Received: 9 May 2022 | Revised: 29 June 2022 | Accepted: 9 July 2022

DOI: 10.1002/jsf2.73

RESEARCH ARTICLE

JSFA Reports

Nutritional composition, biologically active substances and antioxidant activity of young spelt grass extract

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Funding information
co-financed by Ministry of Science and Technological Development of Montenegro, Grant/Award Number: 01-2617/5

Abstract

Background: *Triticum spelta* L. (family *Poaceae*) is an ancient grain, which had been used for nutrition in the past but was eventually substituted, together with other related grains, for bread wheat (*Triticum aestivum* L.). The main reasons for its substitution are higher yield and economic gain in favor of bread wheat. With the belief that the extract of young spelt grass contains a sufficient amount of key nutrients, which contribute to beneficial effects on human health, research was conducted on 27 samples of young spelt grass extracts. For each sample extracted from young spelt grass cultivated under different conditions (irrigation, lighting, harvest), its nutritional composition, the content of biologically active substances and antioxidant activities were determined, with a comparison of the obtained results.

Results: In this two-year study several nutrients, the content of major and trace minerals, antioxidant activity and pigment, and polyphenolic and flavonoid contents were analyzed and how the different growth factors influence them. Young spelt grass extract has a relatively high amount of functional ingredients, such as potassium, phosphorus, manganese, chromium, polyphenols and flavonoids. Chlorophyll a and b and pheophytin were the main pigments in the young spelt grass extract. All of these constituents exhibit antioxidant activity, which is important for a healthy lifestyle and protection against diseases.

Conclusion: This extract is a rich source of some major (potassium and phosphorus) and trace (manganese and chromium) elements, pigments, polyphenols and flavonoids. The antioxidant potential of the young spelt grass extract was positively correlated with their phenolic and flavonoid content. Accordingly, this composition of biologically active substances offers various ways of using this extract in the field of nutrition-like dietary supplements.

KEYWORDS
antioxidant potential, cultivation, extract, spelt

INTRODUCTION

Spelt (*Triticum spelta* L., *Poaceae*) belongs to the ancient wheat species. It is assumed that it was formed around 5000 BC and could be considered as the ancestor of wheat. As people turn more and more to organic production, spelt has gained in importance again. It is cultivated as organic wheat with the use of natural pesticides and fertilizers. The spelt has different physical, nutritional and biological

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properties in relation to wheat. It has a shell, so it is more resistant to various weather types such as frost and low temperatures, as well as pests and other adverse effects. Furthermore, the stem is longer than the wheat stem, so it is prone to "lie down" under the influence of wind. Less fertilizer is required for the cultivation in comparison to the cultivation of wheat.[1]

With the rise of commercial production of wheat, spelt grass loses its importance and is produced in very small quantities, mainly for livestock nutrition. Lately, it is returning to people's daily diet because it is claimed to be even healthier than commercial wheat. Being easier to digest than wheat, it is recommended for people with gastrointestinal problems, children and the elderly. Like wheat, it contains gluten, but in a different chemical form, which is much easier to digest, less harmful and causes allergies to a lesser extent. Spelt consumption is associated with a reduced risk of many chronic diseases, including cancer. This can be related to polyphenolic compounds and their antioxidant activity.[2]

Unlike spelt grains, which are described in detail, the leaf extracts of young spelt grass have been much less studied. Based on so far published results, the extracts of young spelt grass contain a sufficient amount of key nutrients and biologically active compounds, which can be considered to have beneficial effects on human health.[3] In Montenegro, this is the first study of young spelt grass extract composition.

MATERIALS AND METHODS

Young spelt grass cultivation

Young spelt grass was cultivated in an experimental facility in Podgorica (Montenegro) intended for later mass production.

The impact of those special conditions is yet to be determined. The individual and/or the cumulative impact of their changes in the final product was monitored by a series of experimental products obtained under different conditions, as follows:

1. Selection of mode of exposure and procurement of raw materials:
   a. Hydroponic (without substrate);
   b. Spelt seed from a registered agricultural farm near Podgorica was chosen as the raw material for germination.

2. Optimization of environmental conditions:
   a. Temperature: 21 °C;
   b. Humidity: machine for seed germination 60%, greenhouse 50%;
   c. Irrigation method: at 12 h (twice a day), at 8 h (three times a day) and at 6 h (four times a day);
   d. Air circulation: permanent;
   e. Lighting: the ratio of light and darkness 24/0, 16/8 and 6/6/6/6.

3. Optimization of harvesting and start of extraction time:
   a. Harvesting time from the moment of germination to the moment of harvesting: 9 days, 11 days and 12 days;
   b. Extraction time: immediately after harvesting.

TABLE 1 Applied growing conditions of young spelt grass

Sample ID	Lighting (hour)	Irrigation (hour)	Harvest (day)
1	6/6/6/6	6/6/6/6	9th
2	6/6/6/6	8/8/8	9th
3	6/6/6/6	12/12	9th
4	16/8	6/6/6/6	9th
5	16/8	8/8/8	9th
6	16/8	12/12	9th
7	24/0	6/6/6/6	9th
8	24/0	8/8/8	9th
9	24/0	12/12	9th
10	6/6/6/6	6/6/6/6	11th
11	6/6/6/6	8/8/8	11th
12	6/6/6/6	12/12	11th
13	16/8	6/6/6/6	11th
14	16/8	8/8/8	11th
15	16/8	12/12	11th
16	24/0	6/6/6/6	11th
17	24/0	8/8/8	11th
18	24/0	12/12	11th
19	6/6/6/6	6/6/6/6	12th
20	6/6/6/6	8/8/8	12th
21	6/6/6/6	12/12	12th
22	16/8	6/6/6/6	12th
23	16/8	8/8/8	12th
24	16/8	12/12	12th
25	24/0	6/6/6/6	12th
26	24/0	8/8/8	12th
27	24/0	12/12	12th

Applying these conditions, 27 different samples were obtained.

The conditions are shown in Table 1.

The seeds were germinated using seed germination machines (Customized HP-25 hydroponic fodder machine, Henan VIC Machinery Co., Ltd). Slow-rotating machines (Angel Juicer 7500, Angel Co., Ltd) were used for extracting liquid from young spelt grass. Obtained extracts were frozen in a special freezer for quick freezing (Alisa 10, Symio Refrigeration Equipment Co., Ltd, China). After the samples were frozen, they were dried using the method of lyophilization (Christ ALPha 1–2 LLD PLUS, Germany) for three days, at −50°C and 0.07 atm.

Nutritive analyses

The recommended methods of the Association of Official Analytical Chemists were used for the determination of the chemical composition (moisture, ash, lipids and nitrogen content) of obtained extracts.[4–8] At least three replicate determinations were made for each sample.

Proteins were calculated by multiplying the sample's percentage nitrogen content by a factor of 6.25. Available carbohydrates were

calculated by subtracting the total sum of moisture, proteins, lipids and ash from a 100% sample.

Total energy values were calculated in kJ by multiplying the amounts of fat by the factor 37 and protein and carbohydrate by the factor 17.[9]

Determination of mineral content

The amount of 500 mg of each fresh extract was accurately weighted (±0.1 mg) and digested in closed microwave-assisted digestion (Berghof MWS-4, Germany) using 5 ml of nitric acid (≥69%, puriss. p.a., Sigma Aldrich) and 2 ml of hydrogen peroxide (≥30%, for trace analyses, Sigma Aldrich). Digested samples were cooled, filtered and transferred in volumetric flasks by adding deionized water.[10,11] All analyses were carried out in triplicates.

Working standards for measurements of major and trace elements were prepared from Sigma Aldrich solutions of 1000 mg l^{-1} each. For analyses of iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and chromium (Cr) ICPMS technique (Agilent ICPMS 7700, USA) was used, while for analyses of calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and phosphorus (P) it was ICP-OES instrument (Thermo iCAP 6300, Germany).

