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The effects of wheatgrass length on antioxidant activity and total phenolic content in wheatgrass (*Triticum* spp.)

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Abstract: This study was carried out with the aim of determining the effects of wheatgrass lengths on antioxidant activity and total phenolic content of wheatgrass belonging to different cultivars (*Triticum* spp.) in laboratory conditions in 2017. When the wheat seedlings grown in plastic containers under soilless conditions reached three levels of wheatgrass length (5–6, 8–9, and 11–12 cm), the seedlings were cut from the root collar and dried in an oven. As a result of analysis of the dried material, it was found that the cut lengths of wheatgrass significantly ($P < 0.01$) affected antioxidant activity and total phenolic content of wheatgrass. Ten wheat cultivars belonging to *Triticum monococcum* L., *T. durum* L., and *T. aestivum* L. were used. Wheatgrass was cut at three lengths (5–6, 8–9, and 11–12 cm). It was found that the wheatgrass lengths had a significant ($P < 0.01$) effect on antioxidant and total phenolic content of wheatgrass. In addition, in terms of these traits, the most suitable wheatgrass length for cutting changed depending on the wheat species and also cultivars. The highest ($P < 0.01$) antioxidant activity and total phenolic content (TPC) were also determined at three wheatgrass lengths in cultivar Sarıbaşak. The average values obtained from Sarıbaşak wheatgrass were 31.0% for DPPH-free radical scavenging activity and 51.6 mg GAE^{-1} dry weight for TPC. The results of the analysis suggested that Sarıbaşak is the most promising cultivar in terms of both antioxidant activity and TPC in wheatgrass.

Key words: Cultivar, DPPH-free radical scavenging activity, phenolic content, wheatgrass

1. Introduction

Young leaves of wheat plant (*Triticum* spp.), which has the highest cultivation area in the world (http://www.fao.org/faostat/en/) due to its wide adaptability, are evaluated in human consumption and in livestock as grass juice or dried dust (Mujoriya and Bodla, 2011). Today, wheatgrass juice, consumed as a beverage in many countries, is also used to make a dessert in some regions of Turkey (Tangüler et al., 2015).

Wheatgrass is the young grass of a wheat plant. Grass of *Triticum aestivum* contains high concentrations of chlorophyll, amino acids, minerals, vitamins, and enzymes (Walters, 1992; Mujoriya and Bodla, 2011). Wheatgrass juice is known as a superfood because of its benefits for human health (Chomchan et al., 2016).

It is reported that wheatgrass has shown anticancer activity (Singhal et al., 2012; Shakya et al., 2014), antiulcer activity (Srinivas et al., 2013), antiinflammatory activity (Nalini et al., 2011), antioxidant activity (Shakya et al., 2014), antiarthritic activity (Sharma et al., 2013), antimicrobial activity (Sundaresan et al., 2015), and a low-density lipoprotein-lowering effect (Afroz et al., 2014). Wheatgrass juice also supports the production of red blood cells and encourages reliable tissue cell formation (Rana et al., 2011). These positive effects are due to the high level of chlorophyll content (Lai, 1979). Wheatgrass is regarded as "green blood" because of its close similarity to hemoglobin due to its chlorophyll content and treatment effects in clinical conditions arising from hemoglobin deficiency and other chronic conditions (Singhal et al., 2012). Therefore, the wheatgrass juice recently focused on is considered to be a promising herbal drug (Chauhan, 2014).

Comparing wheatgrass and paddy grass, antioxidant activity was higher in paddy grass than wheatgrass (Chomchan et al., 2016). In another study comparing wheat and barley grass, the antioxidant values of wheatgrass were much higher (Wangcharoen and Phimphilai, 2016). These and similar studies show that the antioxidant activity of grass leaves differs according to cereal species, and *Triticum* species are more distinctive and beneficial than others.

