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Comparison between Organic and Conventional Spelt and Wheatgrass juice

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Abstract
This work is aimed to compare antioxidant and scavenger properties of conventionally and organically produced speltgrass. Moreover, the antioxidant status of spelt was compared with wheatgrass. Three spelt varieties were analyzed: Nirvana, Ebners–Rotkorn variety and Eco-10. Antioxidant enzymes, superoxide-dismutase and guiacol-peroxidase, were determined as well as ferric reducing antioxidant power and DPPH-antiradical power. Total soluble proteins and total phenols were determined and also lipid peroxidation as a parameter of oxidative stress. The organically produced spelt was exposed to the higher levels of oxidative stress than conventionally produced spelt, and also DPPH antiradical power was induced by conventional production, which is also demonstrated using the relative antioxidant capacity index. The best antioxidant properties, better than wheat, showed organically produced spelt genotype Ebners–Rotkorn which is determined by the highest phenol content, superoxide-dismutase activity, DPPH antiradical power and ferric reduced antioxidant power and consequently with the lowest lipid peroxidation intensity.

Keywords
grass juice, Triticum aestivum ssp., spelt, Triticum aestivum, antioxidant activity, lipid peroxidase

1 Introduction
Spelt (Triticum aestivum ssp. spelt) is an ancient subspecies of modern bread wheat (Triticum aestivum). For many years, cultivation of spelt declined, due to spelt’s lower yield and its long straw with a tendency to lodge. In recent years, interest in the use of spelt increased, because it is considered as “healthier” and more “natural” grain than modern wheat. 

Spelt possesses a valuable nutritional quality. Specificity of spelt in relation to wheat is in favor of spelt, which possesses more bioactive components in neo-saponified part of the liposoluble fraction [3]. It has higher protein content than wheat, some mineral contents, including selenium and zinc as well as high fiber content [4, 5]. Wheat contains diverse bioactive compounds that may contribute to its antioxidant capacity. These bioactive components include carotenoids, tocopherols, tocotrienols, phe-nolic acids, phytic acid, phytosterols and flavonoids [6, 7]. Previous studies have shown that wheat varieties vary in antioxidant properties as well as in composition in secondary biomolecules including antioxidants [8].

Wheatgrass (aboveground parts of the wheat) was traditionally used in ancient Egypt and perhaps even early Mesopotamian civilizations. Wheatgrass and spelt grass are powerful, concentrated liquid nutrients which have...
been used as home-made remedy or commercially distributed as frozen juice or powdered and freeze dried [9, 10]. Like most plants, it contains chlorophyll, amino acids, minerals, vitamins, and enzymes. It was reported the significant presence of polyphenols and other flavonoids responsible for antioxidant activity as well as a diverse class of organic compounds in the aqueous extract, ranging from saturated and unsaturated fatty acids, alcohols and terpenes [11]. It has also been believed that wheatgrass could strengthen the immune system and increase the life span of cancer patients by regressing the spread of cancer cells [12].

Organic agriculture implies no use of synthetic fertilizers, genetically modified materials, sewage sludge or pesticides. Although the production and need for organic production are recently increasing in the world, it is necessary to find determination method for organic food quality determination with focus on laboratory methods [13]. Many studies found that organic methods produced lower yields and therefore required more land for a given production level [14]. A recent life cycle assessment (LCA) study showed that organic farming was either superior or similar to the conventional integrated production, but with exceptions for some products [15]. It was found that spelt endures well under suboptimal growing conditions [16], and to better utilize nutrients when grown in a low-input system [17], and show more resistance to a number of pathogens [18], than common wheat, thus spelt is recommended for organic agriculture.

Principal Component Analysis (PCA) was applied as a well known pattern recognition technique to the experimental data (used as descriptors) to characterize and differentiate among the observed samples.

Based on results obtained employing all antioxidant capacity (AOC) assays, relative antioxidant capacity index (RACI) has been calculated. Antioxidant efficiency of phenolics present in analyzed samples has been estimated based on phenolic antioxidant coefficients (PAC), determined as a ratio between particular AOC and total phenolic content (TPC), and compared to obtain a more comprehensive insight into the activity of analyzed samples.

