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Utjecaj sorte i otkosa na funkcionalna svojstva pšenične trave (*Triticum aestivum* L.)

Kristić, M., Grubišić, S., Rebekić, A., Rupčić, J., Teklić, T., Lisjak, M.

Poljoprivreda / Agriculture

ISSN: 1848-8080 (Online)

ISSN: 1330-7142 (Print)

<https://doi.org/10.18047/poljo.28.2.5>



Fakultet agrobiotehničkih znanosti Osijek, Poljoprivredni institut Osijek

Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek

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Original scientific paper

Izvorni znanstveni rad

SUMMARY

*Due to its nutritional value, wheatgrass (*Triticum aestivum* L.) is considered to be a functional food, becoming increasingly popular as a supplement to the people's quotidian diet. The study aimed to determine the influence of the number of cuttings and cultivars on the total antioxidant activity (DPPH), the content of chloroplast pigments, vitamin C, phenols, and flavonoids in the wheatgrass juice. Two genotypes of wheatgrass, *T. aestivum* ssp. *aestivum* (variety Katarina) and *T. aestivum* ssp. *sphaerococcum*, respectively, were cut twice during the experiment. In both cuttings, the genotype significantly differed in the flavonoid level and antioxidant activity, while the number of cuttings influenced the content of phenols, vitamin C, and antioxidant activity in the wheatgrass juice. *T. sphaerococcum* had a higher concentration of flavonoids and a significantly lower antioxidant activity when compared to the Katarina wheat variety. On an average, the first cut implicated an increased content of phenols and vitamin C concerning both genotypes, followed by a higher antioxidant value. In the Katarina variety, a significantly higher phenol content and antioxidant activity was detected in the first cut. In the *T. Sphaerococcum*, a decrease in the total content of the examined antioxidants was apparent in the second cut.*

Keywords: antioxidant activity, chloroplast pigments, flavonoids, phenols, wheatgrass

INTRODUCTION

Wheatgrass, as the young wheat shoots (*Triticum aestivum* L.), originates from the family of *Poaceae*. Due to its high nutritional value (Suriyavathana et al., 2016; Grubišić et al., 2019), wheatgrass is considered to be functional foods (Elayath and Iyer, 2012; Chomchan et al., 2016; Kumar et al. 2016; Ogutu et al., 2017; Niroula et al., 2019). Gruenwald (2009) documented a greater consumer preference for natural products in comparison to the synthesized food supplements. The term "functional food" was first used in the 1980s (Čalić et al., 2011), although a wheatgrass' nutritional value has been known since the ancient Egyptian era (Sutar-Kapashikar et al., 2018). Since the 1930s, a discovery by Charles Schnabel has led to the usage of wheatgrass in the human diet (Meyerowitz, 1999; Kumar et al., 2016). Wheatgrass can be consumed in the form of powder,

juice, tablets, or as the raw wheatgrass (Wigmore, 1985; Kumar et al., 2016; Skoczylas et al., 2018). Its high concentration of chlorophyll; of minerals such as iron, calcium, and magnesium; of vitamins A, C, and E; as well as of flavonoids and amino acids, contributes to a high antioxidant capacity (Kulkarni et al., 2006; Suriyavathana et al., 2016; Skoczylas et al., 2018; Rebekić et al., 2019). Since wheatgrass can be easily grown in home cultivation, the aim of this study was to determine the influence of genotypes and cuttings on the content of metabolites with the antioxidant properties in wheatgrass juice. Savsatli (2020) suggested that it is important to determine the appropriate cultivars

Marija Kristić, Mag. Eng. Agr., Assoc. Prof. Miroslav Lisjak (mlisjak@fazos.hr), Sanja Grubišić, Ph. D., Josipa Rupčić, Mag. Eng. agr., Assoc. Prof. Andrijana Rebekić – Faculty of Agrobiotechnical Sciences Osijek, Vladimira Preloga 1, 31000 Osijek.

for the industry. Moreover, the wild wheat relatives are rarely investigated in terms of their genetic potential for a secondary metabolite synthesis. Consequently, they might be of interest for a further wheat breeding in terms of developing the new cultivars with a high functional quality. Considering the potential wheat regrowth and a lack of data on the plant material's functional quality, obtained in further cuttings, this research might contribute to a better knowledge wheatgrass growing and usage.

MATERIAL AND METHODS

Plant material

The research was conducted on the two wheat genotypes, *T. aestivum* ssp. *aestivum* (*vulgare*) L., a cultivar of the Croatian origin (Katarina), and the wild relative *T. aestivum* ssp. *sphaerococcum*. The seed of both wheat genotypes from 2013–14 was donated by the Faculty of Agrobiotechnical Sciences Osijek and collected as a part of the Croatian Science Foundation's project entitled Creating Wheat for the Future - A Search for the New Genes from the Existent Sources, 2014–17.

The preparation of grains for germination

Thirty g of wheat grain (*Triticum aestivum* L.) was washed three times for five minutes with the autoclaved water on a magnetic stirrer. Subsequent to the last water rinse, the grain was placed in the glass autoclaved jars, covered with nets and turned upside down to drain the excess water. The jars with the grain were stored in a dark room for forty-eight hours.