Determination of pigments

For extraction of pigments following procedure was used[12]: 100 mg of the dry extract powder was extracted with 5 ml of ice-cold acetone on an ice bath, using T-25 Ultra-Turrax (Ika-Labortechnik, Germany) homogenizer for 25 s. All extraction procedures were performed in dim light. Obtained acetone extracts were filtered through a 0.2 μm Minisart SRP 15 filter (Sartorius Stedim Biotech GmbH, Germany). After that, extracts were analyzed by HPLC gradient analysis using the following solvents: A: acetonitrile/methanol/water (100/10/5, v/v/v); B: acetone/ethylacetate (2/1, v/v), at a flow rate of 1 ml min^{-1}, employing linear gradient from 10% solvent B to 70% solvent B in 18 min, with a run time of 30 min and photometric detection at 440 nm. The HPLC analysis was performed on an HPLC system with a UV–VIS detector (Dionex UltiMate 3000 Thermo ScientificTM, Germany) using Spherisorb S5 ODS-2 250x4.6 mm column with an S5 ODS-2 50x4.6 mm precolumn (Alltech Associaties, Inc., USA). Pigments were quantified by determining peak areas under the curve in high-performance liquid chromatograms calibrated against known amounts of standards. Each peak was confirmed by the retention time and characteristic spectra of the standards. For the determination of photosynthetic pigments, the following standards were used: α- and β-carotene, lutein, zeaxanthin, pheophytin a, chlorophyll a and b, all from DHI LAB products (Hoersholm, Denmark). All standards were highly purified. The HPLC grade solvents acetone, ethylacetate, methanol and acetonitrile were obtained from Merck.

Determination of total polyphenol and total flavonoid content

Extraction was performed with both distilled water (dH_2O) and 96% ethanol (EtOH) separately. An amount of 0.5 g of each sample was measured, to which 10 ml of solvent was added. After 30 min in an ultrasound bath at 50 °C, the samples were centrifuged at 5000 rpm for 15 min. The supernatants were transferred into 10 ml volumetric flasks and filled with the appropriate solvents. The aqueous extracts were filtered through a 0.45 μm nylon filter.

The total polyphenolic content (TPC) of the extracts was obtained using the spectrophotometric Follin-Ciocalte method.[13] From the aqueous and ethanolic extracts, 0.5 ml was added to 2.5 ml of Folin-Ciocalte reagent, 30 ml of distilled water and 7.5 ml of 20% Na_2CO_3, mixed and filled up to 50 ml with distilled water. The absorbance of the obtained blue-colored mixture was measured spectrophotometricaly (Shimadzu UV-1900, Japan) at 765 nm in comparison to the blank, after 2 h in the dark. As a standard, gallic acid was used. The calibration curve had a range of concentrations of 0–80 mg ml^{-1} and showed good linearity. The results were expressed as mg Gallic Acid Equivalents (GAE) per gram of fresh sample.

For the determination of total flavonoid content (TFC) the following procedure was followed[13]: 0.5 ml of each extract was transferred to 5 ml volumetric flasks, in each 0.15 ml of the 5% $NaNO_2$ was added for 6 min and evenly mixed. After that, 0.15 ml of 10% $AlCl_3$ was added and mixed. After six more minutes, 1 ml of 1 M NaOH was added, and the mixture was filled up to the line with 5 ml of distilled water. Absorbance was measured on a UV–VIS spectrophotometer (Shimadzu UV-1900, Japan) at 510 nm in comparison to the blank solution. The calibration curve was made using a 1000 mM solution of catechin (Sigma Aldrich, Germany) at intervals of 200 mM catechin concentration. The results were expressed as μmol of Catechin Equivalents (CE) per gram of fresh sample.

Antioxidant activity determination

Antioxidant capacity of extracts was determined by running 3 tests.

For the determination of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical potential of the examined samples, in 200 μl of dH_2O as well as EtOH extracts, 2.8 ml of DPPH solution radical was added, and the mixture was mixed very rapidly. The absorbance was measured at 525 nm after the samples rested for 1 h in the dark at room temperature. The calibration curve ranged from 50 to 500 μmol Trolox l^{-1} and was used for the quantification of the antioxidant activity. Results are expressed as μmol Trolox Equivalents (TE) in 1 g of fresh sample.[13]

For the FRAP assay (ferric ion reducing antioxidant power), the solution was prepared with 300 mM acetate buffer (pH 3.6), and 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM solution $FeCl_3x6H_2O$. Before use, it was heated at 37°C. An aliquot of the sample was added to 3 ml of FRAP solution and left for 40 min in the dark. The absorbance of the colored mixture was

read at 593 nm. The antioxidant activity was calculated from the calibration curve ranging from 100 to 700 μmol Trolox l^{-1}. The results were expressed as μmol Trolox Equivalents (TE) in 1 g of fresh sample.[13]

The Trolox equivalent antioxidant capacity (TEAC or ABTS) of the young spelt grass extracts was determined using the ABTS radical discoloration assay. Prepared ABTS solution (14 mM) and potassium peroxydisulfate solution (4.9 mM) in phosphate buffer (pH 7.4) were mixed in the same portions. The solution was left to react overnight (12–16 h) in the dark at room temperature. On the day of analysis, the solution was diluted using the phosphate buffer to the desired absorbance of 0.7 at 734 nm. A sample aliquot of 30 μl was added to 3.0 ml of ABTS solution and after 6 min at 30 °C absorbances were read. The blank was made using 30 μl of phosphate buffer instead of the sample. The calibration curve was in the range of 50–1000 μmol Trolox l^{-1}. The results were expressed as μmol Trolox Equivalents (TE) in 1 g of fresh sample.[13]

In the end, an overall antioxidant potency composite index was determined.[14] An index value of 100 was assigned to the best score for each test. The index score was calculated for all other samples within the test as follows: Antioxidant index score = [(sample score/best score) × 100]. The overall mean index value was determined by dividing the sum of the individual index by the number of tests (three assays in total: DPPH, FRAP and ABTS).

Statistical analyses

Analyses were performed in triplicate. Results are expressed as mean values with the corresponding standard deviation (SD). All the data were analyzed using the SPSS 21-Windows Students version software. Statistical analysis was done by the analysis of variance (ANOVA) followed by Tuckey's test. $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Nutritional composition

The results of the nutrients content and estimated energy values obtained for the 27 samples of *Triticum spelta* L. young leaves extracts are presented in Table 2.

Average dry matter values were 5.97% in the young spelt grass extract harvested on the ninth day from the moment of germination and 5.23% and 5.31% obtained on the eleventh or twelfth day after harvest, respectively.

The total mineral contents (ash) did not vary so considerably and average values were 0.78%, 0.73% and 0.70% fresh weight (FW) in extracts from the ninth, eleventh and twelfth day after harvesting, respectively. The lipid contents for all harvest days varied between 0.23% and 0.25% FW (average values observed in relation to harvest day) which indicated that the young spelt grass extract is a poor source of plant lipids, which is in agreement with the general observation that leafy vegetables are low fat containing food.[15]

Our results showed that extract from spelt grass grown under different conditions had a similar carbohydrate and protein content. The highest carbohydrates (2.79% FW) and protein (2.90% FW) content were in the extract obtained after the harvest on the ninth day, with all-day lighting and irrigation twice a day.

The difference between dry matter, ash, protein and carbohydrate content in the extract from the ninth day was significant compared to extracts from the eleventh and the twelfth harvesting day. Regarding the conditions of lighting and irrigation during the ninth day, for all the above parameters, all-day lighting with irrigation of 12 h was significantly statistically different.

