ISSN 1810-3030 (Print) 2408-8684 (Online)
Journal of Bangladesh Agricultural University
Journal home page: http://baures.bau.edu.bd/jbau, www.banglajol.info/index.php/JBAU

Post-harvest biodegradation of bioactive substances and antioxidant activity in microgreens

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ARTICLE INFO

Article history:
Received: 31 July 2018
Accepted: 10 August 2018

Keywords:
Microgreens, Bioactive substances, Vitamin, DPPH activity, Biodegradation

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Abstract

Low consumption of vegetables due to unavailability and unscrupulous application of chemicals and pesticides leads to malnutrition and chronic diseases. In this regards, microgreens technology could be a boon to mankind because they are grown in chemicals and pesticides free environment and offer functional food along with proper nutrient supply. But a little knowledge has been developed about the consumption period from harvesting, because being perishable in nature their bioactive substances and antioxidant activity get deteriorate. Therefore, the goal of this study was to explore the best consumption period of microgreens for obtaining the maximum bioactive substances (such as chlorophyll, ß-carotene and lycopene), vitamin C and the activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The experiment revealed that after 1st day of harvest the microgreens of four tested varieties (mustard, leaf mustard, radish and cabbage) showed the maximum bioactive substances such as total chlorophyll (8.22, 10.28, 7.62 and 7.63mg/100g respectively), ß-carotene (2.41, 2.88, 2.11 and 2.06 mg/100g respectively), lycopene (4.37, 5.24, 4.91 and 4.44mg/100g respectively), vitamin C (16.23, 13.17, 8.57 and 8.03mg/100g, respectively) and the activity of DPPH (0.75, 1.20, 2.90 and 3.57µg/ml respectively) whereas these substances deteriorated significantly on 3rd or 5th day of harvest. Considering all, it can be concluded that consumption of microgreens immediately after harvesting was the best time getting ample amount of bioactive substances, vitamin C and antioxidant (DPPH) activity.

Introduction

Nowadays, malnutrition is not only a common problem in Bangladesh but also considered one of the crucial global challenges to human being that can be checked (Miller and Welch, 2013). Low consumption of vegetables may be one of the vital reasons behind this problem. Vegetables are the good source of vitamins and bioactive compounds which are known to have protective benefits against malnutrition and oxidative stress-related diseases (Alarcon-Flores *et al.,* 2014; Martinez-Tomas *et al.,* 2012). Several studies have shown that diets rich in vegetables are associated with a reduction in the development of chronic diseases such as cancer, diabetes and cardiovascular disease (Alissa and Ferns, 2017). So, World Health Organization (WHO) stressed the need to increase consumption of fruits and vegetables because they are important components of a healthy diet (WHO, 2005).

In Bangladesh, per capita vegetables consumption is very poor due to low availability of vegetables and unauthorized application of chemicals/pesticides during growing period and post-harvest level that create the marketable vegetables more treacherous for maintaining good health. In this regards, interest in fresh and functional food is on the rise, obligated by the growing interest of purchasers for diets that support health and long life (Ebert, 2013). In these circumstances microgreens production and consumption could be one of the best possible solutions to counteract the stated problems because they also act as a functional food.

Microgreens are a new class of specialty leafy vegetables that are harvested just above the roots after the first true leaves have emerged and are consumed fresh. All microgreens are characterized by a central stem, cotyledonary leaf or leaves, and very young true leaves. Depending on the species, microgreens are harvested between 1 and 3 inches tall at 7-14 days in growth. Although small in size, microgreens can provide surprisingly intense flavors, vivid colors, and crisp textures and can be served as an edible garnish or a new salad ingredient. Infact, microgreens are newly emerging crops contain higher concentrations of bioactive compounds with antioxidants properties and vitamins up to 40 times more than their mature plants in the absence of biofortification and genetic engineering (Fahey *et al.,* 1997; Treadwell *et al.,* 2010; Xiao *et al.,* 2012). Thus, they are considered as functional foods which contain health promoting or disease preventing properties beyond the basic function of supplying nutrients. But, biodegradation of bioactive substances, vitamin and antioxidant properties after harvesting is a crucial problem in microgreens production, handling and consumption. No scientific investigation is undertaken in Bangladesh about the accurate consumption period of microgreens after harvesting which provide the maximum bioactive substances, vitamin and anti-oxidant properties. Therefore, the current research work was designed to investigate the post-harvest difference of bioactive substances, vitamin C and antioxidant properties in microgreens.