Wheatgrass (*Triticum aestivum* L.) growing

The sprouted grains were sown in the plastic trays (15 cm width x 50 cm length x 3 cm height) filled with the Brill Typical 3 substrate (Gebr. Brill Substrate GmbH & Co. KG). The grain was evenly distributed and covered with 0.5 cm of substrate layer. The wheat was grown in the fully controlled conditions in a growth chamber at a temperature of 20°C, with a 12-hour photo period, and watered with 100 mL of water every day. The experiment was conducted in three replications.

The first cutting occurred on the twelfth day subsequent to the sowing, and the second one occurred on the fourteenth day following the first cutting, respectively, on the twenty-sixth day after sowing. A wheatgrass juicer (Wheatgrass BI-30) was used to prepare the juice from the freshly cut leaves.

The determination of chlorophyll and carotenoid content

When determining the chloroplast pigments content, a wheatgrass extract was previously weighed in the 15 ml threaded plastic tubes by adding the MgCO₃ powder to neutralize acidity, along with the 10 ml of acetone. The samples were homogenized on a vortex mixer (for 10 seconds) twice in the 10-minute intervals and then centrifuged at 4000 RPM at 4°C for 10

minutes. The supernatants were transferred into a 2 ml glass cuvette, and absorbances were measured on a spectrophotometer at the wavelengths of 662, 644, and 440 nm. The obtained absorption values (A622, A644, and A440) were included in the Holm-Wettstein's equations for calculating the pigment concentration in mg/dm³ for chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl a+b), and the carotenoid (Car) content (Holm, 1954; Wettstein, 1957). The obtained values were calculated and expressed as mg/ml of the wheatgrass juice.

The determination of total phenols and flavonoids

A stock solution of gallic acid and a saturated solution of Na₂CO₃ were used to determine the total phenols by the Folin-Ciocalteu method (1927). One hundred µl of wheatgrass juice was diluted with 1 ml of 70% ethanol and vortexed. The samples were left in the refrigerator for forty-eight hours and then centrifuged at 4000 RPM at 4°C for twenty minutes. One hundred µl of each standard concentration was transferred into 2 ml plastic tubes, 1.50 ml of distilled water and 100 µl of Folin-Ciocalteu reagent, and 300 µl of saturated Na₂CO₃ solution was added and vortexed. The reaction mixture was incubated for sixty minutes at 37°C, and the absorbance was measured at 765 nm. The total phenols (PHE) concentration was calculated while applying the equation from a calibration curve, derived from the measured absorbances of gallic acid standards and expressed as µg GA/ml of the wheatgrass juice.

To determine the flavonoid content, 200 µl of ethanol extracts was transferred into the 2 ml plastic tubes, whereafter 100 µl of AlCl₃ and 1700 µl of 96% ethanol were added (Ordonez et al., 2006). The samples were homogenized, and the absorbance was measured at the room temperature at 415 nm using a quartz cuvette after sixty minutes. The total flavonoids (FL) concentration was calculated while applying the equation from a calibration curve derived from the measured absorbances of quercetin standards and expressed as µg QC/ml of the wheatgrass juice.

Spectrophotometric determination of vitamin C

The vitamin C concentration was determined according to Roe and Kuether (1943), with minor modifications. One hundred µl of wheatgrass juice was diluted in 5700 µl of distilled water and vortexed. The samples were centrifuged for fifteen minutes at 3000 RCF at 4°C and transferred into the 2 ml plastic tubes. A reagent mix added to the plant extract contained 13.3% of TCA and 2% of the DNPH reagent (2,4 dinitrophenylhydrazine). Subsequent to the addition of the reagent mix, the samples were incubated for three hours at 37°C, 65% of sulfuric acid was added, and the absorbance was read out at 520 nm. The calibration standards were prepared from the stock solution containing 10 mg/100ml of

ascorbic acid. The vitamin C (AA) concentration in the samples was calculated while applying the equation from the calibration curve and expressed as $\mu\text{g AA/ml}$ of the wheatgrass juice.

The determination of antioxidant activity by the DPPH method

The determination of total antioxidant activity was performed by the DPPH method according to Brand-Williams et al. (1995). The ascorbic acid standards were prepared, and the absorbances at 520 nm were measured immediately the addition of 1900 μl of the DPPH reagent (2,2-diphenyl-1-picrylhydrazyl) and 100 μl of 70% ethanol, that is, thirty minutes subsequent to the addition of the DPPH reagent. The wheatgrass juice samples were prepared in four technical replicates by pipetting 40, 60, 80, and 100 μl into the 2 ml plastic tubes, followed by the DPPH reagent. After thirty minutes, the absorbance was measured, and the equation derived from the graph was applied to calculate the wheatgrass juice volume necessary for a fifty-percent inhibition of the DPPH reagent's degradation reaction. The results are expressed as the ml of juice for IC 50%.

Data analysis and processing

The concentration of chloroplast pigments, vitamin C, and antioxidant activity by the DPPH method was examined on a Varian Cary 50 UV-VIS spectrophotometer using the *Cary WinUV* software, while the concentration of phenols and flavonoids was measured on a Shimadzu UV-1800 UV spectrophotometer. The results obtained from the three independent replicates were analyzed using the *SAS Software 9.1.3* (2002 to 2003, SAS Institute Inc., Cary, USA). The analysis of variance (ANOVA), F test, and Fisher's LSD test (least significant difference) were administered.

