

CONCENTRATION OF PROTEINS, BETA-GLUCANS, TOTAL PHENOLS AND ANTIOXIDANT CAPACITY OF SLOVENIAN SAMPLES OF BARLEY

VSEBNOST PROTEINOV, BETA-GLUKANOV, SKUPNIH FENOLOV IN ANTOOKSIDATIVNA VREDNOST SLOVENSKIH VZORCEV JEČMENA

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ABSTRACT

Concentration of proteins, beta-glucans, total phenols and antioxidant capacity of Slovenian samples of barley

Four Slovenian barley samples were analyzed for the content of the ash, proteins, β -glucans and total polyphenols; and for the antioxidant capacity by two methods. Antioxidant capacity was determined by the ferric reducing antioxidant power (FRAP) activity and by the oxygen radical absorbance capacity (ORAC) assay. For the comparison, four foreign barley commercial samples were analyzed, three of German origin and one from Italy. Slovenian samples had equal or lower content of ash, proteins and β -glucans in comparison to foreign samples. A Slovenian barley sample from Šalovci, Prekmurje had the highest content of β -glucans, namely 5.73% in dry weight (DW). The results obtained by both methods of the determination of antioxidant capacity showed significant correlation with the total polyphenols content.

Key words: *Hordeum*, barley, beta-glucans, phenols

IZVLEČEK

Vsebnost proteinov, beta-glukanov, skupnih fenolov in antioksidativna vrednost slovenskih vzorcev ječmena

Štiri vzorce ječmena iz Slovenije smo analizirali na vsebnost pepela, beljakovin, β -glukanov in skupnih fenolov. S pomočjo dveh metod smo tudi ugotavljali antioksidativno zmogljivost. Antioksidativna zmogljivost je bila določena z zmanjšanjem antioksidativne sposobnosti železa (metoda FRAP) in s sposobnostjo absorbance kisikovega radikala (metoda ORAC). Za primerjavo smo analizirali štiri komercialne vzorce tujega ječmena, tri nemške in enega iz Italije. Slovenski vzorci so imeli enako ali nižjo vsebnost pepela, beljakovin in β -glukanov v primerjavi s tujimi. Vendar je slovenski vzorec ječmena iz Šalovcev (Prekmurje) vseboval najvišjo vsebnost β -glukanov, in sicer 5,73% suhe teže (DW). Rezultati obeh metod določanja antioksidativne kapacitete so bili primerljivi in so pokazali pomembno povezavo z vsebnostjo celokupnih fenolov.

Ključne besede: *Hordeum*, ječmen, beta-glukani, fenoli

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1 INTRODUCTION

Interest in growing barley, a crop cultivated by humankind for a long time, has become more intense in recent years in Slovenia, Europe and elsewhere, due to the nutritional value of this ancient crop. Barley was cultivated for centuries for human food, but the areas of growing of barley declined in the middle of the 20th century. However high fibre foods are recently preferred in human nutrition. It should be noted that the cultivation of barley was for many years particularly focused on achieving high dry matter content, high starch, and low content of dietary fibre. This is because barley has been used mostly for either animal feed or for production of barley malt. Cultivars with higher levels of dietary fibre, particularly of β -glucans, are therefore now gaining importance for use in human nutrition and some of these are being introduced to Southern and Central Europe, including Slovenia.

Identification of suitable cultivars high in β -glucan content, evolution of milling and dry separation techniques and using the β -glucan-enriched fractions as an ingredient in bakery products are subjects for further needed research. The methods developed will allow obtaining new, nutritionally valuable barley products with high potential for use in foods.

In recent months it was a quick development of research and published papers on barley β -glucans. SHAIK et al. (2016) reported that in amylose-only starch chemotypes it is showed significant reduction in

starch accumulation with the re-direction to protein and β -glucan accumulation. Ho et al. (2016) newly reported a systematic review of the effects of different high molecular weight β -glucans on cholesterol and cardiovascular disease risk reduction.

MOZA & GUJRAL (2016) reported that in India high altitude barley cultivars (between 1200 m and 3500 m above sea level) contain higher levels of total β -glucans in comparison to the barley cultivars grown in plains (97-126 m altitude). Cultivars grown in higher altitudes may find better utilization in nutraceutical foods. HAN et al. (2016), ZHU et al. (2016) and DIMITROFF et al. (2016) suggested how, understanding genetic background, barley β -glucans based functional food may be developed for the management of obesity. BELOBRAJDIC et al. (2016) suggest a wholegrain barley high molecular weight β -glucan to lover food intake.