In this way, the present study aimed to compare the antioxidant activity of organically and conventionally produced speltgrass and wheat grass. In order to evaluate antioxidant status of spelt and wheat antioxidant enzymes (superoxide-dismutase and guiacol-peroxidase), soluble proteins, total phenol content, 2,2′-Diphenyl-1-Picrylhydrazyl (DPPH) antiradical power, ferric reducing antioxidant power and lipid peroxidation were determined.

2 Materials and Methods

2.1 Chemicals and reagents

2,2′-Diphenyl-1-Picrylhydrazyl (DPPH), 2,4,6-Tripyridyl-Triazine (TPTZ), Nitro Blue Tetrazolium (NBT), Guaiacol (2-methoxyphenol), Folin and Ciocalteu-phenol reagent, 2-Thiobarbituric acid, ferric chloride and mono-basic potassium phosphate were obtained from Sigma-Aldrich (Beograd, Serbia). All reagents and chemicals were of analytical grade.

2.2 Material

The field experiment was carried out on three spelt variety (Triticum aestivum subs. spelt) and one wheat (Triticum aestivum). The spelt variety used in the experiment was Ecol0 and Nirvana, which were supplied from a Serbian market and one variety (Ebners–Rotkorn) which was supplied from an Austrian market. The wheat variety used in the experiment was Europa 90.

2.3 Field experiment

The experiments were conducted at locality Institut “Tamiš” Pančevo (N 44° 56`, E 20° 43`) on the chernozem type of soil in the 2010–2011 vegetation season. The trial was set up in a randomized complete block design with four replicates. Each plot consisted of 10 rows, 10 cm apart and 5 m long (the harvested area was 5 m²). Seedling density was considered as 500 seeds/m². Growth stages of wheat were 30 (BBSH, 2001) [19].

2.4 Extraction procedures

Plant material was previously frozen at -70 °C in SNIJDERS deep freezer (VF360-86). For the freeze-drying process, frozen leaves were lyophilized in a Martin Crist Alpha 1-2 LDplus (osterode, Germany) lyophilizer. The main drying process was performed at p = 0.12 mbar and temperatures from -40 °C to 0 °C for 48 h. The final drying lasted 5 h at p = 0.005 mbar and temperatures from 20 °C to 30 °C. Plant material (1 g dry leaves) was extracted with 25 ml 80 % aqueous ethanol (0.1 M HCl) under 30 min sonication in an ultrasonic bath (Elmasonic S 100-H; Elma Schmidbauer GmbH) at ambient temperature. The extract was rapidly vacuum-filtered through a sintered glass funnel and kept refrigerated. This extract was used for determination of total phenol content, ferric reducing antioxidant power (FRAP) and DPPH antiradical power.

For the determination of lipid peroxidation (LP) and antioxidant enzymes, 2 g of plant material (fresh leaves) was extracted with 10 ml 0.1 M K₂HPO₄ (pH 7.0) under
30 min sonication in an ultrasonic bath at ambient temperature. After 10 minutes of centrifugation at 4 °C and 10,000xg, aliquots of the supernatant were used for enzyme activity determinations. The used centrifuge was BOECO Germany (U-320-R).

2.5 Antioxidant enzymes and soluble proteins
The superoxide dismutase (SOD) activity was assayed according to Giannopolitis and Ries [20], by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT). Glass test tubes containing the mixture were immersed in a bath at 25 °C and illuminated by a fluorescent lamp (Philips MLL 5000W). Identical tubes, which were not illuminated, served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm, using Thermoscientific UV-Visible spectrophotometer (Evolution 220). One unit of SOD was defined as the enzyme activity which inhibited the photoreduction of nitro-blue tetrazolium to the blue formazan by 50 %, and SOD activity of the extracts was expressed as SOD units per mg of protein. Reaction medium was prepared by mixing 2.6 mL 50 mM phosphate buffer (pH 7.8), 100 μL 13 mM methionine, 100 μL 75 μM NBT, 100 μL 0.1 mM EDTA and 50 μL 2 μM riboflavin.

