KSÜ Tarım ve Doğa Derg 27 (2), 417-422, 2024
KSU J. Agric Nat 27 (2), 417-422, 2024
https://doi.org/10.18016/ksutarimdoga.vi.1216114

The Effect of Different Harvest Times on Phenolic Content and Antioxidant Activity in Some Microgreens

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ABSTRACT
Microgreens, which have only become popular during the last decades, are rich in phytochemicals, including phenolic compounds, which act as antioxidants. The study aimed to examine the effects of two different harvest times (cotyledon [embryonic leaves] and 1.5-true leaf stage) of five microgreens on the bioactive compounds in terms of antioxidant capacity and total phenolics. The total phenolic components ranged from 60.9 to 2153.2 mg GAE g^{-1} in cotyledon leaves, whereas the value varied from 96.2 to 2113.9 mg GAE g^{-1} in the true leaves of microgreens. Increases in the phenolic content of the first true leaves in dill and chia were detected as 57.8% and 29.6% compared to the cotyledon leaf. Among the cotyledon microgreens, the maximum phenolic content was detected in the garden cress. The antioxidant capacity of the cotyledon and true leaf stages ranged between 485.4±2.3-1985.67±24.9 µg g^{-1} and 508.87±5.3-2393.56±12.6 µg g^{-1}, respectively. The maximum antioxidant capacity was detected in radish, followed by garden cress. The biggest variation between the cotyledon and first true leaves in the study was observed for red beetroot. This study revealed the alteration in the phenolic content and antioxidant activity of five cultivars based on growth stages of cotyledonary and true leaves in microgreen form.

Food Science

Research Article

Article History
Received : 08.12.2022
Accepted : 27.10.2023

Keywords
Bioactive compound
Cotyledon leaf
Growth stage
Microgreen
Phytonutrient

Farklı Hasat Zamanlarının Bazı Mikroyeşilliklerin Fenolik İçerik ve Antioksidan Aktivitesi Üzerine Etkisi

ÖZET
Son yıllarda popüler hale gelen mikroyeşillikler, antioksidan rolü oynayan fenolik bileşikler de dahil olmak üzere fitokimyasallar açısından zengin besinlerdir. Çalışmanın amacı, beş mikroyeşilliğe uygulanan iki farklı hasat zamanının (kotiledon [embriyonik yapraklar] ve 1.5-gerçek yaprak aşaması) biyoaktif bileşikler üzerindeki etkilerini fenolik içerik ve antioksidan aktivite açısından incelemektir. Toplam fenolik içerik kotiledon yapraklarında 60.9 ile 2153.2 mg GAE g^{-1} arasında değişirken mikroyeşilliklerin gerçek yapraklarında bu değer 96.2 ile 2113.9 mg GAE g^{-1} arasında değişmiştir. Dereotu ve chia bitkilerinde ilk gerçek yaprakların fenolik içeriklerindeki artışlar kotiledon yapraktakine kıyasla %57.8 ve %29.6 olarak tespit edilmiştir. Kotiledon mikroyeşillikler arasında en fazla fenolik içerik bahçe teresinde tespit edilmiştir. Kotiledon ve gerçek yaprak dönemlerinin antioksidan kapasiteleri sırasıyla 485.4±2.3-1985.67±24.9 µg g^{-1} ve 508.87±5.3-2393.56±12.6 µg g^{-1} arasında değişmiştir. En yüksek antioksidan kapasite, turp ve ardından terede tespit edilmiştir. Çalışmada kotiledon ile ilk gerçek yapraklar arasındaki en büyük farklılık kırmızı pancarda gözlemlenmiştir. Bu çalışma, kotiledon ve gerçek yaprağın büyüme aşamalarına dayalı olarak beş mikroyeşillik çeşidinin fenolik içeriği ve antioksidan aktivitesindeki değişimi açıkça ortaya koymuştur.