Positive changes in consumers' life standards present a great opportunity in the development of the functional

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food industry (Hasani et al., 2016). The tendency of consumers to choose more useful products is highly effective in the diversifying of end products. In a study, it was found that wheatgrass juice was superior to apple juice and sour cherry/apple juice in terms of total chlorophyll B mg L^{-1}, total chlorophyll mg L^{-1}, vitamin C g L^{-1}, total polyphenolic index, antioxidant activity, and minerals including P, Ca, Mg, Fe, and Zn (Hasani et al., 2016). Therefore, wheatgrass has a potential to be appreciated as a raw material in the fruit processing industry. Blending wheatgrass juice with kombucha (Sun et al., 2015) or pomegranate (Kashudhan et al., 2017) is recommended as a beverage for more stable and higher antioxidant activity. Wheatgrass-blended kombucha has higher and more stable antioxidant activity and this blending might be recommended for consumption as a novel beverage (Sun et al., 2015). Hence, effective, healthy, and functional therapeutic drinks can be produced from beverages obtained by combining different mixtures (Kashudhan, et al., 2017).

As the possibilities of wheatgrass for utilization in the food industry increase, which has positive effects on health, wheat may become a more valuable plant. The trend of assessing wheat in this direction will also increase the need for quality raw materials. It is important to determine the appropriate cultivars for the industry to meet this need.

This study aimed to determine the antioxidant activity and total phenolic content (TPC) of wheatgrass belonging to different wheat species and promising cultivars in terms of these two traits for the industry.

2. Materials and methods

2.1. Plant material and lab conditions

The wheat materials included five cultivars, Ekiz (*T. aestivum* L.), Konya-2002 (*T. aestivum* L.), Karahan-99 (*T. aestivum* L.), Meram-2002 (*T. durum* L.), and Selçuklu-97 (*T. durum* L.), from the Bahri Dağdaş International Agricultural Research Institute; four cultivars, Altınbaşak (*T. aestivum* L.), Altınöz (*T. aestivum* L.), Adana-99 (*T. aestivum* L.), and Sarıbaşak (*T. durum* L.), from the Eastern Mediterranean Agricultural Research Institute; and a local material (siyez wheat). Siyez wheat (*T. monococcum* L., einkorn) was collected from a siyez wheat field located in the İhsangazi district of Kastamonu in Turkey.

The wheat seeds were germinated in the laboratory (min 20.0 °C and max 23.0 °C) under soil-free conditions and in plastic containers (21 × 11 cm) in 2017. One hundred seeds were placed in each plastic container. The trial was conducted in triplicate and 30 seedlings were used for every replication. This study is focused on revealing the potentials of cultivars in terms of the traits studied. Therefore, the research was carried out under natural light conditions in a laboratory, and no artificial light or fertilizer applications were performed. The seeds were irrigated with distilled water daily. Cutting of all the seedlings took place before the tillering period. The seedlings were cut with scissors at the point of connection with the seed when they reached the planned length (5–6, 8–9, and 11–12 cm). The cut material was dried at 40 °C and then powdered by chopper and kept at –20 °C until analysis (Kulkarni et al., 2006; Rocha et al., 2011).

2.2. Extraction

Each leaf sample, dried and weighed to 0.1 g, was extracted in 10 mL of methanol (80%) at 40 °C using an orbital shaker for 1 h (Kołodziej, 2012). The sample-solvent mixture was centrifuged at 4000 rpm for 20 min. The supernatants were then separated from the mixture. Supernatants of each sample were used in the analysis of antioxidant activity and TPC.

2.3. Antioxidant activity

Antioxidant activity of samples was estimated by DPPH method. The free radical scavenging effects of extracts of wheatgrass on DPPH (2.2-diphenyl-1-picrylhydrazyl) were determined by modifying the method of Brand-Williams et al. (1995). The methanolic solution of DPPH was prepared by dissolving 100 μM of DPPH in 100 mL of methanol (80%), and 0.5 mL of DPPH solution was used for every 1.5 mL of extract solution. These solutions were mixed and left at room temperature for 60 min in the dark. Values of absorbance belonging to samples were measured at a wavelength of 517 nm in a UV-spectrophotometer. The free radical scavenging activity of the sample extracts was calculated by the following formula:

$$\% \text{ DPPH free radical scavenging activity} = [(Ac - Ae) / Ac] \times 100$$

Here, Ac is the absorbance reading of the control and Ae is the absorbance reading in the presence of sample extract.