The guaiacol peroxidase (GPx) activity was measured following the method of Kato and Shimizu [21]. The activity was calculated using the extinction coefficient of 26.6 mM$^{-1}$ cm$^{-1}$ at 470 nm for oxidized tetraguaiacol polymer. One unit of GPx activity was defined as the calculated consumption of 1 μmol of H$_2$O$_2$ min$^{-1}$ mg protein$^{-1}$.

Soluble proteins were determined by the Bradford method (1976) [22]. Reaction medium contained 1 mL 0.07 M Comassie brilliant blue in 3 % HClO$_4$, 1 mL distilled water and 25 μL of sample or standard (bovine serum albumin). After 5 min of incubation, absorbance of samples and standard was recorded at λ=595 nm. Protein concentration was read from calibration curve and finally expressed as mg protein/g dry matter.

2.6 Total phenols content (TPC)
The total phenolic content was determined by a modification of the Foline-Ciocalteu method and the results expressed as mg Gallic acid/100 g dry weight [23, 24]. In 4.2 mL of distilled water, 50 μL of the corresponding extract was added and 250 μL of Folin&Ciocalteu-phenol reagent (previously diluted 1:10 with distilled water). The mixture was neutralized with 20 % sodium-carbonate solution after 5 minutes, and the content transferred into a 1.5 mL cuvette (1 cm path); absorbance was determined after 60 min at 720 nm.

2.7 DPPH antiradical power (DPPH)
The scavenging activity against DPPH radical was evaluated according to the method of Brand-Williams, Cuvelier, and Berset (1995) [25] with minor modifications. The assay mixture contained 2.9 mL DPPH solution (90 μmol/L) and 0.1 mL of the sample solution containing from 1 to 100 mg/mL dry extract; the final volume was 3 mL. The mixture was shaken vigorously on a Vortex mixer, then incubated 60 min at 25 °C in a water bath in the dark, after which the absorbance of the remaining DPPH was determined at 517 nm against a blank. Blank was sample at the same concentration above described containing all reagents except DPPH. For each sample three replicates were carried out. Radical Scavenging Capacity (RSC) was calculated by the Eq. (1):

$$RSC = (A_0 - A_1) / A_0 \times 100$$

where $A_0$ is the control and $A_1$ is the sample solution absorbance. The concentration (in the final reaction medium in each method) that causes a decrease in the initial absorbance (control) by 50 % is defined as IC$_{50}$. The IC$_{50}$ values for all RSC determinations were determined by polynomial fitting of the inhibition values using software ORIGIN 9.1.

DPPH antiradical power defined as:

$$DPPH = (1 / IC_{50}) \times 100.$$  

2.8 Ferric reducing antioxidant power (FRAP)
Total antioxidant capacity was estimated according to the FRAP assay [26]. The FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), 2,4,6-tripyridyl-triazine reagent (10 mM in 40 mM HCl) and FeCl$_3$·6H$_2$O (20 mM) in the ratio of 3:1:1. A sample of 10 μl was mixed with 3 ml of working FRAP reagent and absorbance (593 nm) was measured 4 minutes after vortexing. The test was performed at 37 °C. The FRAP value was calculated using Eq. (3):

$$FRAP value = \Delta A \text{ sample}(0-4 \text{ min}) / \Delta A \text{ standard}(0-4 \text{ min}) .$$

The 100 μM Fe$_2$+ solution was used as a standard; 1 FRAP unit = 100 μM Fe$_2$+. Total antioxidant capacity was expressed in FRAP units.
2.9 Lipid peroxidation (LP)
Lipid peroxidation was estimated based on thiobarbituric acid (TBA) reactivity. Samples were evaluated for malondialdehyde (MDA) production using a spectrophotometric assay for TBA. The extinction coefficient at 532 nm of 153,000 mol$^{-1}$ cm$^{-1}$ for the chromophore was used to calculate the MDA-like TBA produced. The color intensity of the MDA-TBA complex in the supernatant was measured by its absorbance at 532 nm [27].