Gıda Bilimi

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 08.12.2022
Kabul Tarihi : 27.10.2023

Anahtar Kelimeler
Biyoaktif bileşen
Kotiledon yaprak
Büyüme evresi
Mikroyeşillik
Fitokimyasal

Atıf Şekli: Barut Gök, S., Özdüven, F., Eryılmaz Açıkgöz, F. (2024). Farklı Hasat Zamanlarının Bazı Mikroyeşilliklerin Fenolik İçerik ve Antioksidan Aktivitesi Üzerine Etkisi. *KSÜ Tarım ve Doğa Derg 27* (2), 417-422. https://doi.org/10.18016/ksutarimdoga.vi.1216114

To Cite : Barut Gök, S., Özdüven, F., Eryılmaz Açıkgöz, F. (2024). The effect of different harvest times on phenolic content and antioxidant activity in some microgreens. *KSU J. Agric Nat 27* (2), 417-422. https://doi.org/10.18016/ksutarimdoga.vi.1216114

INTRODUCTION

Today, importance is given to healthy nutrition for the prevention of some diseases. As public health awareness increases worldwide, the demand for functional foods with multiple health benefits is also increasing (Marton et al., 2010; Messaoud & Boussaid, 2011; Yaşa et al., 2023). In addition to average nutritional values, microgreens are considered functional foods with human health-promoting and preventing disease properties. These plants are also a good source of mineral substances in the daily diet (Xiao et al., 2012).

Microgreens are also called vegetable confetti, in addition to being an exotic type of edible greens. They are young seedlings of herbs, grains, and vegetables with edible cotyledon leaves or 1.5-2 first true leaves (Xiao et al., 2012; Lenzi et al., 2019) (Fig. 1). They are different from sprouts due to their light requirement and especially a growing medium and have a longer growth cycle of 7-28 days (Di Gioia et al., 2017). In recent years, microgreens have gained the interest of producers due to their short growing cycle, ease of cultivation, production all year round, high potential profitability due to their popularity, and high sustainability in production (Kyriacou et al., 2020). Microgreens may contain higher amounts of phytochemicals, minerals, and vitamins than their mature counterparts. Microgreens are also considered to be a substitute for sprouts due to their rich nutrient content and more intense flavor (Zhang et al., 2021). Previous studies showed that microgreens are good sources of phytonutrients, such as carotenoids and polyphenols (Sun et al., 2013; Xiao et al., 2012; Xiao et al., 2013; Xiao et al., 2015). Although many leafy plants are consumed, limited knowledge is available about their microgreen forms (Yadav et al., 2019). However, most rare research documented the comparative analysis of nutritional components such as minerals, vitamins, and protein content of the microgreen stage alone (Pinto et al., 2015; Ebert et al., 2015). Only a few attempts reported the comparative analysis of bioactive compounds such as phenolics and antioxidants between the cotyledonary and true leaf stages of microgreens (Mishra et al., 2021; Yadav et al., 2019).

The physiological development periods of the products and the effects of growing conditions can also affect a plant's antioxidant capacity (Zhao et al., 2007).

Harvesting microgreens at the right time for the presence of bioactive compounds is one of the main production strategies. The cotyledon leaves, which form as a result of the germination of the seeds, continue their life by consuming the nutrients stored in the cotyledon leaf tissues until the first true leaves emerge. With the continuation of development, the plant starts photosynthesis with the occurrence of chlorophyll synthesis in true leaves. The effect of the nutrient content of the cotyledon leaves, containing stored nutrients, and the first true leaves where photosynthesis begins on the bioactive components will be revealed with this study. Several microgreens had higher concentrations of antioxidants, but the results were not generalizable (Yadav et al., 2019; Di Bella et al., 2020; Zhang et al., 2021).

The present study was undertaken to examine the effects of two different harvest times (cotyledon leaf stage (embryonic leaves) and 1.5 true leaf stage) of five microgreens on the bioactive compounds.

MATERIALS and METHODS

Production of microgreens

The research was carried out in laboratory conditions in April 2021. The research was designed as a randomized block experimental design (three replications). The plants used in the research were: garden cress (*Lepidium sativum* L.), radish (*Raphanus sativus* L.), red beetroot (*Beta vulgaris* L.), dill (*Anethum graveolens* L.), and chia (*Salvia hispanica* L.). Preservatives and pesticides were not used on the seeds of the plants used in the research.