Comparing the obtained results with literature data on the composition of the most commonly investigated similar products, wheat grass and barley grass extract, the average ash content in analyzed young spelt grass was about 10% and 40% higher, respectively. In addition, the average fat content in spelt grass extracts was about 1.5 times higher than in extract obtained from wheat grass and about 1.3 times higher than in extract obtained from barley grass. The obtained results showed that the protein content, compared with literature data for wheat and barley grass, was almost twice as higher, while carbohydrate amounts were similar to those in wheatgrass extracts, but about 20% lower than in barley grass extract. These differences indicate that the extract of young spelt grass has a significantly higher amount of dry matter compared to wheat and barley.[16,17]

Based on the estimated nutrient composition, the energy value of 100 g of fresh young spelt grass extract was calculated. The average value of the ninth-day extract was 93.3 kJ $(100\ g)^{-1}$ which was about 15% higher than in extracts obtained in the remaining two days of harvest.

Minerals

The results for the major mineral content obtained for the 27 samples of *Triticum spelta* L. young leaves extracts are presented in Table 3.

All samples had low amounts of sodium (average 40.4 mg $(100\ g)^{-1}$ FW) and sufficient concentrations of potassium (average 236, 206 and 209 mg $(100\ g)^{-1}$ FW for each harvesting day, respectively). Average magnesium content was low and in the range of 14.3–15.7 mg $(100\ g)^{-1}$ FW. Variations in calcium content were in the range of 7.1–8.6 mg $(100\ g)^{-1}$ FW, while phosphorus average content was 75–79 mg $(100\ g)^{-1}$ in fresh extracts.

Compared to barley grass, the spelt grass had six times higher sodium and twenty times higher magnesium content. However, barley grass is about seven times richer than spelt in calcium.[17] Potassium, calcium and magnesium content was about 6, 3.5 and 8 times higher than in wheatgrass, respectively.[16,18]

The differences between the content of potassium, calcium and phosphorus in the extract on the ninth day were significant in relation to the extracts on the eleventh and twelfth day of harvest. Observing the influence of lighting and irrigation conditions during the ninth day,

TABLE 2 Proximate composition (mean % ± standard error) and energy value of fresh spelt grass extracts

Sample ID	Dry matter %	Ash %	Fat %	Protein %	Total carbohydrate %	Energy value kJ (100 g)$^{-1}$
1	5.10 ± 0.25^{a}	0.65 ± 0.03^{a}	0.14 ± 0.02^{a}	2.02 ± 0.24^{a}	2.29 ± 0.10^{ab}	78.5^{a}
2	5.40 ± 0.33^{ab}	0.75 ± 0.05^{bcd}	0.17 ± 0.02^{ab}	2.18 ± 0.35^{ab}	2.30 ± 0.17^{a}	82.5^{ab}
3	5.95 ± 0.36^{abc}	0.80 ± 0.02^{b}	0.22 ± 0.02^{ab}	2.46 ± 0.19^{abc}	2.47 ± 0.2^{ab}	92.0^{abc}
4	5.25 ± 0.26^{a}	0.66 ± 0.06^{a}	0.20 ± 0.03^{ab}	2.14 ± 0.26^{a}	2.25 ± 0.15^{ab}	82.1^{a}
5	5.45 ± 0.45^{ab}	0.66 ± 0.08^{a}	0.23 ± 0.02^{ab}	2.23 ± 0.33^{a}	2.33 ± 0.11^{abc}	86.1^{abc}
6	6.45 ± 0.36^{bcd}	0.83 ± 0.03^{bc}	0.29 ± 0.03^{ab}	2.79 ± 0.26^{bcd}	2.54 ± 0.25^{abc}	101.2^{bc}
7	6.80 ± 0.19^{cd}	0.84 ± 0.05^{cd}	0.31 ± 0.04^{bc}	2.85 ± 0.24^{cd}	2.75 ± 0.37^{bc}	106.7^{cd}
8	6.50 ± 0.22^{bcd}	0.92 ± 0.07^{bcd}	0.35 ± 0.03^{cd}	2.86 ± 0.14^{cd}	2.37 ± 0.28^{ab}	101.9^{cd}
9	6.85 ± 0.36^{d}	0.89 ± 0.07^{d}	0.32 ± 0.04^{d}	2.90 ± 0.27^{d}	2.79 ± 0.22^{c}	108.7^{d}
Average	*$5.97 \pm 0.31^{\alpha}$*	*$0.78 \pm 0.05^{\alpha}$*	*0.25 ± 0.03*	*$2.48 \pm 0.25^{\alpha}$*	*$2.45 \pm 0.21^{\alpha}$*	*93.3^{α}*
10	4.85 ± 0.31	0.69 ± 0.08	0.17 ± 0.03	2.15 ± 0.26	1.84 ± 0.15	74.2
11	5.00 ± 0.28	0.76 ± 0.09	0.20 ± 0.03	2.22 ± 0.18	1.82 ± 0.27	76.1
12	5.10 ± 0.30	0.72 ± 0.06	0.22 ± 0.02	2.23 ± 0.15	1.93 ± 0.24	78.8
13	4.85 ± 0.19	0.65 ± 0.07	0.20 ± 0.01	2.08 ± 0.11	1.92 ± 0.17	75.4
14	4.75 ± 0.21	0.60 ± 0.04	0.22 ± 0.02	1.98 ± 0.20	1.95 ± 0.19	74.9
15	5.45 ± 0.24	0.71 ± 0.04	0.18 ± 0.03	2.42 ± 0.15	2.14 ± 0.21	84.2
16	5.60 ± 0.21	0.83 ± 0.06	0.24 ± 0.03	2.47 ± 0.17	2.06 ± 0.29	85.9
17	5.40 ± 0.31	0.75 ± 0.05	0.31 ± 0.01	2.46 ± 0.16	1.88 ± 0.17	85.3
18	6.10 ± 0.44	0.89 ± 0.09	0.36 ± 0.04	2.71 ± 0.21	2.14 ± 0.27	95.8
Average	*$5.23 \pm 0.28^{\beta}$*	*$0.73 \pm 0.06^{\beta}$*	*0.23 ± 0.02*	*$2.30 \pm 0.18^{\beta}$*	*$1.96 \pm 0.22^{\beta}$*	*81.2^{β}*
19	5.05 ± 0.34	0.61 ± 0.09	0.20 ± 0.03	2.27 ± 0.26	1.97 ± 0.20	79.5
20	5.30 ± 0.25	0.75 ± 0.03	0.24 ± 0.03	2.39 ± 0.14	1.92 ± 0.27	82.1
21	5.20 ± 0.17	0.69 ± 0.07	0.20 ± 0.02	2.39 ± 0.15	1.92 ± 0.31	80.8
22	5.00 ± 0.30	0.60 ± 0.07	0.24 ± 0.04	2.16 ± 0.19	2.00 ± 0.28	79.7
23	5.45 ± 0.29	0.65 ± 0.07	0.23 ± 0.04	2.36 ± 0.22	2.21 ± 0.11	86.3
24	5.25 ± 0.31	0.71 ± 0.06	0.23 ± 0.05	2.35 ± 0.25	1.96 ± 0.25	81.8
25	5.30 ± 0.19	0.78 ± 0.02	0.23 ± 0.04	2.36 ± 0.11	1.93 ± 0.26	81.5
26	5.40 ± 0.29	0.75 ± 0.03	0.25 ± 0.02	2.42 ± 0.15	1.98 ± 0.20	84.1
27	5.85 ± 0.33	0.76 ± 0.07	0.30 ± 0.04	2.68 ± 0.16	2.11 ± 0.14	92.6
Average	*$5.31 \pm 0.27^{\beta}$*	*$0.70 \pm 0.06^{\beta}$*	*0.24 ± 0.03*	*$2.38 \pm 0.18^{\alpha\beta}$*	*$2.00 \pm 0.22^{\beta}$*	*83.2^{β}*

Note: Data sharing the same letter (a, b, c, d) or (α, β) in the same column are not significantly different, $p > 0.05$, Tuckey's multiple range tests.

for all the above parameters, all-day lighting with irrigation of 12 h was significantly statistically different.