2.10 Statistical analysis
All determinations were performed in quadruplicate. Statistical comparisons between samples were performed with Student’s t-test for independent observations. Differences were considered significant at $p < 0.05$. The antioxidant test results were investigated with multivariate analysis. The correlation matrix was calculated, giving the correlation coefficients between each pair of variables, i.e. the analytical parameters tested. Each term of the matrix is a number ranging from -1 to +1: the + or - sign indicates a positive or negative interdependence between variables (direction), and the absolute value indicates the strength of the interdependence. Correlations between different parameters were considered significant at $p < 0.05$ level. Principal component analysis (PCA) was used to discover the possible correlations among measured parameters, while Cluster analysis (CA) is used to classify objects into groups.

The data were processed statistically using the software package STATISTICA 10.0 (StatSoft Inc., Tulsa, OK, USA).

3 Results and discussion
The widely used spectrophotometric assays, such as FRAP and DPPH, were applied in parallel with GPx, SOD and LP to measure AOC of antioxidant and scavenging activities of organic and conventional spelt and wheatgrass, which TPC was determined using FC assay was shown in Table 1. A large variation in phenolic content was noticed.

3.1 Soluble proteins and lipid peroxidation
Soluble protein content ranged from 18.45 (Eco-10 spelt, conventional) to 25.00 mg/g (Nirvana spelt, conventional). Lipid peroxidation (MDA content) ranged from 14.03 (Nirvana spelt, conventional) to 18.18 nmol MDA/mg protein (Eco-10 spelt, organic). Soluble protein content and LP was negatively correlated. Results are given in Table 1.

It was observed that conventionally produced Nirvana possessed 9.40 % higher soluble protein content than organically produced one. Between organically and conventionally produced Eco-10 no significant difference was observed. In both investigated spelt genotypes (Eco-10 and Nirvana), lipid peroxidation was higher in organically produced spelt for 11.95 % and 16.46 %, respectively.

Evidence suggests that organic cropping systems generally produce lower, more variable yields than systems employing synthetic fertilizers and chemical crop protection measures. According to Palmer et al. [28], the major limiting factor for yields in organic production systems is limiting nitrogen supply. Our results are in agreement with mentioned observations. Organic production of spelt did not provoke accumulation of soluble proteins, but contrary, the highest accumulation of soluble proteins was observed in conventionally produced spelt genotype Nirvana. Lipids are highly susceptible to oxidation and lipid peroxidation products, such as MDA quantity, are potential biomarkers for oxidative stress status in vivo [29]. Proteins are also the direct target for Reactive Oxygen Species (ROS) because of their high concentrations. Predictably, the lipid peroxidation intensity was significantly lower in conventionally produced spelt than in the organic spelt where protective measures did not apply.

### Table 1 Experimental results of antioxidant and scavenging activities of organic and conventional spelt and wheatgrass