The seeds of the plants were sown on April 6 in a 38x24x6 cm seedling tray filled with peat. Seed sowing was carried out quite densely and by the broadcasting method. Some specifications of the used peat are 160-260 mg L^{-1} N, 180-280 mg L^{-1} P2O5, 200-150 mg L^{-1} K2O, 80-150 mg L^{-1} Mg, pH: 6, 70% organic matter, and 35% C.

Sample preparation

Harvesting was carried out by cutting microgreens from the root collar area with a sharp, sterile knife. After the harvest, the plants were rinsed with distilled water, quickly frozen at -20±1 °C without losing time, and stored in these conditions until analysis. The seed sowing, germination, cotyledon leaf plant harvest, and true leaf plant harvest dates of the plants are given below (Table 1). Cotyledon-leaf plant harvest date was 8-14 days after sowing, depending on the plant type and the true-leaf plant harvest date was 14-16 days after sowing (Fig. 1, Fig. 2a, Fig. 2b).

Table 1. Seed sowing, germination, cotyledon-leaf plant harvest, and true-leaf plant harvest dates.
Çizelge 1. Tohum ekimi, çimlenme, kotiledon yaprak ve gerçek yaprak hasat tarihleri.

Microgreens	Scientific name	Seed Sowing Date	Germination Date	Cotyledon Leaf Plant Harvest Date	True Leaf Plant Harvest Date
Garden Cress	*Lepidium sativum* L.	April 6	April 8	April 14	April 22
Red Beetroot	*Beta vulgaris* L.	April 6	April 9	April 20	April 22
Dill	*Anethum graveolens* L.	April 6	April 11	April 20	April 22
Radish	*Raphanus sativus* L.	April 6	April 8	April 14	April 20
Chia	*Salvia hispanica* L.	April 6	April 10	April 15	April 20

Temperature (°C) and humidity (%) were measured during the study in controlled climate room conditions and the average temperature was 22 °C and average humidity was 65%.

Figure1. Microgreens used in the research
Şekil 1. Çalışmada kullanılan mikroyeşillikler

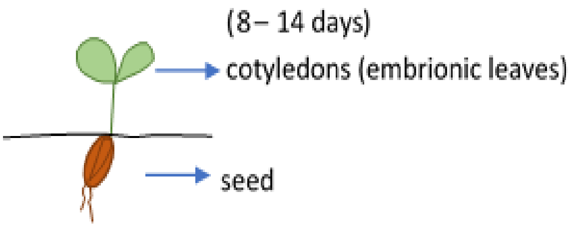

Figure2a. Cotyledon-leaf plant harvest
Şekil 2a. Kotiledon-yaprak hasadı

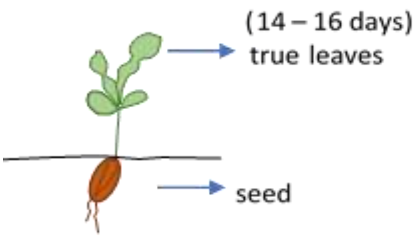

Figure2b. True-leaf plant harvest
Şekil 2b. Gerçek yaprak hasadı

Total phenolic content assay

The total phenolics in microgreens were extracted by shaking with 80% MeOH at room temperature for 24 h. The suspension was centrifuged at 10,000 rpm for 10 min at 25±1 °C, and the supernatant was collected. Phenolics were detected using the Folin–Ciocalteu reagent method by reading the absorbance at 765 nm with a UV–Vis spectrophotometer (UV-2500, Shimadzu, Japan) according to the method of Ainsworth and Gillespie (2007). Gallic acid was used as the standard, and the results are shown as milligrams of gallic acid equivalent (GAE)/100 g fresh microgreen weight (FW).

DPPH radical scavenging activity assay

The antioxidant capacity of different microgreen extracts (ME) was determined according to the method of Brand-Williams et al. (1995). Different concentrations of extracts (40-160 µL) were placed in 9-mL tubes, and 600 µl of molar DPPH* were added to each tube; the total volume was completed to 6 mL with MeOH. After mixing and incubating the tubes for 30 min at room temperature in the dark, absorbance was read at 517 nm against the control. By using the absorbance value, the % inhibition of DPPH radicals (I%) for each of the extracts was calculated by using equation (3). In equation (1), the absorption of the control (methanol instead of SE) is expressed as A control (0.340), and the absorption of the analyzed sample is expressed as A sample.