The results of trace mineral contents in fresh spelt grass extracts are presented in Table 4.

The values for copper content in analyzed samples were low, and average values on a harvest day basis were in the range of 0.068–0.074 mg (100 g)$^{-1}$ FW. Zinc average content in spelt grass extracts was 0.37 mg (100 g)$^{-1}$ FW, while the manganese content was 0.32 mg (100 g)$^{-1}$ FW. Young spelt grass extracts are not good sources of iron, and on average, they had 0.58 mg (100 g)$^{-1}$ FW of this mineral. The values for total chromium content in fresh extracts were in the range of 2.3–3.8 μg (100 g)$^{-1}$ FW.

A statistical comparison leads to the conclusion that in the case of the content of microelements, there is a significant difference in the content of iron and chromium in the extract obtained from a grass harvested on the ninth day from seed germination in relation to the other two. In this extract, all analyzed microelements, except chromium, are mostly present in the one obtained with permanent 24-h lighting and irrigation for 12 h.

Although not a significant dietary source of copper, young spelt grass extract contained about four times more copper and twice as zinc compared to the extract from grass obtained by germinating wheat seeds from India. A similar conclusion is drawn for the content of iron, manganese and chromium. Namely, the analyzed extracts had significantly higher amounts of these minerals compared to the amounts in wheatgrass extract. The comparison concludes that there was 30% more iron, about 50% more manganese and even more than five times more chromium.[16,18]

TABLE 3 Major mineral composition of fresh spelt grass extracts (mean mg (100 g)$^{-1}$ ± standard error)

Sample ID	Na	K	Mg mg (100 g)$^{-1}$	Ca	P
1	37.5 ± 2.2^{ab}	202 ± 3^{ab}	12.2 ± 1.2^{a}	7.4 ± 0.4	67 ± 1^{ab}
2	36.2 ± 2.6^{abc}	227 ± 3^{cde}	12.5 ± 1.0^{ab}	9.1 ± 0.8	74 ± 2^{cd}
3	39.3 ± 2.5^{cd}	235 ± 3^{cde}	13.6 ± 1.4^{bc}	7.8 ± 0.4	77 ± 1^{bc}
4	32.5 ± 1.9^{a}	184 ± 3^{a}	11.2 ± 0.9^{a}	7.5 ± 0.4	64 ± 1^{a}
5	38.8 ± 2.8^{abc}	213 ± 2^{abc}	13.2 ± 0.9^{ab}	7.9 ± 0.6	71 ± 1^{abc}
6	44.1 ± 3.0^{abc}	258 ± 3^{bcd}	15.5 ± 1.0^{ab}	5.8 ± 0.1	88 ± 2^{cde}
7	51.5 ± 2.9^{d}	300 ± 4^{de}	18.9 ± 0.7^{d}	10.5 ± 0.2	93 ± 3^{de}
8	38.3 ± 2.5^{ab}	228 ± 4^{abc}	16.8 ± 0.6^{cd}	10.1 ± 0.4	81 ± 1^{cd}
9	49.1 ± 1.9^{d}	278 ± 3^{e}	18.6 ± 0.7^{d}	11.4 ± 0.1	96 ± 3^{e}
Average	$40.8 \pm 2.5^{\alpha\beta}$	$236 \pm 3^{\alpha}$	$14.7 \pm 0.9^{\alpha\beta}$	$8.6 \pm 0.4^{\alpha}$	$79 \pm 2^{\alpha}$
10	32.7 ± 1.9	173 ± 2	11.0 ± 0.7	5.1 ± 0.7	63 ± 2
11	39.9 ± 2.0	226 ± 3	13.9 ± 1.0	6.4 ± 0.9	77 ± 2
12	43.0 ± 2.7	231 ± 3	15.2 ± 1.2	7.0 ± 0.4	72 ± 2
13	34.0 ± 2.9	168 ± 3	12.3 ± 1.4	5.5 ± 0.5	66 ± 1
14	38.6 ± 2.4	193 ± 2	13.2 ± 1.1	6.3 ± 0.6	69 ± 3
15	37.9 ± 2.1	202 ± 2	12.8 ± 0.7	5.4 ± 0.4	77 ± 4
16	43.9 ± 1.8	235 ± 3	17.3 ± 0.6	9.9 ± 0.8	88 ± 4
17	35.2 ± 2.8	194 ± 4	16.4 ± 0.9	10.1 ± 0.4	76 ± 3
18	38.9 ± 2.7	231 ± 5	16.4 ± 0.9	9.3 ± 0.2	82 ± 2
Average	$38.2 \pm 1.9^{\alpha}$	$206 \pm 2^{\beta}$	$14.3 \pm 0.9^{\alpha}$	$7.2 \pm 0.6^{\beta}$	$75 \pm 3^{\alpha\beta}$
19	38.0 ± 1.9	175 ± 3	13.4 ± 0.8	5.9 ± 0.6^{a}	72 ± 3
20	45.1 ± 1.8	247 ± 5	16.1 ± 1.1	6.0 ± 0.5^{a}	87 ± 1
21	44.9 ± 2.3	226 ± 2	15.7 ± 1.1	5.9 ± 0.3^{a}	80 ± 2
22	39.9 ± 2.2	175 ± 3	13.8 ± 1.0	5.0 ± 0.3^{a}	66 ± 2
23	45.2 ± 2.3	212 ± 3	15.6 ± 1.0	6.4 ± 0.4^{a}	83 ± 4
24	39.5 ± 2.5	191 ± 2	13.3 ± 1.2	6.8 ± 0.4^{a}	78 ± 2
25	43.4 ± 2.9	205 ± 2	17.4 ± 0.7	8.4 ± 0.8^{b}	80 ± 2
26	35.2 ± 3.5	194 ± 2	16.4 ± 0.8	8.8 ± 0.6^{b}	76 ± 2
27	48.0 ± 3.4	259 ± 4	19.8 ± 0.9	10.6 ± 0.5^{c}	93 ± 3
Average	$42.2 \pm 2.5^{\beta}$	$209 \pm 3^{\beta}$	$15.7 \pm 1.0^{\beta}$	$7.1 \pm 0.5^{\beta}$	$79 \pm 2^{\beta}$

Note: Data sharing the same letter (a, b, c, d, e) or (α, β) in the same column are not significantly different, $p > 0.05$, Tuckey's multiple range tests.

Compared to young barley grass extracts from Egypt, iron and zinc were twice less, while the amount of manganese was as much as 40 times higher.[17]

Extract of young spelt grass contained high mineral contents, principally potassium, phosphorus, manganese and chromium which proved that fresh spelt grass extract is a good dietary source of mentioned elements (minimum 7.5% of the reference daily intake values [RDI]).[9] Table 5 shows the content of mentioned minerals in fresh spelt grass extract compared with RDI.

It is known that potassium contributes to the normal functioning of the nervous system and maintenance of normal blood pressure. In addition, phosphorus contributes to the normal function of cell membranes, the maintenance of normal teeth and like manganese, contributes to the normal energy-yielding metabolism and the maintenance of normal bones. Furthermore, manganese contributes to the normal formation of connective tissue and the protection of cells from oxidative stress. Chromium contributes to normal macronutrient metabolism and the maintenance of normal blood glucose levels.[19] Also, manganese is essential for antioxidant activity. This mineral was associated with the antioxidant enzyme superoxide dismutase.[20]

The mentioned health benefits of these elements confirm the assumption that the extract of young spelt grass should be included in the human diet in order to preserve and improve good health status.