Materials and Methods

Initially four microgreens (mustard, leaf mustard, radish and cabbage) were selected from fifteen varieties by performing sensory attribute test (Xiao *et al.* 2015). Surface sterilized seeds of selected microgreens were then shown in pre drawn pots containing soil plus cowdung (1:1). The seedlings were grown for 12 days under ambient environmental conditions (Fig. 1) and after harvesting they were stored in air tight polybag in refrigerator with 4–6°C temperature. The bioactive substances (chlorophyll, ß-carotene and lycopene), vitamin C and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) activity were assayed on 1st (immediately after harvesting), 3rd and 5th day of harvesting which was considered as treatments. Treatments were replicated for three times. The entire experiment was conducted in Plant Physiology laboratory, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh.

Fig. 1. Growing of microgreens in growth chamber

Determination of chlorophyll, ß-carotene and lycopene: The leaf sample (1g of each microgreens) was extracted with 10 ml of chilled acetone solution in dark. After centrifugation at 4000 rpm for 10 minutes the absorbance of supernatants was taken at 453, 505, 645 and 663 nm wave length and calculated according to the equation depicted in Barros *et al.,* (2010).

Vitamin C content and DPPH activity assay: Vitamin C (vit C) content was determined by following a procedure described by Xaio *et al.,* (2012) with 2.6-dichloroindophenl and measuring the content by titrimetric method. Antioxidant activity was assayed using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) with some modifications (Sanja *et al.*,2009). Radical scavenging activity was calculated using the following formula,

$$\% \text{ radical scavenging activity} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

Here, OD= Optical density

Statistical analysis

The collected data were statistically analyzed by using Minitab 17. Tukey's LSD test was applied to compare the treatment means at 0.05 level of confidence.

Results

Variation of chlorophyll content

Chlorophyll (Chl) content (a, b and total) was the maximum at 1st day of harvest (just after harvesting) which degraded with time. Mustard, leaf mustard, radish and cabbage contained Chl a (5.72, 6.33, 4.86 and 4.68 mg/100g, respectively), Chl b (2.50, 3.95, 2.76 and 2.95 mg/100g, respectively) and total Chl (8.22, 10.28, 7.62 and 7.63 mg/100g, respectively) at 1st day of harvest. On 3rdday of harvest these varieties showed degradation in Chl a at 18.70, 12.00, 16.26 and 8.55%, respectively, Chl b at 4.8, 12, 15.58 and 11.19%, respectively and total Chl at 14.60, 23.93, 16.14 and 9.96%, respectively whereas on 5th day reduction was recorded in Chl a at 20.45, 25.90, 25.30 and 26.92%, respectively, Chl b at 11.2, 49.87, 26.45 and 24.75%, respectively and total Chl at 17.64, 35.12, 25.72 and 26.08%, respectively in comparison to 1st day of harvest (Table 1).

Table 1. Post-harvest change in chlorophyll content (mg/100g FW) of tested microgreens

	Chlorophyll a			Chlorophyll b			Total chlorophyll		
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5
Mustard	5.72±.06^{a}	4.55±.07^{b}	4.55±.08^{b}	2.50±.06^{a}	2.38±.02^{ab}	2.22±.02^{b}	8.22±.12^{a}	7.07±.11^{b}	6.77±.07^{b}
Leaf mustard	6.33±.21^{a}	4.69±.31^{b}	4.69±.10^{c}	3.95±.49^{a}	2.25±.06^{b}	1.98±.08^{c}	10.28±.70^{a}	7.82±.28^{b}	6.67±.07^{c}
Radish	4.86±.25^{a}	3.63±.37^{b}	3.63.12^{c}	2.76±.54^{a}	2.33±.02^{b}	2.03±.10^{c}	7.62±.79^{a}	6.39±.37^{b}	5.66±.01^{c}
Cabbage	4.68±.06^{a}	3.42±.08^{b}	3.42.06^{c}	2.95±.06^{a}	2.26±.04^{b}	2.22±.05^{c}	7.63±.12^{a}	6.87±.12^{b}	5.64±.02^{c}

Each value presents the means ± SE gained from three independent experiments. Values marked with the different letter within the columns differ significantly @ 5% level of probability. Here, FW= Fresh weight.