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment</th>
<th>SPC (mg/ml)</th>
<th>GPx (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>LP (nmol/mg protein)</th>
<th>TPC (mg/ml)</th>
<th>DPPH (nmol/l)</th>
<th>FRAP (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Eco-10</td>
<td>Organic</td>
<td>20.00$^a$</td>
<td>5.64$^b$</td>
<td>10.33$^c$</td>
<td>18.18$^c$</td>
<td>698.10$^c$</td>
<td>2.75$^c$</td>
<td>27.20$^c$</td>
</tr>
<tr>
<td>2 Eco-10</td>
<td>Conv.</td>
<td>18.45$^a$</td>
<td>5.94$^a$</td>
<td>11.38$^b$</td>
<td>16.24$^b$</td>
<td>720.20$^b$</td>
<td>1.70$^b$</td>
<td>25.25$^b$</td>
</tr>
<tr>
<td>3 Nirvana</td>
<td>Organic</td>
<td>22.65$^a$</td>
<td>6.96$^a$</td>
<td>12.23$^b$</td>
<td>16.34$^b$</td>
<td>713.20$^b$</td>
<td>2.37$^b$</td>
<td>31.35$^b$</td>
</tr>
<tr>
<td>4 Nirvana</td>
<td>Conv.</td>
<td>25.00$^a$</td>
<td>3.80$^a$</td>
<td>9.44$^a$</td>
<td>14.03$^a$</td>
<td>750.40$^a$</td>
<td>1.54$^a$</td>
<td>32.75$^a$</td>
</tr>
<tr>
<td>5 Ebners-R</td>
<td>Organic</td>
<td>20.25$^a$</td>
<td>5.88$^a$</td>
<td>19.38$^b$</td>
<td>14.40$^b$</td>
<td>877.20$^b$</td>
<td>1.52$^b$</td>
<td>39.35$^b$</td>
</tr>
<tr>
<td>6 Europa 90</td>
<td>Organic</td>
<td>22.20$^a$</td>
<td>9.05$^a$</td>
<td>9.26$^a$</td>
<td>16.09$^a$</td>
<td>816.70$^a$</td>
<td>2.36$^a$</td>
<td>32.55$^a$</td>
</tr>
</tbody>
</table>

Values with the same letter are not statistically different at the $p < 0.05$ level (post hoc Tukey’s HSD test); SPC (mg/ml) - soluble protein content; GPx (U/mg protein)- guaiacol peroxidase activity; SOD (U/mg protein) - superoxide dismutase activity; LP MDA (nmol/mg protein) - lipid peroxidation enzyme, TPC (catechin eq (mg/100g dw))- total phenolic content.
3.2 Soluble proteins and lipid peroxidation
The SOD activity ranged from 9.26 (wheat, organically produced) to 19.38 U/mg protein (Ebners-R spelt, organically produced). GPx activity ranged from 3.8 (Nirvana spelt, organic) to 9.05 U/mg protein (wheat, organically produced). No significant correlations between antioxidant enzymes and with LP and soluble proteins were found. Both antioxidant enzymes were significantly higher in organically produced spelt Nirvana, for 29.56 % (SOD) and for 83.16 % (GPx). Results are given in Table 1.

SOD catalyzes the dismutation of superoxide radical in all living tissues, including plants, leading to hydrogen-peroxide production. Peroxidases are haem-containing enzymes that use hydrogen peroxide as the electron acceptor to catalyze a number of oxidative reactions. The conversion of superoxide into hydrogen peroxide and decomposition of hydrogen-peroxide presents the first line of cellular defense to prevent deleterious biological oxidation by oxygen radical generated during cellular metabolism. Despite the presence of an efficient antioxidant system, oxidative stress still occurs in plant cells, either due to uncontrolled production or inefficient scavenging of ROS [30]. The reaction of antioxidant system depends on a few crucial factors such as duration to which the cell gets exposed to the oxidative stress and ecological context of oxidative stress as well as the ratio between antioxidants and also between ROS ($O_2^-\cdot$, $H_2O_2$, $•OH$). In fact, the antioxidant defenses in living cells can respond to prooxidative conditions, when oxidative stress is not very strong, with a compensatory mechanism that increases the antioxidant activities [31]. If oxidative stress is persisting, or its level is very high, the protein damage became profound and a decreased SOD activity may occur. Our results that antioxidant enzymes were induced in organic production are in agreement with results of Palmer et al. [28] who also indicated that environment drivers may affect biochemical indicators and yields in organic systems more than in conventional systems.

3.3 Total phenol content, DPPH and FRAP
Total phenol content ranged from 698.10 (Eco-10 spelt, organic) to 877.20 mg/100g dw catechin eq. (Ebners-R spelt, organic). FRAP values ranged from 25.25 (Eco-10 spelt conventionally produced) to 39.35 FRAP units (Ebners-R spelt, organic). DPPH antiradical power ranged from 1.52 (Ebners-R spelt, organic) to 2.75 (Eco-10 spelt, organic). Results are given in Table 1.