On the other hand, our results are in accordance with observations that cereal grasses shoots have a lower content of some microelements (Cu, Zn, Fe). Possible reason is that availability of these

TABLE 4 Content of Cu, Zn, Mn and Fe (mean mg (100 g)$^{-1}$ ± standard error) and Cr (mean μg (100 g)$^{-1}$ ± standard error) in fresh spelt grass extracts

Sample ID	Cu mg (100 g)$^{-1}$	Zn mg (100 g)$^{-1}$	Mn mg (100 g)$^{-1}$	Fe mg (100 g)$^{-1}$	Cr μg (100 g)$^{-1}$
1	0.071 ± 0.011^{ab}	0.34 ± 0.06^{a}	0.27 ± 0.06^{a}	0.52 ± 0.09^{a}	3.0 ± 0.4
2	0.078 ± 0.008^{ab}	0.36 ± 0.02^{ab}	0.26 ± 0.03^{a}	0.57 ± 0.09^{ab}	3.2 ± 0.3
3	0.066 ± 0.012^{ab}	0.35 ± 0.05^{a}	0.30 ± 0.02^{a}	0.55 ± 0.06^{a}	4.0 ± 0.5
4	0.062 ± 0.009^{a}	0.32 ± 0.01^{a}	0.25 ± 0.04^{a}	0.50 ± 0.05^{ab}	2.1 ± 0.3
5	0.070 ± 0.009^{ab}	0.33 ± 0.06^{ab}	0.27 ± 0.05^{a}	0.52 ± 0.04^{ab}	5.1 ± 0.5
6	0.079 ± 0.012^{ab}	0.46 ± 0.07^{ab}	0.36 ± 0.05^{a}	0.74 ± 0.06^{abc}	3.7 ± 0.5
7	0.072 ± 0.014^{b}	0.42 ± 0.05^{ab}	0.39 ± 0.07^{b}	0.70 ± 0.08^{bc}	5.6 ± 0.5
8	0.084 ± 0.014^{ab}	0.38 ± 0.04^{a}	0.40 ± 0.03^{b}	0.70 ± 0.03^{bc}	3.6 ± 0.4
9	0.084 ± 0.010^{b}	0.46 ± 0.06^{b}	0.41 ± 0.04^{b}	0.74 ± 0.05^{c}	3.4 ± 0.4
Average	*0.074 ± 0.011*	*0.38 ± 0.05*	*0.32 ± 0.04*	*0.61 ± 0.06*$^{\alpha}$	*3.8 ± 0.4*$^{\alpha}$
10	0.059 ± 0.008	0.29 ± 0.06	0.25 ± 0.06	0.47 ± 0.06	2.8 ± 0.4
11	0.062 ± 0.007	0.36 ± 0.06	0.31 ± 0.02	0.55 ± 0.09	2.2 ± 0.3
12	0.072 ± 0.010	0.30 ± 0.04	0.26 ± 0.03	0.46 ± 0.09	2.4 ± 0.4
13	0.062 ± 0.010	0.39 ± 0.06	0.36 ± 0.06	0.61 ± 0.08	2.8 ± 0.1
14	0.069 ± 0.011	0.37 ± 0.07	0.29 ± 0.02	0.51 ± 0.05	2.7 ± 0.2
15	0.071 ± 0.012	0.35 ± 0.06	0.27 ± 0.05	0.53 ± 0.09	2.4 ± 0.4
16	0.077 ± 0.010	0.37 ± 0.04	0.39 ± 0.05	0.62 ± 0.06	3.4 ± 0.1
17	0.073 ± 0.008	0.32 ± 0.05	0.37 ± 0.04	0.57 ± 0.07	3.6 ± 0.3
18	0.082 ± 0.009	0.37 ± 0.05	0.37 ± 0.04	0.69 ± 0.06	3.7 ± 0.5
Average	*0.070 ± 0.009*	*0.35 ± 0.05*	*0.32 ± 0.04*	*0.56 ± 0.07*$^{\beta}$	*2.9 ± 0.3*$^{\beta}$
19	0.069 ± 0.009	0.35 ± 0.04	0.27 ± 0.03	0.52 ± 0.06	1.9 ± 0.1^{a}
20	0.074 ± 0.012	0.40 ± 0.04	0.34 ± 0.05	0.61 ± 0.04	2.4 ± 0.3^{a}
21	0.066 ± 0.015	0.33 ± 0.06	0.28 ± 0.05	0.49 ± 0.04	2.0 ± 0.3^{b}
22	0.062 ± 0.011	0.31 ± 0.06	0.29 ± 0.04	0.49 ± 0.02	2.3 ± 0.2^{a}
23	0.071 ± 0.008	0.39 ± 0.07	0.33 ± 0.03	0.57 ± 0.06	1.6 ± 0.2^{b}
24	0.065 ± 0.009	0.37 ± 0.05	0.30 ± 0.03	0.55 ± 0.08	1.4 ± 0.3^{a}
25	0.075 ± 0.008	0.38 ± 0.05	0.38 ± 0.06	0.55 ± 0.04	3.1 ± 0.1^{b}
26	0.063 ± 0.010	0.32 ± 0.01	0.37 ± 0.04	0.57 ± 0.06	3.2 ± 0.2^{b}
27	0.064 ± 0.011	0.40 ± 0.08	0.42 ± 0.07	0.68 ± 0.07	2.3 ± 0.1^{b}
Average	*0.068 ± 0.010*	*0.36 ± 0.05*	*0.33 ± 0.04*	*0.56 ± 0.05*$^{\beta}$	*2.3 ± 0.2*$^{\beta}$

Note: Data sharing the same letter (a, b, c) or (α, β) in the same column are not significantly different, $p > 0.05$, Tuckey's multiple range tests.

TABLE 5 Content of minerals in 100 g fresh extract compared with reference daily intake (RDI)

Mineral	RDI	Mean value (mg (100 g)$^{-1}$)	% RDI
K	2000 mg	217	10.9
P	700 mg	78	11.1
Mn	2 mg	0.32	16.0
Cr	40 μg	3.0	7.5

elements from seeds during plant growth was not enough since the concentration of these minerals depends on their bioavailability in the substrate and water.[21]

Pigments

Three classes of pigments namely: xanthophylls, chlorophylls and carotenes were identified and quantified. The results are presented on an FW basis in Table 6.

Two xanthophyll pigments – lutein and zeaxanthin were identified and quantified in young spelt grass extracts. Violaxanthin, antheraxanthin and neoxanthin were not found in any of the samples. In the analyzed samples, lutein and zeaxanthin were present in a ratio of 1:1.5. This ratio is not characteristic of other edible green leafy plants in which the amount of lutein is significantly predominant.[22] The lutein content was measured in the range 558–1700 μg (100 g)$^{-1}$ FW, while the amount of zeaxanthin ranged from 630 to 2105 μg (100 g)$^{-1}$ FW.

TABLE 6 Content of pigments (mean mg [100 g^{-1}] ± standard error) in fresh spelt grass extracts