Level of ß-carotene content

Mustard, leaf mustard, radish and cabbage showed the maximum ß-carotene which was 2.41, 2.88, 2.11 and 2.06 mg/100g, respectively at 1st day of harvest. On 3rd day 16.18, 17.71, 19.91 and 9.22% reduction was observed while it was 22.40, 49.65, 28.91 and 16.50% on 5th day in mustard, leaf mustard, radish and cabbage, respectively in compared to 1st day of harvest (Fig. 2**)**.

Variation of lycopene content

The maximum lycopene content was recorded at 1st day of harvest which was 4.37, 5.24, 4.91 and 4.44mg/100g in mustard, leaf mustard, radish and cabbage, respectively. In comparison with 1st day of harvest, 3rd day of harvest showed a significant reduction (10.76, 19.66, 27.49 and 9.68% in mustard, leaf mustard, radish and cabbage, respectively) in lycopene content whereas 5th day of harvest showed reduction of 14.17, 41.03, 30.55 and 13.51% in mustard, leaf mustard, radish and cabbage, respectively (Fig. 3**)**.

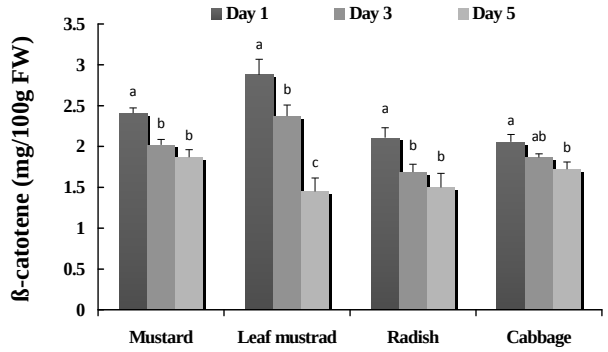

Fig. 2. Temporal variation in ß-carotene content of tested microgreens. The vertical bars represent the mean ±SE. Here, FW= Fresh weight

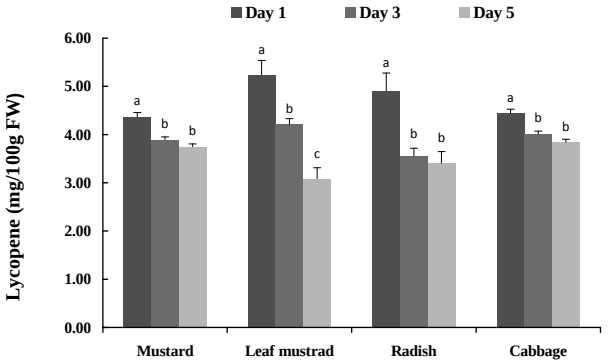

Fig. 3. Degradation in lycopene content of tested microgreens over time. The vertical bars represent the mean±SE. Here, FW= Fresh weight

Reduction in vitamin C content

Mustard, leaf mustard, radish and cabbage showed the maximum vitamin C which was 16.23, 13.17, 8.57 and 8.03mg/100g, respectively at 1st day of harvest. On 3rd day 14.36, 19.51, 26.49 and 25.28% reduction was observed while it was 31.05, 31.13, 44.34 and 41.84% on 5th day in mustard, leaf mustard, radish and cabbage, respectively compared to day 1 harvest (Fig. 4).

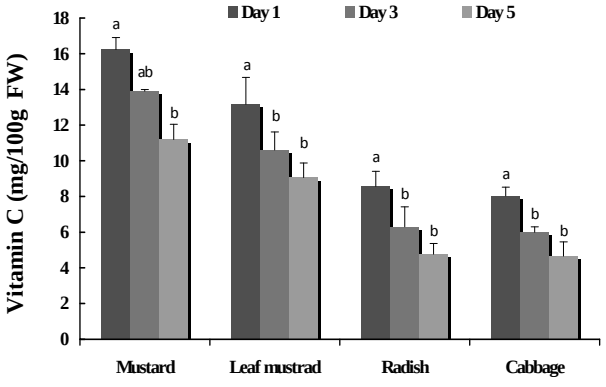

Fig. 4. Temporal dilapidation in vitamin C content of tested microgreens. The vertical bars represent the mean±SE. Here, FW= Fresh weight

Change in DPPH radical scavenging activity

DPPH radical scavenging activity was the maximum at immediately after harvesting. On 1st day of harvest the DPPH radical scavenging activity with an IC_{50} value was .75, 1.20, 2.90 and 3.57 µg/ml in mustard, leaf mustard, radish and cabbage, respectively which showed a decreased trend at 46.67, 72.5, 67.93 and 68.06% on 3rd day and 136, 141.67, 110.35 and 98.88% on 5th day in mustard, leaf mustard, radish and cabbage, respectively in comparison to day 1 (Fig. 5). The lowest IC_{50} value means had the highest DPPH radical scavenging activity.