2.4. Total phenolic content (TPC)

The TPC of samples was determined using the Folin–Ciocalteu method (Waterhouse, 2002). Mixtures of 20 μL of sample (supernatant), 1580 μL of distilled water, 100 μL of Folin–Ciocalteu reagent, and 300 μL of Na_2CO_3 (sodium carbonate) were shaken in test tubes at 50 °C for 15 min. Subsequently, these mixtures were kept in the dark for 1 h and then values of absorbance belonging to samples were measured at 765 nm of wavelength in the spectrophotometer. The total phenolic content was calculated as mg of gallic acid equivalents (GAE) per gram of dry weight of the samples by the calibration curve obtained using the spectrophotometer.

2.5. Statistical analysis

Statistical analysis of the values was performed in triplicate according to a completely randomized design in split plots. The statistical program JMP (a software program created by SAS Institute Inc. and used for statistical analysis) was used for the analysis of the data and Tukey's honestly significant difference multiple comparison test was applied to compare the means.

3. Results

The elapsed time for the cutting depending on wheat cultivars and wheatgrass length is given in Table 1. Among the 10 cultivars, the cultivar Ekiz showed faster growth in reaching the planned cutting length than the others. This situation is a positive trait in terms of earliness. However, this cultivar had the lowest values for antioxidant activity and TPC among all cultivars.

When the data obtained according to wheat cultivars were sorted by species, the mean values belonging to *T. monococcum* L. (einkorn), *T. durum* L., and *T. aestivum* L. were calculated as 22.6%, 25.8%, and 19.5% for antioxidant activity and 34.0, 39.2, and 30.7 mg GAE g^{-1} dry weight for TPC values, respectively ($P < 0.01$) (Figure 1).

The wheat cultivars used in the study showed significant ($P < 0.01$) differences in terms of the antioxidant activity and TPC of their grasses. The highest antioxidant values were obtained from the cultivars Sarıbaşak (31.0%) and Selçuklu-97 (25.8%), and siyez wheat (22.6%). The highest TPC was obtained from Sarıbaşak (51.6 mg GAE g^{-1} dry weight) (Tables 2 and 3).

The effect of wheatgrass length on these traits was also significant ($P < 0.01$). As the cutting length increased, both TPC and antioxidant values of the cultivars increased. When the wheatgrass length reached 8–9 and 11–12 cm, there was no difference in antioxidant activity. The average antioxidant activity of the wheatgrass was the lowest when the wheatgrass length was 5–6 cm. On the other hand, the highest value for TPC (36.4 mg GAE g^{-1} dry weight) was found in 11–12 cm length while the lowest TPC value was realized in 5–6 cm length (30.9 mg GAE g^{-1} dry weight).

On the other hand, the effect of wheatgrass length for cutting on antioxidant activity and TPC varied depending on the cultivars. This difference was found statistically significant ($P < 0.01$). When the wheat cultivars with the highest average values were evaluated according to wheatgrass lengths, it was observed that the highest values were obtained only from the Sarıbaşak in terms of both antioxidant activity and TPC. Sarıbaşak was also the least affected cultivar by the length in terms of TPC. In the same way, the highest values were obtained in terms of antioxidant activity and TPC when the grass length reached 8–9 cm in siyez wheat and 11–12 cm in cultivar Selçuklu-97.

Considering the averages of the cultivars, it was determined that there was a very significant correlation ($P < 0.01$) between antioxidant activity and TPC ($R = 0.946$) (correlation coefficient = 0.946) in wheatgrass. The R^2 value was calculated as 0.894. A high value of R^2 indicates a very strong correlation (Figures 2 and 3).

Table 1. The elapsed time (days) for the cutting depending on wheat cultivars and grass length.