Sample ID	α-Carotene μg (100 g)$^{-1}$	β-Carotene μg (100 g)$^{-1}$	Lutein μg (100 g)$^{-1}$	Zeaxanthin μg (100 g)$^{-1}$	Pheophytin a μg (100 g)$^{-1}$	Chlorophyll a μg (100 g)$^{-1}$	Chlorophyll b μg (100 g)$^{-1}$
1	7.8 ± 0.9	61.3 ± 7.8	624 ± 11	766 ± 8	3715 ± 35	2862 ± 11	8830 ± 25[a]
2	16.7 ± 1.2	120.9 ± 9.9	1463 ± 29	1847 ± 12	6788 ± 28	6039 ± 18	18,742 ± 28[ab]
3	26.5 ± 1.2	132.0 ± 12.5	817 ± 19	1144 ± 24	6146 ± 35	4978 ± 22	14,884 ± 33[ab]
4	12.8 ± 1.0	68.9 ± 12.7	610 ± 14	838 ± 21	5862 ± 21	2402 ± 19	10,732 ± 39[ab]
5	16.5 ± 0.9	131.0 ± 9.0	897 ± 18	1094 ± 15	7263 ± 24	3788 ± 10	15,753 ± 41[ab]
6	41.2 ± 1.4	233.8 ± 13.5	1700 ± 25	2105 ± 11	8201 ± 29	6449 ± 11	28,096 ± 44[ab]
7	18.1 ± 1.1	124.2 ± 12.9	953 ± 24	1119 ± 22	6999 ± 31	4238 ± 22	18,721 ± 35[ab]
8	19.7 ± 1.3	134.9 ± 11.4	1382 ± 29	1657 ± 17	8111 ± 40	5528 ± 14	19,526 ± 39[ab]
9	36.3 ± 1.7	268.0 ± 14.9	1270 ± 36	1750 ± 11	8807 ± 33	6503 ± 13	24,766 ± 28[b]
Average	21.7 ± 1.2	141.7 ± 11.5	1079 ± 23	1369 ± 16	7210 ± 31	4754 ± 16	17,784 ± 35
10	16.3 ± 2.2	164.9 ± 11.1	706 ± 11	888 ± 15	5008 ± 24	3221 ± 11	11,039 ± 38
11	13.8 ± 2.5	111.4 ± 9.7	606 ± 14	753 ± 16	4714 ± 24	2861 ± 10	10,726 ± 55
12	16.8 ± 0.9	142.7 ± 8.1	570 ± 25	630 ± 14	4336 ± 11	3433 ± 15	10,076 ± 24
13	13.7 ± 1.7	130.9 ± 8.6	703 ± 31	899 ± 10	6371 ± 24	3225 ± 24	13,021 ± 29
14	17.4 ± 2.0	73.1 ± 7.7	634 ± 25	801 ± 8	7008 ± 20	3399 ± 24	11,756 ± 33
15	15.2 ± 1.1	112.4 ± 11.0	834 ± 19	1150 ± 15	4976 ± 36	5141 ± 20	12,586 ± 41
16	32.2 ± 1.1	233.4 ± 14.5	977 ± 19	1224 ± 14	6366 ± 15	5331 ± 19	16,170 ± 24
17	21.3 ± 1.1	167.2 ± 11.0	1141 ± 19	1417 ± 10	6356 ± 24	4201 ± 29	18,121 ± 56
18	24.8 ± 1.6	144.3 ± 9.3	1510 ± 21	1848 ± 11	6937 ± 25	6010 ± 25	24,700 ± 25
Average	19.1 ± 1.6	142.3 ± 10.1	853 ± 20	1068 ± 13	5786 ± 23	4091 ± 20	14,244 ± 36
19	5.9 ± 0.8	90.5 ± 11.0	558 ± 8	700 ± 17	4072 ± 21	3808 ± 14	8550 ± 21
20	19.9 ± 1.7	105.2 ± 14.5	757 ± 15	1025 ± 14	5829 ± 23	4358 ± 15	11,947 ± 20
21	22.8 ± 1.9	195.9 ± 10.0	1003 ± 24	1228 ± 21	6168 ± 10	4079 ± 25	15,021 ± 18
22	20.7 ± 2.0	233.4 ± 9.6	1115 ± 24	1372 ± 20	7507 ± 11	4757 ± 14	18,519 ± 30
23	4.8 ± 1.0	75.9 ± 11.2	747 ± 11	1047 ± 11	5330 ± 20	4921 ± 17	12,826 ± 14
24	20.6 ± 1.8	195.1 ± 15.5	1093 ± 19	1360 ± 15	7096 ± 17	4504 ± 20	16,027 ± 25
25	23.0 ± 1.1	134.5 ± 15.7	1224 ± 22	1913 ± 14	7267 ± 18	7077 ± 21	16,849 ± 29
26	28.0 ± 1.2	209.3 ± 14.7	1112 ± 14	1502 ± 17	6217 ± 19	5732 ± 19	15,728 ± 14
27	30.3 ± 1.4	170.3 ± 11.3	1253 ± 28	1493 ± 17	6965 ± 25	4955 ± 18	20,355 ± 33
Average	19.5 ± 1.4	155.7 ± 12.6	984 ± 18	1293 ± 17	6272 ± 18	4910 ± 18	15,091 ± 23

Note: Data sharing the same letter (a, b) or in the same column are not significantly different, $p > 0.05$, Tuckey's multiple range tests.

Dietary carotenoids, especially xanthophylls, enjoyed significant scientific attention because of their characteristic biological activities, including anti-allergic, anti-cancer and anti-obese actions. Lutein is one of the major xanthophylls present in green leafy vegetables and it is known to selectively accumulate in the macula of the human retina.[23] As an antioxidant and as a blue light filter, lutein can protect the eyes from oxidative stress, of which a non-protection can lead to age-related macular degeneration and cataracts.[24–26]

Chlorophyll a and chlorophyll b are the predominant forms of chlorophyll in higher plants that play a key role as primary photosynthetic pigments that allow plants to absorb energy from light. Carotenoides, on the other hand, play a role in the photoprotection of chlorophyllous pigments.[27,28]

The chlorophyll a/b ratio was found to be similar in all extracts (1:3–1:4), although the chlorophyll a and b contents varied greatly (chlorophyll a 2402–7077 μg (100 g)$^{-1}$ FW and chlorophyll b 8550–28,096 μg (100 g)$^{-1}$ FW). The results also indicated that significant amounts of chlorophyll a were converted into pheophytin a, probably during the lyophilization process.

α- and β-carotene were found in all samples in the range 4.8–41.2 μg (100 g)$^{-1}$ FW and 61.3–268.0 μg (100 g)$^{-1}$ FW respectively. Compared to wheat and barley grass harvested on the 10th day of vegetation, young spelt grass extract contained on average about 70% less α-carotene and 50% more β-carotene.[29] An increased intake of β-carotene-rich food in daily diet may be one of the strategies for improving vitamin A status instead of synthetic vitamin A.

TABLE 7 Content of polyphenols (mean mg GAE g^{-1} ± standard error) and flavonoids (mean mg CE g^{-1} ± standard error) in fresh spelt grass extracts