RESULTS AND DISCUSSION

Wheatgrass juice is known as the "green blood" (Padalia et al., 2010) due to its high chlorophyll content. Devi et al. (2020) reported that the chlorophyll content

in wheatgrass depends on the day of the cut. In their research, wheatgrass was cut on the seventh, tenth, and thirteenth day after sowing, whereby the highest chlorophyll content was determined on the tenth day after sowing. As reported by Anwar et al. (2015), the number of cuttings and the growing conditions also influenced the content of chlorophyll in the leaves of wheat seedlings. They have found the highest chlorophyll content in the wheatgrass juice obtained from the plants grown in the open field, after the second cut. In addition, the plants grown in the open-field conditions contained more chlorophyll if compared to those grown in the semi-controlled laboratory conditions. In our research, the juice prepared from the seedlings after the first cut a significantly higher content of phenols, vitamin C and carotenoids, as well as a higher antioxidant activity according to DPPH method as an average for both genotypes, (Table 1).

Phenolic compounds are known as a plant's secondary metabolites, due to which the wheatgrass' antioxidant potential has a great effect on the suppression of cardiovascular diseases, inflammation, and cancer (Sutar-Kapashikar et al., 2018). Qamar et al. (2018) concluded that both wheat and barley grass are the good sources of natural antioxidants considering their high phenol and flavonoid content. Agrawal et al. (2015) investigated the influence of the seedling height on the antioxidant activity in wheatgrass. The highest value of the aforementioned parameter was obtained when young plants were between 23 and 25 cm high, while the lowest value was measured in the plants reaching 17–20 cm in height. Here, a significant decrease of phenols (35%) and antioxidant activity (41%) in the wheatgrass juice of the Katarina genotype was detected in second cutting (Fig. 1). The same pattern was reproduced in *T. sphaerococcum*, in which a significant decrease of phenols (31%), flavonoids (9%), ascorbic acid (80%), and antioxidant activity (25%) occurred in second cut, indicating a declining nutritional value of the wheatgrass juice obtained from the regrown plants (Fig. 2).

Table 1. The influence of genotype and the number of cuttings on an antioxidant activity (DPPH; ml juice for IC50%), phenols (PHE; $\mu\text{g GA/ml juice}$), flavonoids (FL; $\mu\text{g QC/ml juice}$), vitamin C (AA; $\mu\text{g/ml juice}$), and the chloroplast pigments (Chl, Car; mg/ml juice). The data are the averages of three replicates; ANOVA. F test. The values marked with different lowercase letters (a, b, and c) differ according to the LSD test $p \leq 0.05$. The values marked with different capital letters (A, B, and C) are distinguished according to the LSD test $p \leq 0.01$.

Tablica 1. Utjecaj sorte i otkosa na antioksidativnu aktivnost (DPPH; ml soka za IC50%), fenole (PHE; $\mu\text{g GA/ml soka}$), flavonoide (FL; $\mu\text{g QC/ml soka}$), vitamin C (AA; $\mu\text{g/ml soka}$) i kloroplastne pigmente (Chl, Car; mg/ml soka). Podatci su prosjek triju ponavljanja; ANOVA. F test. Vrijednosti označene različitim malim slovima (a, b, c) razlikuju se prema LSD testu $p \leq 0,05$. Vrijednosti označene različitim velikim slovima (A, B, C) razlikuju se prema LSD testu $p \leq 0,01$.

		PHE	FL	AA	DPPH	Chl a	Chl b	Chl a+b	Car	Ratio Chl a/b	Ratio Chl a+b/Car
Variety/Sorta	Katarina	899	415 ^B	4.3	32.5 ^B	0.28	0.09 ^b	0.37	0.08	3.0	4.4 ^B
	<i>T. sphaerococcum</i>	878	487 ^A	4.5	38.6 ^A	0.30	0.10 ^a	0.40	0.08	3.0	4.8 ^A
	F Value	0.10	15.59	0.72	9.44	3.07	5.17	3.63	0.00	1.71	45.02
	P	0.7567	0.0019	0.4114	0.0097	0.1051	0.0422	0.0810	0.9680	0.2155	<.0001
Cutting/Otkos	1 st cutting/ 1. otkos	1064 ^A	450	6.2 ^A	28.6 ^B	0.29	0.10	0.39	0.09 ^a	3.1 ^b	4.5 ^B
	2 nd cutting/ 2. otkos	712 ^B	452	2.6 ^B	42.5 ^A	0.28	0.10	0.38	0.08 ^b	3.0 ^a	4.7 ^A
	F Value	28.05	0.02	237.65	50.63	1.49	0.13	1.04	5.18	6.53	16.78
	P	0.0002	0.8991	<.0001	<.0001	0.2450	0.7297	0.3284	0.0420	0.0253	0.0015
A * B	F Value	0.23	1.04	119.27	2.34	13.68	19.16	15.32	20.17	3.65	3.50
	P	0.6419	0.0210	<.0001	0.1520	0.0030	0.0009	0.0021	0.0007	0.0801	0.0860

Özköse et al. (2016) examined the influence of fertilization and the number of cuttings on the chlorophyll and carotenoid content in the juice of different perennial turfgrasses, *T. durum*, *T. aestivum*, *Lolium perenne* L., and *Festuca arundinacea* Schreb, respectively. In all the examined turfgrasses, the content of chloroplast pigments in the second cutting significantly decreased, resulting in a lower organic matter production, as compared to the first cutting, while fertilization decreased the chlorophyll content but did not influence the carotenoid content.