No significant differences between organic and conventional spelt were observed considering total phenol content and FRAP values. Nevertheless, organic produced spelt showed significantly lower DPPH than conventional, for 38.05 % (Eco-10) and 35.15 % (Nirvana). A significant positive correlation was found between total phenol content and FRAP values ($r=0.861; p < 0.05$). DPPH was also positively correlated, but insignificantly, with TPC and FRAP. Significant negative correlation was found between LP and DPPH ($r= -0.869, p < 0.05$). Correlation matrix is presented in Table 2.

According to Grinder-Pedersen et al. [32], different food production methods may result in differences in the content of secondary metabolites such as polyphenolic compounds. Polyphenolic compounds are strong natural antioxidants and also part of the plant defence system with variety of functions [33]. The presence and content of different polyphenols are intraspecifically dependant and also affected by cultivation, harvesting conditions such as growing conditions, degree of ripeness and variety of the plants. Only a limited number of studies have investigated the effect of cultivation technique on the content of flavonoids, and the results are inconsistent [32]. Chemicals used in conventional production such as synthetic herbicides, pesticides, insecticides and fertilizers may both decrease and increase the production of polyphenolic compounds in plants [34, 35]. Organic food production is characterized by the absence or limited use of synthetic herbicides, pesticides, and insecticides and a lower use of fertilizers. Our results considering Eco-10 and Nirvana spelt genotypes pointed out no significant effect of the cultivation technique on total phenol content and FRAP values. These results are in accordance with previously reported studies of polyphenol content in three different strawberry cultivars in the organic

| Table 2 Correlation matrix for antioxidant and scavenging activities of organic and conventional spelt and wheatgrass |
|-----------------|-------|-------|-------|-------|-------|-------|
|                  | GPx   | SOD   | LP    | TPC   | DPPH  | FRAP  |
| SPC              | -0.173| -0.350| -0.485| 0.026 | 0.087 | 0.358 |
| GPx              | -0.085| 0.345 | 0.258 | -0.570| 0.033 |       |
| SOD              | -0.377| 0.641 | 0.469 | 0.673 |       |       |
| LP               | -0.611| -0.869*| -0.699|       |       |       |
| TPC              | 0.464 | 0.861*|       |       |       |       |
| DPPH             |       |       |       |       | 0.442 |       |

*The correlations marked with asterix were statistically significant at $p < 0.05$ level.
SPC (mg/ml) - soluble protein content; GPx (U/mg protein) - guaiacol peroxidase activity; SOD (U/mg protein) - superoxide dismutase activity; LP MDA (nmol/mg protein) - lipid peroxidation enzyme, TPC (catechin eq (mg/100g dw)) - total phenolic content.
and conventional systems where no difference was seen [35]. Nevertheless, DPPH was lower in conventionally produced spelt genotypes what can be explained by the direct effect of certain chemicals used in production or their metabolites on this DPPH radical scavenging, or by the accumulation specific polyphenols with high scavenging potential towards DPPH radical. The highest value for all three antioxidant parameters were detected for organic Ebners-R spelt variety.

### 3.4 Relative antioxidant capacity index (RACI) and phenolic antioxidant coefficients (PAC)

In various methods for AOC activity determination, efficiency of AOs can vary due to the different chemical mechanisms lying behind the assays. In order to achieve a more reliable comparison between the samples analyzed, two additional parameters such as a relative antioxidant capacity index (RACI), calculated by assigning equal weight to all AOC assays applied (including TPC as a measure of total reducing activity), and phenolic antioxidant coefficients (PAC), calculated as a ratio between particular AOC capacity and total phenolic content, were introduced.

As seen in Fig. 1, the highest values of RACI were ascribed to spelt Ebners-R and wheat Europa 90 (0.501 and 0.447). Low but still positive values of RACI were obtained for organically grown spelt Nirvana (0.119) and spelt Eco-10 (0.057). The negative values of RACI were calculated for spelt Nirvana (-0.695) and spelt Eco-10 (-0.430), which were conventionally raised.