	TPC (mg GAE g^{-1})		TFC (mg CE g^{-1})	
Sample ID	EtOH	dH_2O	EtOH	dH_2O
1	39.7 ± 1.5	91.4 ± 1.1	8.5 ± 0.9^{a}	35.3 ± 1.1^{a}
2	40.2 ± 1.4	99.4 ± 1.4	34.2 ± 0.7^{ab}	43.6 ± 0.9^{a}
3	69.6 ± 1.6	108.4 ± 1.9	23.8 ± 0.9^{ab}	87.1 ± 1.2^{ab}
4	64.4 ± 1.0	100.5 ± 1.4	19.2 ± 1.1^{ab}	81.6 ± 1.4^{ab}
5	59.1 ± 1.8	98.8 ± 1.7	22.8 ± 1.2^{ab}	88.1 ± 1.1^{ab}
6	65.4 ± 1.9	110.5 ± 1.5	13.5 ± 1.1^{ab}	96.2 ± 1.2^{a}
7	72.8 ± 2.0	118.7 ± 1.8	43.0 ± 1.2^{b}	105.4 ± 1.7^{ab}
8	68.9 ± 1.5	117.8 ± 1.7	48.5 ± 1.4^{ab}	116.1 ± 1.7^{ab}
9	73.3 ± 1.7	118.5 ± 2.0	27.5 ± 1.1^{ab}	129.0 ± 1.4^{b}
Average	*61.5 ± 1.6*	*107.1 ± 1.6*$^{\alpha}$	*26.8 ± 1.1*	*86.9 ± 1.3*
10	54.0 ± 1.9	88.4 ± 2.0	22.2 ± 1.5	67.6 ± 1.3
11	49.4 ± 1.7	86.7 ± 1.4	10.8 ± 1.4	64.6 ± 1.5
12	55.6 ± 1.8	95.8 ± 1.7	22.3 ± 1.3	78.5 ± 1.2
13	51.5 ± 1.4	87.1 ± 1.8	19.0 ± 1.1	65.3 ± 1.1
14	63.3 ± 1.2	112.4 ± 1.8	30.0 ± 1.4	96.8 ± 1.1
15	40.3 ± 1.1	97.0 ± 1.4	23.5 ± 1.3	46.5 ± 1.5
16	52.6 ± 1.4	62.7 ± 1.5	35.1 ± 1.4	69.6 ± 2.0
17	58.7 ± 1.5	98.9 ± 1.8	22.3 ± 1.1	80.7 ± 1.9
18	70.5 ± 1.4	112.0 ± 1.6	27.1 ± 1.1	122.2 ± 1.8
Average	*55.1 ± 1.5*	*93.4 ± 1.7*$^{\beta}$	*23.6 ± 1.3*	*76.9 ± 1.5*
19	56.1 ± 1.2	92.9 ± 1.4	19.7 ± 0.9	74.8 ± 1.7
20	60.9 ± 1.7	107.9 ± 1.9	21.3 ± 1.5	94.9 ± 1.7
21	46.8 ± 1.4	100.7 ± 1.8	14.9 ± 1.5	66.0 ± 1.1
22	56.5 ± 1.8	86.5 ± 1.7	18.8 ± 1.0	88.2 ± 1.3
23	63.3 ± 1.4	112.4 ± 1.6	30.0 ± 1.0	96.8 ± 1.3
24	48.0 ± 1.2	94.7 ± 1.3	28.7 ± 1.4	64.8 ± 1.2
25	68.6 ± 1.8	108.0 ± 1.7	27.8 ± 1.3	84.8 ± 1.5
26	44.1 ± 1.4	98.1 ± 1.9	18.4 ± 1.1	65.7 ± 1.8
27	58.3 ± 1.4	102.5 ± 2.0	25.2 ± 1.5	91.9 ± 1.7
Average	*55.8 ± 1.5*	*100.4 ± 1.7*$^{\beta}$	*22.8 ± 1.2*	*80.9 ± 1.3*

Note: Data sharing the same letter (a, b) or (α, β) in the same column are not significantly different, $p > 0.05$, Tuckey's multiple range tests.
Abbreviations: CE, catechin equivalent; GAE, galic acid equivalents; TFC, total flavonoid content; TPC, total polyphenol content.

Only in the case of chlorophyll b significant differences between the extract on the ninth day was observed in relation to the extracts on the eleventh and twelfth day of harvest.

Total polyphenols and total flavonoids

Polyphenolic compounds as secondary metabolites may be present in different amounts which may depend on the food production methods.[30] They are strong natural antioxidants with a lot of functions in the plant defense system.[31] The presence of polyphenols largely depends on the applied growing conditions, degree of maturity and diversity of plants. A small number of studies have investigated the impact of cultivation techniques on flavonoid content, and existing research results are inconsistent.[30]

Results of the TPC (Total polyphenol content) and TFC (Total flavonoid content) assays are shown in Table 7.

From the TPC assay, it was concluded that the polyphenolic concentration in the ethanol and aqueous extracts is highest in those from grass harvested on the ninth day, and amounts to 61.5 and 107.1 mg GAE g^{-1} fresh sample respectively. Obtained results for TPC were about 50% higher than those reported in the literature for wheat grass.[32]

TABLE 8 Antioxidant capacity of fresh spelt grass extracts (mean μM TE g^{-1} ± standard error)

	FRAP (μM TE g^{-1})		DPPH (μM TE g^{-1})		ABTS (μM TE g^{-1})	
Sample ID	EtOH	dH_2O	EtOH	dH_2O	EtOH	dH_2O
1	71.9 ± 2.2^{ab}	136.7 ± 2.6	249.9 ± 3.7	623.2 ± 3.9^{a}	149.9 ± 2.2	240.7 ± 3.9^{ab}
2	81.0 ± 2.8^{a}	132.6 ± 3.5	258.1 ± 4.2	678.8 ± 3.1^{abc}	152.3 ± 2.2	266.8 ± 3.8^{ab}
3	102.3 ± 2.9^{ab}	122.0 ± 3.5	529.0 ± 1.9	733.0 ± 3.3^{bcd}	261.8 ± 2.5	280.2 ± 3.0^{ab}
4	96.6 ± 3.0^{ab}	117.1 ± 2.4	639.5 ± 4.5	628.4 ± 2.9^{ab}	251.0 ± 3.0	268.8 ± 2.8^{a}
5	95.4 ± 2.2^{ab}	106.3 ± 1.9	488.9 ± 3.3	772.8 ± 4.4^{bcd}	234.9 ± 3.1	248.0 ± 2.7^{ab}
6	112.9 ± 2.5^{ab}	115.5 ± 2.4	551.5 ± 2.4	896.6 ± 3.9^{abcd}	290.9 ± 3.5	266.4 ± 3.3^{ab}
7	112.2 ± 3.0^{ab}	121.7 ± 2.2	616.1 ± 2.6	967.0 ± 3.7^{cd}	288.3 ± 4.2	283.6 ± 3.3^{ab}
8	117.1 ± 2.9^{ab}	120.6 ± 3.1	535.0 ± 3.3	835.9 ± 4.1^{abcd}	275.0 ± 3.5	289.1 ± 3.4^{ab}
9	123.5 ± 2.0^{b}	156.1 ± 3.4	672.7 ± 3.7	960.4 ± 4.0^{d}	308.9 ± 3.7	289.3 ± 3.8^{b}
Average	*101.4 ± 2.6*$^{\alpha}$	*125.4 ± 2.8*	*504.5 ± 3.3*	*788.5 ± 3.7*	*245.9 ± 3.1*	*270.3 ± 3.3*$^{\alpha}$
10	98.9 ± 2.5	90.7 ± 2.9	437.0 ± 2.8	615.5 ± 3.6	207.1 ± 3.0	224.6 ± 3.4
11	83.0 ± 2.5	83.0 ± 2.8	425.5 ± 3.6	687.5 ± 3.5	190.0 ± 3.1	191.5 ± 3.4
12	89.3 ± 2.5	98.9 ± 2.2	499.8 ± 3.8	760.4 ± 3.9	221.9 ± 2.5	242.8 ± 3.5
13	85.4 ± 2.5	92.2 ± 2.2	399.6 ± 4.1	638.7 ± 3.8	199.3 ± 2.4	214.9 ± 3.4
14	81.8 ± 2.7	136.8 ± 2.2	540.1 ± 3.5	808.2 ± 4.1	218.0 ± 2.4	262.7 ± 2.9
15	75.8 ± 2.1	139.5 ± 2.0	298.7 ± 3.8	659.5 ± 2.9	153.1 ± 2.2	246.9 ± 2.8
16	93.0 ± 2.7	118.7 ± 2.8	442.4 ± 3.6	725.2 ± 3.6	189.3 ± 2.8	259.3 ± 3.7
17	95.0 ± 2.8	105.3 ± 2.4	482.2 ± 3.1	732.2 ± 4.0	225.7 ± 3.1	233.8 ± 2.4
18	119.0 ± 3.1	115.3 ± 2.5	567.3 ± 3.5	770.4 ± 3.3	286.7 ± 3.1	269.0 ± 2.9
Average	*91.2 ± 2.6*$^{\beta}$	*108.9 ± 2.2*	*454.7 ± 3.5*	*710.9 ± 3.6*	*210.1 ± 2.7*	*238.4 ± 3.2*$^{\beta}$
19	87.9 ± 3.0	88.9 ± 1.9	484.8 ± 3.5	620.1 ± 3.8	214.6 ± 3.9	235.3 ± 3.0
20	84.3 ± 2.4	121.9 ± 3.1	524.7 ± 2.9	757.9 ± 3.7	239.6 ± 3.5	271.9 ± 3.1
21	75.4 ± 2.1	105.0 ± 2.4	422.2 ± 3.9	824.7 ± 4.0	170.0 ± 3.5	242.3 ± 3.4
22	90.5 ± 2.3	81.5 ± 2.4	498.0 ± 4.0	705.0 ± 3.0	230.5 ± 2.7	203.0 ± 3.3
23	81.8 ± 2.4	136.8 ± 2.8	540.1 ± 4.1	808.2 ± 3.5	218.0 ± 3.5	262.7 ± 2.7
24	85.1 ± 2.2	104.5 ± 2.8	388.0 ± 3.3	659.9 ± 3.3	175.4 ± 3.4	242.0 ± 2.4
25	91.7 ± 2.8	118.2 ± 2.0	595.7 ± 3.1	799.2 ± 3.8	241.7 ± 3.4	235.9 ± 2.6
26	67.5 ± 2.7	114.8 ± 1.9	389.3 ± 3.1	628.3 ± 3.5	156.6 ± 3.3	236.4 ± 2.6
27	88.9 ± 2.9	100.0 ± 2.0	531.8 ± 2.9	908.5 ± 4.0	215.9 ± 2.9	252.1 ± 2.3
Average	*83.7 ± 2.5*$^{\beta}$	*108.0 ± 2.4*	*486.1 ± 3.4*	*745.8 ± 3.6*	*206.9 ± 3.3*	*242.4 ± 2.8*$^{\beta}$