Cultivar	Length of wheatgrass		
	5–6 cm	8–9 cm	11–12 cm
Siyez	5	7	9
Sarıbaşak	6	8	12
Selçuklu- 97	6	7	9
Meram 2002	6	8	11
Karahan 99	6	7	9
Konya 2002	6	7	9
Ekiz	5	6	9
Altınbaşak	6	8	12
Adana-99	6	7	9
Altınöz	6	8	10

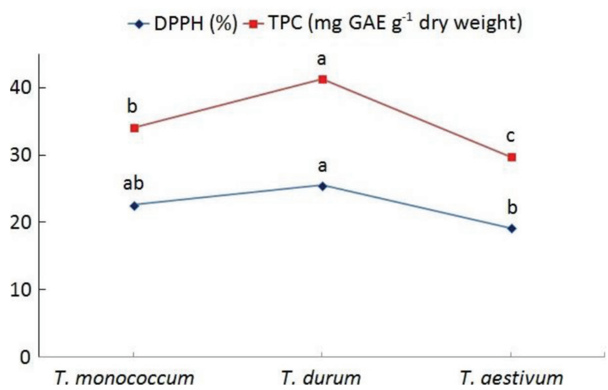

Figure 1. The average values of DPPH-free radical scavenging activity and total phenolic content (TPC) according to *Triticum* species ($P < 0.01$).

4. Discussion

Studies on various plant species such as *Camellia sinensis* (Benzie and Szeto, 1999), *Chenapodium quinoa* (Nsimba et al., 2008), *Rubus fruticosus* (Ryu et al., 2016), and *Triticum aestivum* (Kulkarni et al., 2006) revealed correlations between antioxidant capacity and TPC. A similar correlation was found in our present study as well as in previous studies on wheatgrass. Shakya et al. (2014) reported that methanol extracts were good solvent extracts and these extracts showed a positive correlation between total phenolics and flavonoids and other phytochemical constituents and the antioxidant properties. However, the level of this correlation varies depending on the studies.

Table 2. DPPH-free radical scavenging activity values (%) and standard deviation values (SD) of wheatgrass according to wheat cultivars and grass length (GL).

Cultivar (C)	Grass Length (GL)						Average	Statistical calculations
	5–6 cm	SD	8–9 cm	SD	11–12 cm	SD		
Siyez	18.3 c–f	1.99	26.0 a–e	2.85	23.4 a–f	4.52	22.6 ab	CV = 17.8% C** (F = 5.7) GL** (F = 9.9) C × GL** (F = 3.3)
Sarıbaşak	36.0 a	1.73	29.8 a–c	5.56	27.3 a–d	4.86	31.0 a	
Selçuklu-97	19.0 c–f	3.17	26.7 a–d	5.56	31.6 ab	1.71	25.8 ab	
Meram-2002	19.5 b–f	3.56	16.9 d–f	3.77	25.4 a–e	3.45	20.6 b	
Karahan-99	19.9 b–f	3.83	21.9 b–f	1.59	17.1 d–f	3.83	19.6 b	
Konya-2002	18.4 c–f	3.45	18.9 c–f	2.28	24.9 a–f	4.93	20.7 b	
Ekiz	19.3 b–f	5.60	15.8 d–f	4.94	21.0 b–f	3.85	18.7 b	
Altınbaşak	15.5 d–f	4.44	20.6 b–f	4.38	21.5 b–f	1.71	19.2 b	
Adana-99	12.7 f	1.48	22.0 b–f	4.11	17.9 c–f	0.75	17.5 b	
Altınöz	13.5 ef	0.49	16.7 d–f	4.74	25.5 a–e	2.26	18.6 b	
Average	19.2 b		21.5 ab		23.6 a		21.4	

*, ** level of significance, P < 0.05*, P < 0.01**; means with the same letter are not statistically significant; CV – coefficient of variation, F – calculated F value.

Table 3. Total phenolic content (TPC) values (mg GAE g^{-1} dry weight) and standard deviation values (SD) of wheatgrass according to wheat cultivars and grass length (GL).