Inhibition %$=((A_{(control)}-A_{(sample)})/(A_{(control)}))\times 100$ (1)

Inhibition values were graphed against different concentrations for each extract, and linear regression analysis was applied to obtain the equation defining the curve. By using Eq.(1), the EC_{50} value was calculated. The EC_{50} value is the amount of antioxidants necessary to decrease the initial DPPH* concentration by 50%.

Statistical analysis

The results of the experiments were evaluated using SPSS 16 statistical software. ANOVA variance analysis and Duncan multiple comparison tests were conducted on the research results.

RESULTS AND DISCUSSION

Total Phenolic Content (TPC)

The analysis of variance results for the total phenolic content (TPC) of microgreens is presented in Table 2. Variation for TPC of cotyledon and true leaf extracts of microgreens was observed among the cultivars. Results expressed that the TPC ranged from 60.9 to 2153.2 mg Gallic acid equivalent (GAE) g^{-1} in the cotyledon leaf, whereas the value varied from 96.2 to 2113.9 mg GAE g^{-1} in the true leaf of microgreens. Cotyledon leaves of garden cress, red beetroot, and radish were found to have significantly higher sources for TPC than the first true leaves of these microgreens. On the contrary, an increase in the phenolic content of first true leaves in dill and chia was detected as 57.8% and 29.6% compared to the cotyledon leaf stage. Among the cotyledon leaf microgreens, the maximum phenolic content was detected in garden cress (2153.2±9.5 mg GAE g^{-1}), followed by red beetroot (905.2±8.3 mg GAE g^{-1}). Similarly, the highest phenolic content of the true leaf microgreens was detected in garden cress (2113.9±8.5 mg GAE g^{-1}) followed by red beetroot (253.2±7.5 mg GAE g^{-1}). However, decreasing trends of 1.8% and 72.02% were detected, respectively. The findings are in accordance with the results of Mishra et al. (2021), who detected the TPC of six Indian mustard genotypes. Although the results for TPC of cotyledon leaf and true leaf stages were not statistically different, the true leaf extracts of four genotypes had higher TPC than their cotyledon leaf extracts. The variation in the phenolics of the studied microgreens could be attributed to the presence/abundance/absence of different kinds of phenolics from cultivar to cultivar. In addition to this, Navarro et al. (2008) & Delgado et al. (2004) reported that the concentration of phenolic compounds in the plant varieties depends on the growth steps. In fact, the young plant has a unique blend of phytonutrients giving it a much higher bioavailability of nutritional components than in mature stages (Yadav et al., 2019). However, the phenylalanine ammonia-lyase (PAL) activity could also be important in the plant based on the variety and growth stage (Medda et al., 2020). The first enzyme in the synthesis of most phenolics is phenylalanine ammonia-lyase (PAL), which catalyzes the deamination of L-phenylalanine, and the cinnamic acid thus formed is further used for the synthesis of other phenolic compounds like phenolic acids, lignin, flavonoids and condensed tannins in the plant (Medda et al. 2020).

Table 2 TPC of different microgreen extracts (mg GAE g FW^{-1}).
Çizelge 2. Farklı mikroyeşillik ekstraklarının toplam fenolik içerikleri (mg GAE g FW^{-1})

		Garden Cress	Red Beetroot	Dill	Radish	Chia
Cotyledon		2153.2±9.5 ^{a}	905.2±8.3 ^{a}	60.9±2.6 ^{b}	247.6± 5.0^{a}	81.2±0.6 ^{b}
True leaf		2113.9±8.5 ^{b}	253.2±7.5 ^{b}	96.2±4.5 ^{a}	175.6±8.1 ^{b}	105.2±2.3 ^{a}
	CV %	0.06	0.32	2.89	1.33	2.00
	LSD	5.17	6.57	7.98	9.93	6.57
	Prob > F	0.0009**	0.001**	0.0027**	0.0010**	0.0040**

Values are the mean of three replicates ± standard deviation; different letters in the same column represent statistically different results.

