2.74^{a}	6.62 ± 5.00^{a}	0.006*
Alkaloids (mg/100gm)	10.37 ± 1.14	9.40 ± 0.43	10.69 ± 0.81	0.22^{NS}

Data expressed as Mean ±Standard deviation of Triplicates

*: indicates p-value is <0.05 by ANOVA

^{NS}: indicates no significance difference between means

a,b and c :the same superscript in row indicates the same to each other and different superscripts in row indicates difference with each other, are significantly different (p<0.05) by DMRT

A significant difference (p<0.05) was observed between the plant stages in total phenolics and flavonoid content. Whereas no significance difference was observed between the samples for alkaloid content.

In a similar study which compared different stages of plant, i.e., microgreens, baby greens and mature greens of lettuce, found that the phenolic content was higher in microgreen (78%) than the adult plant. According to the study, it was observed that as the plant grows the phytonutrient composition gradually decreases, this can be due to the formation of more metabolic component synthesis during the germination process and then during the embryo development. But as the plant grows, these metabolites start to decrease. *(*Martínez-Ispizua *et al.,* 2022)

Antioxidant Activity: DPPH method is the most reliable and quick method to assess the scavenging activity of natural compounds. This method is based on the principle that the DPPH reduced to DPPH-H (non-radical form) by the hydrogen donating antioxidant compound. Table 3 represents the radical scavenging activity of microgreens, baby greens and mature greens with ascorbic acid as standard.

From the above table 3, it was observed that the percentage inhibition activity increases as the concentration increases. The concentration ranges from 200 mcg/ml to 1000 mcg/ml. The percentage scavenging activity was found to be 77.56 mcg/ml, 74.73 mcg/ml and 72.29 mcg/ml for microgreens, baby greens and mature greens in the concentration of 20mcg/ml and 85.04 mcg/ml, 84.35 mcg/ml and 78.47 mcg/ml in the concentration of 1000 mcg/ml respectively. The results depict that the percentage scavenging activity of plant decreases as it grows. Figure 1 depicts the difference in the percentage scavenging activity of ascorbic acid compared to the microgreens, baby greens and mature greens. The data from the fig 1 shows that, microgreens have slightly higher antioxidant activity as compared to the baby greens which again have higher water activity than mature greens.

Table 3: DPPH radical scavenging activity for different stages of plant

Concentration (mcg/ml)	Percentage of inhibition			
	Ascorbic acid	Microgreens	Baby greens	Mature greens
200	97.02	77.56	74.73	72.29
400	97.48	79.31	77.56	74.89
600	97.79	80.61	81.76	75.95
800	98.40	83.89	82.98	76.49
1000	98.45	85.04	84.35	78.47

In a similar study by Martínez-Ispizua *et al.,* 2022*,* the antioxidant activity was found out to be 71.5% and 71.1% for microgreens and baby greens respectively, and a lower antioxidant activity was found to be in adult stage (55.6%). The difference in the antioxidant activity was due to the difference in the phenolic and vitamin C content of microgreens and baby greens to that of mature greens.

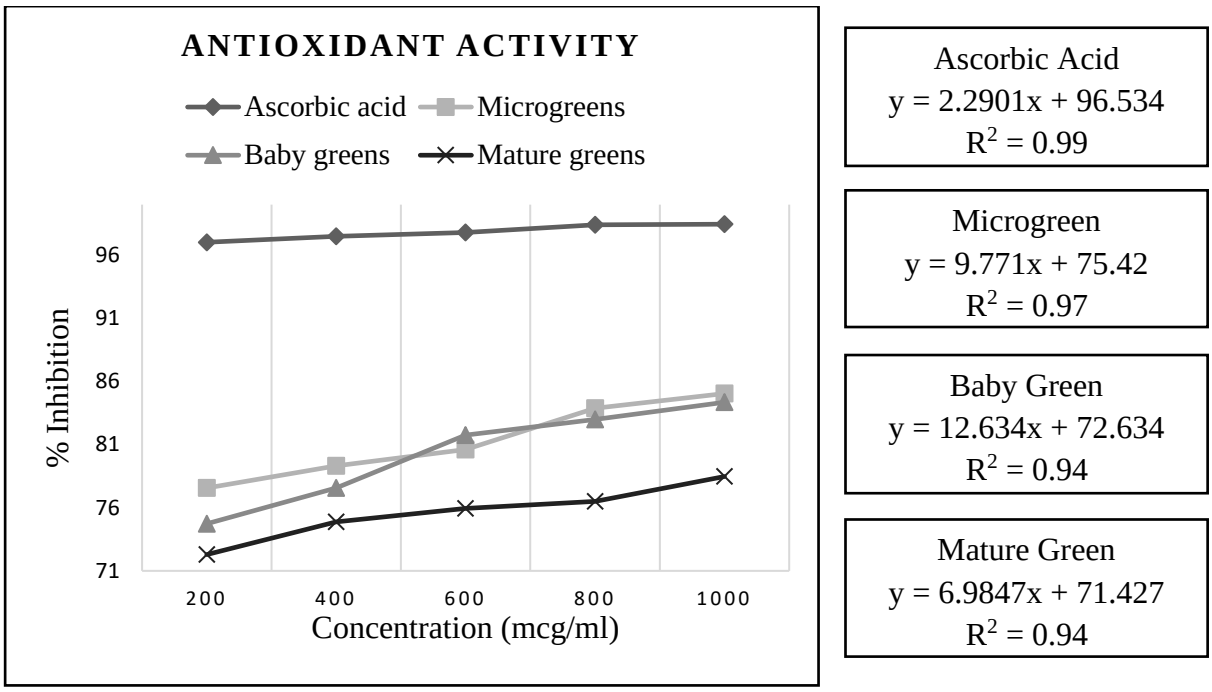

Fig 1: Antioxidant activity (percentage inhibition) of plant stages

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Received: 10th Jule 2022; August: 23th January 2023; First distribution: 18th September 2023