Cultivar (C)	Grass length (GL)						Average	Statistical calculations
	5–6 cm	SD	8–9 cm	SD	11–12 cm	SD		
Siyez	27.4 f–i	1.99	42.7 a–d	2.85	32.0 c–i	4.52	34.0 bcd	CV = 11.6% C** (F = 47.5) GL** (F = 15.3) C × GL** (F = 7.3)
Sarıbaşak	55.0 a	1.73	48.7 ab	5.56	51.2 ab	4.86	51.6 a	
Selçuklu-97	29.8 d–i	3.17	40.8 b–e	5.56	44.3 a–c	1.71	38.3 b	
Meram-2002	25.1 g–i	3.56	23.4 g–i	3.77	34.9 c–h	3.45	27.8 e	
Karahan-99	41.2 b–e	3.83	32.8 c–i	1.59	27.9 f–i	3.83	33.9 bcd	
Konya-2002	26.5 f–i	3.45	35.6 c–g	2.28	42.3 a–e	4.93	34.8 bc	
Ekiz	31.5 c–i	5.60	21.8 i	4.94	33.4 c–i	3.85	28.9 de	
Altınbaşak	26.5 f–i	4.44	33.9 c–i	4.38	32.0 c–i	1.71	30.8 cde	
Adana-99	22.1 hi	1.48	29.7 e–i	4.11	27.9 f–i	0.75	26.5 e	
Altınöz	23.6 g–i	0.49	25.2 g–i	4.74	38.4 b–f	2.26	29.1 de	
Average	30.9 c		33.5 b		36.4 a		33.6	

*, ** level of significance: P < 0.05*, P < 0.01**; means with the same letter are not statistically significant; CV – coefficient of variation, F – calculated F value.

The strong level of correlation obtained from the current study showed similarities to the results obtained by Kulkarni et al. (2006) and Rao et al. (2013).

Variation in the values of TPC and antioxidant activity of wheatgrass powder depending on different drying methods was determined as 23.3–24.7 mg GA g^{-1} dry weight and 33.0%–58.3%, respectively (Singh, 2016). Significant differences (P < 0.05) were found in terms of these traits in another study comparing 2 siyez wheat and 4 bread wheat cultivars (Karakaş, 2016). According to Karakaş (2016), the total phenol content ranged from 5.12 to 27.14 mg GAE g^{-1} dry weight and antioxidant activity ranged from 13.98 to 63.03 mg L^{-1} in the used wheat materials. The highest antioxidant values were obtained

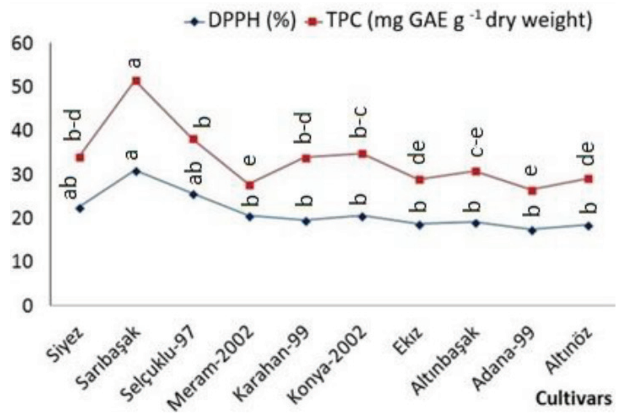

Figure 2. DPPH-free radical scavenging activity and total phenolic content (TPC) of wheatgrass of different cultivars ($P < 0.01$).

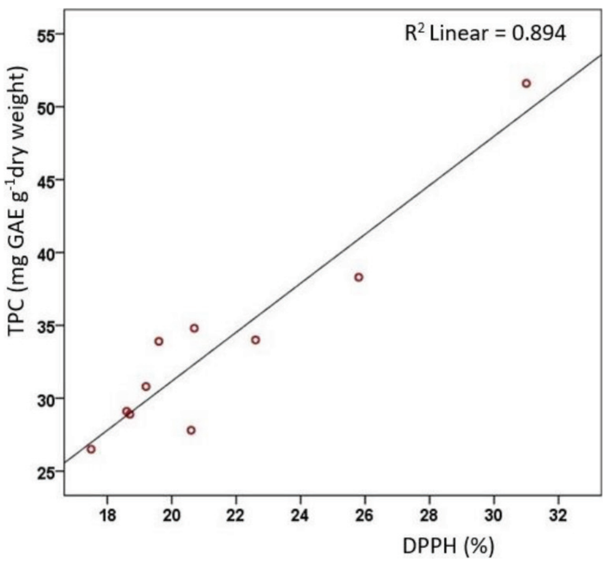

Figure 3. Correlation ($P < 0.01$) between DPPH-free radical scavenging activity and total phenolic content (TPC) in wheatgrass.