Note: Data sharing the same letter (a, b, c, d) or (α, β) in the same column are not significantly different, $p > 0.05$, Tuckey's multiple range tests.
Abbreviations: ABTS, trolox equivalent antioxidant capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric ion reducing antioxidant power; TE, trolox equivalent.

The flavonoid content followed the content of total polyphenols in EtOH extracts and was higher about four times than in wheat grass.[32]

Also, in the case of TPC and TFC, the conditions of 24-h lighting and irrigation every 12 h gave an extract with the highest content of analyzed components.

Antioxidant capacity

Antioxidant activities of extracts obtained from young spelt grass were examined through their abilities to scavenge synthetic DPPH and ABTS radicals, as well as their power to reduce ferric (FRAP). All analyses were performed in triplicates and obtained results were expressed as μM TE g^{-1} of fresh weight (Table 8).

FRAP test is quick and easy to perform, based on a repeatable reaction, which measures the reducing power that is linearly related to the molar concentration of antioxidants.[33] This linear dependence leads to the conclusion that the total phenol content can be a reliable indicator of the antioxidant activity of the samples.

Results of the FRAP test showed the antioxidant activity of the aqueous extracts was slightly higher compared to the ethanolic and varied from 83.7 μmol TE g^{-1} (average value for harvest twelfth day after germination) to 101.4 μmol TE g^{-1} of the fresh sample (average

TABLE 9 Antioxidant composite index (ACI) of the extracts was obtained from the grass harvested on the ninth day after seed germination which grew under different lighting and irrigation conditions

	FRAP index		DPPH index		ABTS index		ACI (%)	
Sample ID	EtOH	dH_2O	EtOH	dH_2O	EtOH	dH_2O	EtOH	dH_2O
1	58	88	37	65	49	83	48	79
2	66	85	38	71	49	92	51	83
3	83	78	79	76	85	97	82	84
4	78	75	95	65	81	93	85	78
5	77	68	73	80	76	86	75	78
6	91	74	82	93	94	92	89	86
7	91	78	92	100	93	98	92	92
8	95	77	80	87	89	100	88	88
9	100	100	100	100	100	100	100	100

Abbreviations: ABTS (TEAC), trolox equivalent antioxidant capacity; ACI, antioxidant composite index; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric ion reducing antioxidant power.

value for harvest ninth day after germination). For aqueous extracts, average values varied from 108.0 to 125.4 μmol TE g^{-1} of fresh samples, respectively. The highest values (123.5 μM TE g^{-1} in EtOH and 156.1 μM TE g^{-1} in dH_2O) were recorded in the young spelt grass extract obtained under conditions of permanent lighting and irrigation two times per day (every 12 h) and harvested nine days after seed germination.

For evaluation of the free radical scavenging properties of the extracts, DPPH radical assay and the ABTS radical cation assay were used.

To determine the antioxidant activity of various plant extracts, a relatively stable organic radical DPPH is very often used.[34] Results of the DPPH test showed that the antioxidant activity of dH_2O extracts was significantly higher than EtOH. The average values for free radical scavenging ability varied between 454.7 μM TE g^{-1} (11th day) to 504.5 μM TE g^{-1} (9th day) in ethanol extracts using the DPPH antioxidant method. The same was for aqueous extracts. Values ranged from 710.9 μM TE g^{-1} (11th day) to 788.5 μM TE g^{-1} (9th day). Similar to before, in this case, the conditions of all-day lighting, irrigation every 12 h and harvesting after nine days gave an extract with the highest result, which indicates the strongest activity.

Results of the ABTS test showed that the antioxidant activity of the ethanol extracts varied from 206.9 to 245.9 mmol TE g^{-1} of fresh sample. As it was for the DPPH, aqueous extracts showed slightly higher values. Also, in this case, the extract of grass harvested on the ninth day, which was grown under conditions of permanent lighting and watered every 12 h, showed the strongest activity.

The results obtained from all three tests indicate that the antioxidant capacity of the analyzed samples, as already assumed, follows and depends on the content of TP and TF. Correlations between the results obtained with all three antioxidant tests were positive, as well as the correlation between antioxidant activity with TP and TF content ($r \sim 0.99$, $p < 0.05$).

The results obtained for FRAP, DPPH and ABTS tests were significantly higher than those obtained in the same studies on fresh wheatgrass juice.[35]

The overall antioxidant activity of the extracts obtained from the grass harvested on the ninth day after seed germination is expressed as an antioxidant composite index (ACI) (Table 9). A statistical-mathematical model is used to estimate ACI values, which is a modern tool for ranking the display of antioxidant activity of various plants. The main advantage of using ACI is that the value expressed as a percentage includes all segments of antioxidant activity obtained in various antioxidant tests. Another advantage of using the ACI index instead of three tests is that it simplifies the process of comparing the antioxidant properties of different foods. The antioxidant composite index (ACI) of young spelt grass extracts (Table 9) was in the following descending order:

$$\mathrm{EtOH} - 9 > 7 > 6 > 8 > 4 > 3 > 5 > 2 > 1$$

$$\mathrm{dH_2O} - 9 > 7 > 8 > 6 > 3 > 2 > 1 > 4,5.$$

CONCLUSIONS

This study is the first comprehensive study offering detailed information on nutritional composition, major and trace elements, pigments, phenolic, flavonoids and antioxidant activity of extracts of young spelt grass (*Triticum spelta* L.) cultivated in Montenegro. This extract is a rich source of some major (potassium and phosphorus) and trace (manganese and chromium) elements, pigments (especially chlorophyll and pheophytin), polyphenols and flavonoids. The antioxidant potential of young spelt grass extract was positively correlated with their phenol and flavonoid content. Accordingly, this composition of biologically active substances offers various ways of using this extract in the field of nutrition-like dietary supplements.

When it comes to the applied growing conditions, it is concluded that by the cultivation of young spelt grass for nine days after seed

germination, with permanent lighting and watering every 12 h, an extract of the richest nutritional composition was obtained.

FUNDING INFORMATION

Data for this research were obtained as a result of the implementation of program activities on the project "*Triticum spelta* L. extract as a dietary supplement" co-financed by the Ministry of Economic Development of Montenegro under the Grant agreement number 01–2617/5.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are available in the manuscript.

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How to cite this article: Jančić D, Šuković D, Rešetar J, Delić L, Nikolić M. Nutritional composition, biologically active substances and antioxidant activity of young spelt grass extract. JSFA Reports. 2022;2(8):385–97. https://doi.org/10.1002/jsf2.73