DPPH radical scavenging activity

The analysis of variance results for the antioxidant activity of microgreens is presented in Table 3. The DPPH radical scavenging activities of methanol extracts obtained from the cotyledon, and true leaf stages ranged between 485.4±2.3-1985.67±24.9 µg g^{-1} and 508.87±5.3-2393.56±12.6 µg g^{-1}, respectively. Cotyledon leaves of all microgreens, except red beetroot, exhibited higher antioxidant activity than the first true leaves. Among the cotyledon microgreens, the maximum antioxidant activity was detected in radish (485.4±2.3 µg g^{-1}) followed by garden cress (698.04±12.8 µg g^{-1}). In accordance with these results, Xiao et al. (2015) reported that the highest concentration of total phenolics was found in China rose radish regarding the phytonutrient concentrations of the studied microgreen species (Xiao et al., 2015). The biggest alteration between the cotyledon and the first true leaves in the present study was observed for red beetroot. Similarly, the highest concentration of antioxidant capacity in young lettuce seedlings was observed in 7 days following germination and rapidly decreased by more than 60% in about 14 days. There were comparisons of antioxidant contents and capacity between microgreens and their mature counterparts (Pinto et al., 2015; Choe et al., 2018; Zhang et al., 2021). Many studies showed higher nutritional quality and higher concentration of antioxidants in microgreens than in mature plants. Furthermore, the results were not generalizable (Zhang et al., 2021). However, this is the first study that reveals the variation in bioactive compounds, such as antioxidant activity, in microgreens in terms of cotyledonary and first true leaves.

CONCLUSION

Studies demonstrated that microgreens contain much higher levels of functional components such as phenolics and antioxidants than the amounts found in mature leaves. Although much research has focused on the mineral content and some properties of the plants at different growth stages, there is limited knowledge about the change in bioactive compounds of different growth stages in terms of microgreens and mature leaves. However, no studies reported the change in bioactive compounds of cotyledonary leaf and true leaf stages in terms of microgreens. Cotyledon leaves of garden cress, red beetroot, and radish were significantly higher sources of total phenolic components than the first true leaves of microgreens. However, cotyledon leaves of all microgreen species, except red beetroot, exhibited higher antioxidant activity than their first true leaves. The variation in phenolics of studied microgreens could be attributed to the presence/abundance/absence of the different kinds of phenolics from cultivar to cultivar. This study demonstrated the changes in the phenolic components and antioxidant capacity of five cultivars in different growth stages of the microgreen form.

Table 3 The antioxidant activity of different microgreen extracts (EC_{50} µg g^{-1})
Çizelge 3. Farklı mikroyeşillik ekstraktlarının antioksidan aktivitesi (EC_{50} µg g^{-1})

		Garden Cress	Red Beetroot	Dill	Radish	Chia
Cotyledon		698.04± 12.8 ^{b}	1567.83.2±183.1 ^{a}	1985.67±24.9 ^{a}	485.4±2.3 ^{b}	1124.25±170.5 ^{a}
True leaf		1129.13±15.5 ^{a}	508.87±5.3 ^{b}	2393.56±12.6 ^{b}	791.33±47.9 ^{a}	1327.74±57.0 ^{a}
	CV %	2.19	12.84	0.40	5.56	6.55
	LSD	7.30	468.20	30.44	124.60	282.32
	Prob > F	0.0014**	0.0104*	0.0003**	0.0088*	0.09^{ns}

Values are mean of three replicates ± standard deviation; different letters in the same column represent statistically different results.

Author Contributions

SBG: Conceptualization; data curation; formal analysis; investigation; methodology; software; supervision; validation; visualization; writing – original draft; writing. FÖ: Data curation; formal analysis; investigation; methodology. FEA: Conceptualization; data curation; formal analysis; investigation; methodology; supervision; writing – original draft; writing.

Conflict of Interest

The authors declare that they have no conflict of interest.

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