from both siyez wheats. Values of antioxidant activity in our study were below the values reported by Singh (2016), but TPC values were above the values reported by Singh (2016) and Karakaş (2016). In addition, Karakaş (2016) found that siyez wheat was superior to the other in terms of antioxidant value. Similar to these results, siyez wheat was involved in the statistical group with the highest antioxidant activity in the present study.

The lowest values found for *T. aestivum* in the present study are similar to findings reported by Saini et al. (2017). In research conducted by Saini et al. (2017), the highest antioxidant activity was obtained from the species *T. dicoccum*, followed by *T. durum* and *T. aestivum*. Not only the wheatgrass of *T. dicoccum* but also its grains are rich in antioxidants and TPC. Serpen et al. (2008) determined that grains of emmer wheat (*Triticum dicoccum* Schrank) genotypes had higher total antioxidant activity, total phenolics, and flavonoids among emmer and einkorn (*Triticum monococcum* L.) wheat landraces and 2 bread wheat cultivars grown in Turkey. The fact that *T. dicoccum*, which is tetraploid ($2n = 4x = 28$), has higher values in terms of these traits is parallel to the results obtained from the tetraploid wheats, which are also superior in our study.

In our study, the highest average antioxidant value was obtained from 8–12 cm and the highest TPC was obtained from 11–12 cm of wheatgrass length. During research conducted by Agrawal et al. (2015), wheatgrass was cut at different lengths between 17.8 and 30.5 cm, and then the grass juice prepared was compared in terms of mineral substances and antioxidants. The highest nutrient and antioxidant levels were determined at a length of 22.9–25.4 cm ($P < 0.05$). Our study differs from this study, which shows that the antioxidant content is also high at a time when the wheatgrass is longer. Özköse et al. (2016) determined high values of DPPH (90.3%–94.4%) and TPC (289–443 mg GAE L^{-1}) in grass juice obtained from wheat. The differences in these previous studies could be due to the cutting length, the genetic characteristics of the cultivars, and environmental conditions.

In our work, antioxidant values and TPC also increased as the wheatgrass length increased in general. The increase in wheatgrass length could be linked with the increase in the age of the seedling along with the growing plant. Generally, as the time elapsed for cutting in wheat (*T. aestivum* L.) increases, the phenolic components also increase (Kardas and Durucasu, 2014). On the other hand, there are also research results showing that high antioxidant activity could be obtained from 15-day-old wheatgrass belonging to *T. aestivum* and *T. durum* (Kulkarni, 2006; Kardas and Durucasu, 2014).

It should be considered that high yield in grass juice of quality wheat cultivars can be obtained via some cultural practices such as carbon dioxide (Karaşahin, 2015), seaweed treatments (Karaşahin, 2017), adequate cutting length (Ergün, 2011; Agrawal et al., 2015), or suitable cutting time (Kulkarni, 2006; Kardas and Durucasu, 2014; Lae and Oo, 2014).

In this study, the wheatgrass lengths for cutting significantly influenced the antioxidant activity and TPC of the wheatgrass. The highest average values were obtained in the levels of wheatgrass length of 8–9 cm and 11–12 cm for DPPH-free radical scavenging activity and wheatgrass length of 11–12 cm for TPC. The effect of wheatgrass length showed differences depending on wheat cultivars. The highest values in terms of these two traits were obtained from Selçuklu-97 and siyez wheat, and in particular Sarıbaşak. The results of the study indicate that especially the Sarıbaşak cultivar may be selected for wheatgrass production owing to its high values. Moreover, the Sarıbaşak cultivar could be considered for use in programs for breeding new cultivars that have wheatgrass with higher antioxidant levels and TPC for health.

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