On an average, for both cuttings, the two genotypes examined significantly differed in the flavonoid content, chlorophyll b, and total chlorophyll and carotenoid ratio, with the higher values being established in

the wild *T. sphaerococcum* relative (Table 1). A higher antioxidant activity was detected in the wheatgrass juice of the Katarina variety. Ghumman et al. (2017) also discovered significant differences in the chlorophyll content between a strong (PBW343) and a weak wheat variety (RAJ3765). The strong wheat variety measured a significantly higher chlorophyll content in the wheatgrass juice powder (7.05 mg/g) when compared to the weak wheat variety (6.69 mg/g). Indeed, bearing in mind the established significant influence of the genotype itself on the different functional metabolites' content and the wheatgrass juice's antioxidative properties, a necessarily better knowledge about the genetic background of the plant's secondary metabolism and growing conditions optimization may be suggested.

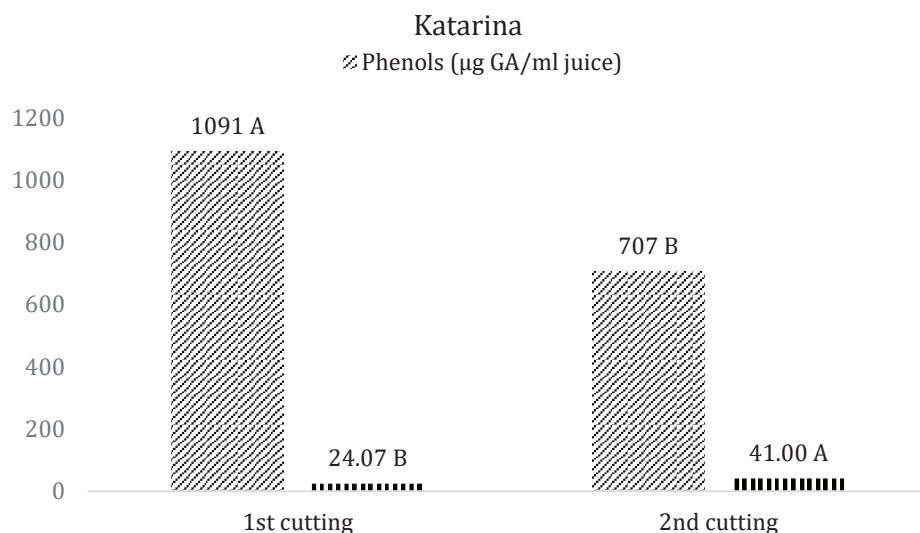


Figure 1. The influence of cutting on the total content of phenols and an antioxidant activity by the DPPH method in the wheatgrass juice of the Katarina genotype. The data are averages of three replicates. The values marked with different capital letters (A, B, and C) are distinguished according to the LSD test $p \leq 0.01$.

Grafikon 1. Utjecaj otkosa na ukupni sadržaj fenola i antioksidativnu aktivnost po DPPH metodi u soku pšenične trave genotipa Katarina. Podatci su prosjek triju ponavljanja. Vrijednosti označene različitim velikim slovima (A, B, C) razlikuju se prema LSD testu $p \leq 0,01$.

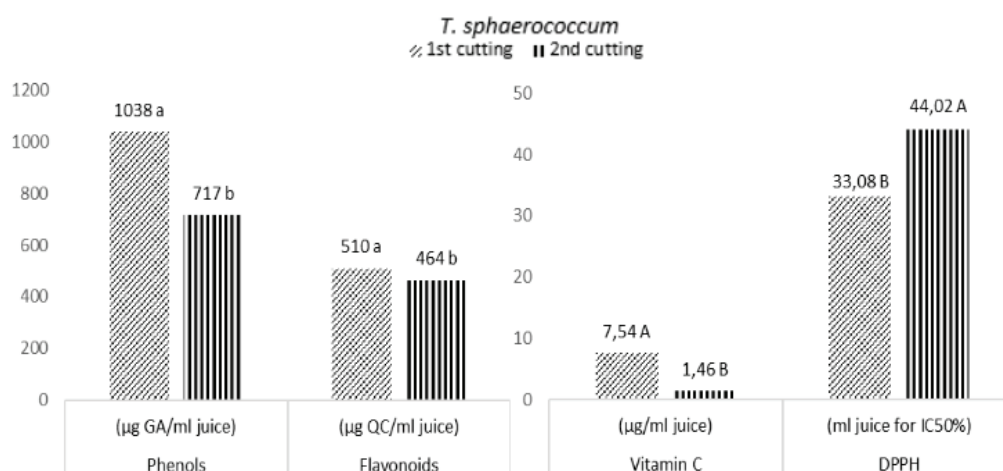


Figure 2. The influence of cutting on the total content of phenols, flavonoids, vitamin C and an antioxidant activity by the DPPH method in the wheatgrass juice of *T. sphaerococcum*. The data are the averages of three replicates. The values marked with different lowercase letters (a, b, and c) are distinguished according to the LSD test $p \leq 0.05$. The values marked with different capital letters (A, B, and C) are distinguished according to the LSD test $p \leq 0.01$.