The PAC calculation enables the comparison of the effectiveness of phenolics present in analysed samples and also provides specific insight into differences between applied AOC assays. Variations of both PAC\textsubscript{SOD} and PAC\textsubscript{FRAP} were remarkable, while variations in PAC\textsubscript{LP} and PAC\textsubscript{GPX} were much lower. The lowest values of PAC\textsubscript{DPPH} have been ascribed to samples with higher values of RACI, i.e. high AOC value. High PAC of organic and conventional spelt and wheatgrass did not seem to be always associated with high AOC value (Fig. 2).

#### 3.5 PCA analysis

The orientation of the vector describing the variable in factor space indicates an increasing trend of these variables, and the length of the vector is proportional to the square of the correlation values between the fitting value for the variable and the variable itself. The angles between corresponding variables indicate the degree of their correlations (small angles corresponding to high correlations).

The PCA of the presented AOC data explained that the first two components accounted for 74.32 % of the total variance (50.54 and 23.78 %, respectively) in the seven variables (AOC assays, TPC and SPC). Considering the map of the PCA performed on the data, LP (which contributed 23.1 % of total variance, based on correlations), exhibited a positive score according to the calculation of the first principal component, while SOD (13.8 %), TPC (19.0 %), FRAP (22.2 %) and DPPH (18.4 %) showed a negative influence for the first principal component (Fig. 3). The positive contribution to the second principal component was observed for SPC (21.1 % of total variance, based on correlations), while the negative contribution to the second principal component calculation was observed for GPX (34.7 %), SOD (13.4 %) and TPC (13.6 %). The points shown in the PCA graphics, which are geometrically close to each other indicate the similarity of patterns that represent these points. The first principal component is determined by the influence of LP, DPPH, FRAP, TPC and SOD, and it represents the differences in AOC of the samples. The most intensive AOC activity was observed for Ebners-R, while the lowest FRAP value was obtained for Eco-10. Nirvana and Europa samples were close to obtained AO activity. The second PC shows the differences in protein content. Conventionally grown samples were characterized by higher soluble protein content (Nirvana), while organically grown samples showed the higher GPX value.

The presented results represent a platform for the future research, which will be focused on the more detailed exploration of wheat and spelt grass phytochemical potential and corresponding biological activities.

#### 4 Conclusion

Differences in antioxidant status of all three investigated Spelt genotypes (Eco-10, Nirvana and Ebners–Rotkorn) were observed between each other as well as in comparison with wheat. The highest superoxide-scavenging activity,
ferric reducing antioxidant power, total phenol content, DPPH antiradical power and the lowest lipid peroxidation intensity was observed in Ebners–Rotkorn genotype, even better than organically produced wheat. Comparative analysis of organically and conventionally produced spelt genotypes Eco-10 and Nirvana indicated that DPPH-antiradical power was induced in both genotypes by conventional production. Regardless the lower DPPH, and slightly increased LP, organically produced spelt possess high antioxidant capacity that can resist towards oxidative stress by induction of antioxidant enzymes. The presented results represent a platform for the future research, which will be focused on the more detailed exploration of wheat and spelt grass phytochemical potential and corresponding biological activities.

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Nomenclature

- **AOC**: antioxidant capacity
- **DPPH**: Diphenyl-1-Picrylhydrazyl
- **GPx**: guaiacol peroxidase
- **FC**: Folin Ciocalteu - phenol reagent
- **FRAP**: ferric reducing antioxidant power
- **LCA**: life cycle assessment
- **LP**: lipid peroxidation
- **MDA**: malondialdehyde
- **NBT**: Nitro Blue Tetrazolium
- **PAC**: phenolic antioxidant coefficients
- **PCA**: Principal Component Analysis
- **SOD**: superoxide dismutase
- **TBA**: thiobarbituric acid
- **TPC**: Total phenols content
- **TPTZ**: 2,4,6-Tripyridyl-Triazine

**Fig. 2** Phenolic antioxidant coefficients (PAC) of organic and conventional spelt and wheatgrass

* Samples are numbered according to Table 1.

**Fig. 3** PCA ordination of AOC variables based on component correlations
References