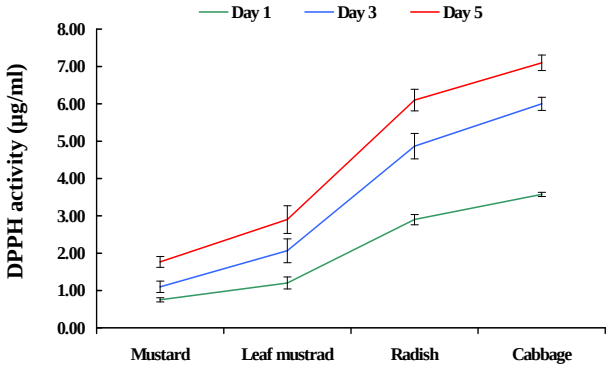

Fig. 5. Temporal dilapidation in DPPH activity of tested microgreens. The lines represent the mean±SE

Discussion

Cause of biodegradation of bioactive substances, vitamin C and antioxidant properties: Syntheses of bioactive substances (pigments) is believed to require proper supply of minerals, water and light influx that regulate several physiological and biochemical reactions and maintain enzymatic activity. Being harvested microgreens faces lack of minerals and water uptake as well as light harvesting efficiency that might seize the paths of physiological and biochemical reactions and enzymatic activity leading to further no production and/or degradation of bioactive substances. Degradation of chlorophyll was rationalized by the need of a senescing/harvested cell to detoxify the potentially phyto-toxic pigment (Hortensteiner and Krautler, 2011). The harvested microgreens do respire instead of photosynthesis that begins to produce several harmful toxic pigments as well as ROS. The bioactive substances like chlorophyll, ß-carotene and lycopene might take part in sequestration of toxic pigments in vacuoles and detoxify the ROS by degrading themselves. Vitamin C is thermo sensitive and non-stable vitamin. Though the harvested microgreens were stored in less than 10 ^{0}C, enzymatic activity for formation of ascorbic acid might be seized due to lack of proper supply of essentials. Besides due to constant respiration a huge ROS is produced within the cell. So to protect them, they might use endogenous vitamin C that leads to degradation of vit C. DPPH radical scavenging activity also decreased with time. Plants protect them from oxidative damage by their own antioxidant properties that might lead to lessen

the DPPH radical scavenging activity with time. Further molecular and biochemical experiments is needed for better understanding.

Health benefits of bioactive substances, vit C and antioxidant: Microgreens are the reservoir of bioactive substances such as chlorophyll, β-carotene and lycopene etc. Chlorophyll is an abundant source of nutrients along with wound-healing, anti-inflammatory (Lanfer-Marquz *et al.* 2005) and antioxidant properties that decreasesthe binding of carcinogens to DNA and thus helps in remove from body (Ferruzzi and Blakeslee, 2007). β-carotene and lycopene defendour body by detoxifying disease-causing free radicals and helps to maintain the health of our skin, eyes and immune system (Burri 1997; Kang *et al.* 2003). Usually, young edible microgreens are an excellent source of vitamin C, an antioxidant that protect our body from the harmful effects of free radicals thus cancer (Stratton and Godwin, 2011). Common cold and skin infection are successful cured by vitamin C **(**Hodges *et al*. 1969; Heimer *et al*. 2009**).** Microgreens also perform as a radical scavenger which was determined by DPPH radical scavenging activity. DPPH itself a radical that fixes with other free radicals and lead the free radical eliminate from body thus certify healthy life and longevity.

It may be concluded that, microgreens would be a good, cheap and immediate source for bioactive substances, vit C and antioxidant for maintaining good health and healthy life. But being perishable in nature, the study recommends consuming microgreens as soon as possible after harvesting.

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