Grafikon 2. Utjecaj otkosa na ukupni sadržaj fenola, flavonoida, vitamina C i antioksidativnu aktivnost po DPPH metodi u soku pšenične trave *T. sphaerococcum*. Podatci su prosjek triju ponavljanja. Vrijednosti označene različitim malim slovima (a, b, c) razlikuju se prema LSD testu $p \leq 0,05$. Vrijednosti označene različitim velikim slovima (A, B, C) razlikuju se prema LSD testu $p \leq 0,01$.

Similarly, Benincasa et al. (2015) found significant differences between the examined wheat varieties in the phenol content and an antioxidant activity. In their research, the cultivar *T. durum* cv. had the highest values of the aforementioned parameters, followed by the *T. dicoccum* cv. Augeo, *T. dicoccum* cv. Rosso Rubino, *T. monoccum* cv. Monlis, *T. aestivum* cv. Orso, *T. spelta* cv. Pietro, *T. dicoccum* cv. Zephyr and *T. spelta* cv. Giuseppe.

They concluded that all examined *Triticum* species have a potential to be used as functional foods. Niroula et al. (2019) found that the phenol content in wheatgrass (*Triticum aestivum* L.) and barley grass (*Hordeum vulgare*) increases day by day and reaches its maximum between the tenth and the thirteenth day and then decreases. In wheatgrass, the highest values of an antioxidant activity were measured between the tenth and fifteenth day.

Table 2. The influence of variety on an antioxidant activity (DPPH; ml juice for IC50%), phenols (PHE; $\mu\text{g GA/ml}$ juice), flavonoids (FL; $\mu\text{g QC/ml}$ juice), vitamins C (AA; $\mu\text{g/ml}$ juice), and the chloroplast pigments (Chl, Car; mg/ml juice) in the first cutting. The data are the averages of three replicates; ANOVA. F test. The values marked with different lowercase letters (a, b, and c) are distinguished according to LSD test $p \leq 0.05$. The values marked with different capital letters (A, B, and C) are distinguished according to the LSD test $p \leq 0.01$.

Tablica 2. Utjecaj sorte na antioksidativnu aktivnost (DPPH; ml soka za IC50%), fenole (PHE; $\mu\text{g GA/ml}$ soka), flavonoide (FL; $\mu\text{g QC/ml}$ soka), vitamin C (AA; $\mu\text{g/ml}$ soka) i kloroplastne pigmente (Chl, Car; mg/ml soka) u prvome otkosu. Podatci su prosjek triju ponavljanja; ANOVA. F test. Vrijednosti označene različitim malim slovima (a, b, c) razlikuju se prema LSD testu $p \leq 0,05$. Vrijednosti označene različitim velikim slovima (A, B, C) razlikuju se prema LSD testu $p \leq 0,01$.

1 st cutting/ 1. otkos	PHE	FL	AA	DPPH	Chl a	Chl b	Chl a + b	Car	Ratio Chl a/b	Ratio Chl a + b/Car
Katarina	1091	389 ^B	4.82 ^B	24.07 ^B	0.31	0.10	0.41	0.10 ^a	3.06	4.28 ^B
<i>T. sphaerococcum</i>	1038	510 ^A	7.54 ^A	33.08 ^A	0.28	0.09	0.38	0.08 ^b	3.08	4.76 ^A
F Value	0.17	22.0	175.32	67.83	2.26	3.56	2.54	10.88	0.40	38.99
P	0.6947	0.0034	0.0001	0.0002	0.1832	0.1083	0.1619	0.0164	0.5528	0.0008

Chomchan et al. (2016) reported significant differences in the content of bioactive components in two examined juices made of wheatgrass (*Triticum aestivum* L.) and rice grass (*Oryza sativa*). The wheatgrass juice had a higher content of chlorophyll, ascorbic acid, and total phenols, while a higher concentration of carotenoids was detected in the rice juice. As reported by Durairaj et al. (2015), the wheatgrass extracts are rich in phenolic compounds, with a total phenol content amounting to 210.15 $\mu\text{M GA/g}$ and the flavonoid content amounting to 160.25 $\mu\text{M QC/g}$. Wangcharoen and Phimphilai (2016) detected the higher values of chlorophyll content and total phenols in the barley juice if compared to the

wheatgrass juice, in which 45 $\mu\text{g}/100$ ml of chlorophyll and 2.80 mg GA/100 ml of phenols were found. Özköse et al. (2016) also examined the content of total phenols, flavonoids, vitamin C, and an antioxidant activity in the multiple wheatgrass genotypes. A higher phenol content was detected in *Triticum durum* L. in both the first (443 mg GA/l) and the second pruning (422 mg GA/l) when compared to *Triticum aestivum* L. Oppositely, flavonoid content in the second pruning of *Triticum aestivum* L. was significantly higher if compared to the *Triticum durum* L., which also had a lower vitamin C content in the first pruning. The antioxidant activity was affected by neither the genotypes nor the number of prunings.

Table 3. The influence of variety on an antioxidant activity (DPPH; ml juice for IC50%), phenols (PHE; $\mu\text{g GA/ml}$ juice), flavonoids (FL; $\mu\text{g QC/ml}$ juice), vitamin C (AA; $\mu\text{g/ml}$ juice), and the chloroplast pigments (Chl, Car; mg/ml juice) in the second cutting. The data are the averages of three replicates; ANOVA. F test. The values marked with different lowercase letters (a, b, and c) are distinguished according to the LSD test $p \leq 0.05$. The values marked with different capital letters (A, B, and C) are distinguished according to the LSD test $p \leq 0.01$.

Tablica 3. Utjecaj sorte na antioksidativnu aktivnost (DPPH; ml soka za IC50%), fenole (PHE; $\mu\text{g GA/ml}$ soka), flavonoide (FL; $\mu\text{g QC/ml}$ soka), vitamin C (AA; $\mu\text{g/ml}$ soka) i kloroplastne pigmente (Chl, Car; mg/ml soka) u drugome otkosu. Podatci su prosjek triju ponavljanja; ANOVA. F test. Vrijednosti označene različitim malim slovima (a, b, c) razlikuju se prema LSD testu $p \leq 0,05$. Vrijednosti označene različitim velikim slovima (A, B, C) razlikuju se prema LSD testu $p \leq 0,01$.

2 nd cutting/ 2. otkos	PHE	FL	AA	DPPH	Chl a	Chl b	Chl a + b	Car	Ratio Chl a/b	Ratio Chl a + b/Car
Katarina	706	440	3.78 ^A	41.00	0.24 ^b	0.08 ^b	0.32 ^B	0.07 ^b	3.03	4.61 ^b
<i>T. sphaerococcum</i>	717	464	1.46 ^B	44.02	0.31 ^a	0.11 ^a	0.43 ^A	0.09 ^a	2.89	4.88 ^a
F Value	0.09	0.83	31.59	0.65	12.77	16.05	14.03	9.42	3.37	11.09
P	0.7742	0.3973	0.0014	0.4525	0.0117	0.0071	0.0096	0.0220	0.1162	0.0158

According to the data figured in Table 2, *T. sphaerococcum* stood out in the first cut with a significantly higher flavonoid and vitamin C content, while a higher carotenoid content and an antioxidant activity were detected in the juice of the Katarina variety. There was no significant difference, however, between the two tested genotypes

in the first cut with regard to the phenol content. In the second cut, a significantly higher vitamin C content in the Katarina variety wheatgrass juice of of was found (Table 3). Also, the wild relative of *T. sphaerococcum* had a significantly higher chloroplast pigment content if compared to the Katarina genotype. Based on the data obtained, a juice

prepared from a wild relative had a higher nutritional value if compared to the Katarina genotype with respect to the composition of biologically active compounds. Since the values of a majority of biologically active and nutritionally important compounds declined in the second cutting in both tested genotypes, a wheatgrass recultivation cannot be recommended.

CONCLUSION

Two wheatgrass genotypes grown in the controlled conditions, *Triticum aestivum* L. (Katarina cultivar) and *T. sphaerococcum*, were tested for the content of biologically active compounds (chloroplast pigments, ascorbic acid, phenols, and flavonoids) and for an antioxidative activity in the juice, obtained by the first and the second cut following a regrowth. In a comparison of the first to the second cut, a significant influence of genotypes on the level of flavonoids and chlorophyll a and a significant influence of the number of cutting on the phenols and vitamin C was manifested. Both tested genotypes had a higher concentration of biologically active compounds in the first cutting. Also, a wild relative, *T. sphaerococcum*, had a significantly higher content of flavonoids, vitamin C, and antioxidant activity when compared to the Katarina cultivar. Consequently, a necessity to acquire a better knowledge of the genetic background of plant's secondary metabolism and growing conditions optimization in wheatgrass production may be suggested. Based on the obtained results, a wheatgrass regrowth, commonly applied in so-called "wheatgrass home growing" cannot be recommended for the controlled or for the semi-controlled growth conditions too.

ACKNOWLEDGEMENT

This research is part of the project entitled *Genotypic Specificity of Wheatgrass (Triticum aestivum L.), a Highly Nutritional Food Supplement*, UIP-2017-05-4292, funded by the Croatian Science Foundation, led by Andrijana Rebekić, Ph. D.

REFERENCES

- Agrawal, A., Gupta, E., Chaturvedi, R. (2015). Determination of minerals and antioxidant activities at different levels of jointing stage in the juice of wheat grass-the green wonder. *Indian Journal of Pure & Applied Biosciences*, 3(2), 311-316.
- Anwar, D. A., Mohammadi, T., MMF, A. (2015). Wheatgrass juice and its nutritional value as affected by sprouting conditions. *Arab Universities Journal of Agricultural Sciences*, 23(1), 37-49. <https://doi.org/10.21608/ajs.2015.14558>
- Benincasa, P., Galieni, A., Manetta, A. C., Pace, R., Guiducci, M., Pisante, M., Stagnari, F. (2015). Phenolic compounds in grains, sprouts and wheatgrass of hulled and non-hulled wheat species. *Journal of the Science of Food and Agriculture*, 95(9), 1795-1803. <https://doi.org/10.1002/jfsa.6877>
- Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, 28(1), 25-30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Chomchan, R., Siripongvutikorn, S., Puttarak, P., Rattanapon, M. R. (2016). Investigation of phytochemical constituents, phenolic profiles and antioxidant activities of ricegrass juice compared to wheatgrass juice. *Functional Foods in Health and Disease*, 6(12), 822-835. <https://doi.org/10.31989/ffhd.v6i12.290>
- Čalić, S., E. Friganović, V. Maleš, A. Mustapić (2011). Funkcionalna hrana i potrošači. *Praktični menadžment*, 2(2), 51-57.
- Devi, C. H., Bains, K., Kaur, H. and Ram, H. (2020). Nutritional composition, bioactive compounds and free radical scavenging activity of wheatgrass (*Triticum aestivum* L.) as influenced by harvesting stages and cultivation method. *Indian Journal of Natural Products and Resources*, 11(2), 118-123.
- Durairaj, V., Hoda, M., Shakya, G., Babu, S. P. P., Rajagopalan, R. (2014). Phytochemical screening and analysis of antioxidant properties of aqueous extract of wheatgrass. *Asian Pacific journal of tropical medicine*, 7, 398-404. [https://doi.org/10.1016/S1995-7645\(14\)60265-0](https://doi.org/10.1016/S1995-7645(14)60265-0)
- Elayath, N., Iyer, U. (2012). Wheatgrass (*Triticum aestivum* L.) as functional food—bridging the translational research gap in nutrition. *Energy (Kcal)*, 354, 0-3.
- Folin, O., Ciocalteu, V. (1927). On tyrosine and tryptophane determinations in proteins. *Journal of biological chemistry*, 73(2), 627-650. [https://doi.org/10.1016/S0021-9258\(18\)84277-6](https://doi.org/10.1016/S0021-9258(18)84277-6)
- Ghumman, A., Singh, N., Kaur, A. (2017). Chemical, nutritional and phenolic composition of wheatgrass and pulse shoots. *International journal of food science & technology*, 52(10), 2191-2200. <https://doi.org/10.1111/ijfs.13498>
- Grubišić, S., Orkić, V., Guberac, S., Petrović, S., Lisjak, M., Kristić, M., Rebekić, A. (2019). Optimalan način sjetve pšenice (*Triticum aestivum* L.) za uzgoj pšenične trave. *Poljoprivreda*, 25(2), 31-37. <https://doi.org/10.18047/poljo.25.2.5>
- Gruenwald, J. (2009). Novel botanical ingredients for beverages. *Clinics in dermatology*, 27(2), 210-216. <https://doi.org/10.1016/j.clindermatol.2008.11.003>
- Holm, G. (1954). Chlorophyll mutations in barley. *Acta Agriculturae Scandinavica*, 4(1), 457-471. <https://doi.org/10.1080/00015125409439955>
- Kulkarni, S. D., Tilak, J. C., Acharya, R., Rajurkar, N. S., Devasagayam, T. P. A., Reddy, A. V. R. (2006). Evaluation of the antioxidant activity of wheatgrass (*Triticum aestivum* L.) as a function of growth under different conditions. *Phytotherapy Research*, 20(3), 218-227. <https://doi.org/10.1002/ptr.1838>
- Kumar, N. S., Murali, M., Nair, A. M., Nair, A. S. (2016). Green blood therapy of wheat grass-Nature's finest medicine'-A literature review. *Journal of Pharmaceutical and Biological Sciences*, 11(2), 57-64. <https://doi.org/10.9790/3008-1102045764>
- Meyerowitz, S. (1999). Wheatgrass: nature's finest medicine: the complete guide to using grasses to revitalize your health (6th ed.). USA: Sproutman Publications, pp 245.

18. Niroula, A., Khatri, S., Khadka, D., Timilsina, R. (2019). Total phenolic contents and antioxidant activity profile of selected cereal sprouts and grasses. *International Journal of Food Properties*, 22(1), 427-437. <https://doi.org/10.1080/10942912.2019.1588297>
19. Ogutu, F. O., Makori, S. I., Maringa, C. W., Lemtukei, D., Okiko, G., Luvita, S. (2017). Wheat Grass: A Functional Food. *Food Science and Quality Management*, 65, 33-38.
20. Ordonez, A. A. L., Gomez, J. D., Vattuone, M. A., Isla, M. I. (2006). Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food chemistry*, 97(3), 452-458. <https://doi.org/10.1016/j.foodchem.2005.05.024>
21. Özköse, A., Arslan, D., Aysenur, A. (2016). The comparison of the chemical composition, sensory, phenolic and antioxidant properties of juices from different wheatgrass and turfgrass species. *Notulae Botanicae Horti Agrobotanici*, 44(2), 499-507. <https://doi.org/10.15835/nbha44210405>
22. Padalia, S., Drabu, S., Raheja, I., Gupta, A., Dhamija, M. (2010). Multitude potential of wheatgrass juice (Green Blood): An overview. *Chronicles of young scientists*, 1(2), 23-28.
23. Qamar, A., Saeed, F., Tahir-Nadeem, M., Hussain, A. I., Niaz, B., Ullah Khan, A., Afzaal M., Badar Ul Ain, H., Imran, M. (2018). Exploring the phytochemical profile of green grasses with special reference to antioxidant properties. *International Journal of Food Properties*, 21(1), 2566-2577. <https://doi.org/10.1080/10942912.2018.1540990>
24. Rebekić, A., Grubišić, S., Orkić, V., Guberac, S., Lisjak, M., Mišković Špoljarić, K. (2019). Wheatgrass (*Triticum aestivum* L.) – natural food supplement. *Proceedings of 54th Croatian & 14th international symposium on agriculture*, Zagreb, Croatia, 2019. 209-213.
25. Roe H. Joseph and Kuether A. Carl (1943). The Determination of Ascorbic Acid in Whole Blood and Urine Through The 2,4-Dinitrophenylhydrazine Derivative of Dehydroascorbic Acid. *The Journal of Biological Chemistry*, 147, 399-407. [https://doi.org/10.1016/S0021-9258\(18\)72395-8](https://doi.org/10.1016/S0021-9258(18)72395-8)
26. Savsatli, Y. (2020). The effects of wheatgrass length on antioxidant activity and total phenolic content in wheatgrass (*Triticum spp.*). *Turkish Journal of Agriculture and Forestry*, 44(3), 271-277. <https://doi.org/10.3906/tar-1811-33>
27. Skoczylas, Ł., Korus, A., Tabaszewska, M., Gędoś, K., Szczepańska, E. (2018). Evaluation of the quality of fresh and frozen wheatgrass juices depending on the time of grass harvest. *Journal of Food Processing and Preservation*, 42(1), e13401. <https://doi.org/10.1111/jfpp.13401>
28. Suriyavathana, M., Roopavathi, I. (2016). Phytochemical Characterization of *Triticum Aestivum* (Wheat Grass). *Journal of Pharmacognosy and Phytochemistry*, 5(1), 283.
29. Sutar-kapashikar, P. S., Gawali, T. R., Koli, S. R., Khot, A. S., Dehankar, S. P., Patil, P. D. (2018). Phenolic Content in *Triticum Aestivum*: A Review. *International Journal of New Technology and Research*, 4(12), 01-02. <https://doi.org/10.31871/IJNTR.4.12.08>
30. Wangcharoen, W., Phimphilai, S. (2016). Chlorophyll and total phenolic contents, antioxidant activities and consumer acceptance test of processed grass drinks. *Journal of Food Science and Technology* 53(12):4135-4140. <https://doi.org/10.1007/s13197-016-2380-z>
31. Wettstein, D. (1957). Chlorophyll-letale und der submikroskopische Formwechsel der Plastiden. *Experimental cell research*, 12(3), 427-506. [https://doi.org/10.1016/0014-4827\(57\)90165-9](https://doi.org/10.1016/0014-4827(57)90165-9)
32. Wigmore, A. (1985). *The Wheatgrass Book*. USA: Hippocrates Health Institute, Inc. pp. 123.

UTJECAJ SORTE I OTKOSA NA FUNKCIONALNA SVOJSTVA PŠENIČNE TRAVE (*Triticum aestivum* L.)

SAŽETAK

Zbog kvalitete nutritivnoga sastava, pšenična se trava (*Triticum aestivum* L.) smatra funkcionalnom hranom te postaje sve popularnija kao dodatak svakodnevnoj prehrani ljudi. Cilj istraživanja bio je utvrditi utjecaj broja otkosa i sorte na ukupnu antioksidativnu aktivnost (DPPH), sadržaj kloroplastnih pigmenata, vitamina C, fenola i flavonoida. U pokusu su ispitana dva genotipa pšenične trave, *Triticum aestivum* ssp. *aestivum* (kultivar Katarina) i *Triticum aestivum* ssp. *sphaerococcum*. U obama otkosima utvrđen je značajan utjecaj genotipa na sadržaj flavonoida i antioksidacijsku aktivnost, dok je broj otkosa značajno utjecao na fenole, vitamin C i antioksidacijsku aktivnost. *T. sphaerococcum* imao je veću koncentraciju flavonoida i značajno nižu antioksidativnu aktivnost u usporedbi s kultivarom Katarina. U prosjeku je za obje sorte u prvome otkosu utvrđen značajno veći sadržaj fenola i veći sadržaj vitamina C, što je bilo popraćeno i većom antioksidativnom aktivnošću. Kod kultivara Katarina značajno veći sadržaj fenola i antioksidativna aktivnost utvrđeni su u prvome otkosu. Kod *T. sphaerococcum* utvrđen je značajan pad sadržaja ispitivanih antioksidanasa u drugome otkosu.

Ključne riječi: antioksidativna aktivnost, kloroplastni pigmenti, flavonoidi, fenoli, pšenična trava

(Received on July 29, 2022; accepted on November 24, 2022 – Primljeno 29. srpnja 2022.; prihvaćeno 24. studenoga 2022.)