After the separation in barley after milling to ten milling fractions, the highest β -glucan content was determined in the bran fraction (WIEGE et al. 2016). It was a strong positive correlation between β -glucan and protein content. Fractions containing high level of β -glucan have as well high concentration of protein and ash (WIEGE et al. 2016).

Croatian barley samples were screened for total high molecular weight β -glucans content (KRSTANOVIC et al. 2016), but such kind of research was not yet performed in Slovenia.

2 MATERIALS AND METHODS

2.1 Samples

Barley samples analysed are presented in Table 1.

Table 1: Description of studied barley samples
Preglednica 1: Opis preučevanih vzorcev ječmena

Sample	Description
A	Winter barley sample from Šentjernej, Dolenjska, Slovenia
B	Winter barley sample from Trbonje, Dravograd, Slovenia
C	Winter barley sample from Šalovci, Prekmurje, Slovenia
D	Winter barley sample from Murska Sobota, Prekmurje, Slovenia
E	Winter barley sample from Nienstadt, Germany
F	Winter barley sample from Germany, cultivated in Jable, Slovenia
G	Naked barley sample from Asheberg, Germany
H	Winter barley sample from Lazio, Italy

Samples A-D are samples of barley grain, each of barley domestic cultivars was grown for years by farmers in different parts of Slovenia. The origin of barley

cultivars is not known. Samples E-F are commercial barley samples available in Slovenia, originating, as far as it is known, in Germany or Italy.

2.2 Methods

2.2.1 Determination of moisture, ash, proteins and β -glucans

Constituents of barley samples were determined using the standard methods.

For the determination of moisture: Method ICC No. 109/1, was used.

In short: This method can be taken as the standard for the development of methods which are specifically suited to the practical determination of the moisture content of wheat, rice (hulled paddy), barley, maize or whole maize meal, millet, rye and oats, as grains, ground grains, semolina and flour. It is not to be used for the settlement of commercial disputes. Measurement of moisture loss when the material, ground if necessary without change of moisture content, is equilibrated in an anhydrous atmosphere at a temperature between 45 and 50 °C and at a pressure of 1.3 ... 2.7 KPa (10 ... 20 mm Hg).

For the determination of ash: Method ICC No. 104, was used.

In short: Samples were ground and placed into crucibles, and crucibles placed into a muffle furnace. The ashing was carried out at 900 °C, and is completed when the cool residue was white or nearly white. As the ash quantity has to be related to dry matter, the moisture content of the test sample has to be determined separately.

For the determination of proteins: Method ICC 105/2. Kjeldhal Method was used.

In short: The organic matter of the sample is oxidized with concentrated sulfuric acid in the presence of a catalyst: the product of the reaction $(\text{NH}_4)_2\text{SO}_4$ is treated by alkali; free ammonia is distilled and titrated.

For the determination of β -glucans: Method ICC No.166 was used.

In short: β -D-glucan is determined using highly purified lichenase and β -D-glucosidase. β -D-glucan is specifically hydrolyzed by lichenase to oligosaccharides, which are quantitatively cleaved to glucose by β -glucosidase. Glucose is measured using glucose oxidase - peroxides - buffer mixture. Method is rapid procedure for direct, quantitative measurement of (1->3) (1->4)- β -D-glucan (β -D-using highly purified lichenase and β -glucosidase). β -D-glucan is specifically hydrolyzed by lichenase to oligosaccharides, which are then quantitatively cleaved to glucose by β -glucosidase. Glucose is measured using glucose oxidase-peroxidase-buffer mixture.

2.2.2 Extraction method

In this study, 80% methanol extracts from barley were used for the determination of total phenolic content and antioxidant property. Barley samples (1 g) were extracted with 6 ml acidified methanol (HCl/methanol/water, 1:80:10, v/v/v) at room temperature (25 °C) for 2 h using orbital shaker. The mixture was centrifuged at 3000g for 10 min. The supernatant was used for determination of total phenolic content and antioxidant capacity. Several studies have shown that 80% methanol is an effective solvent in extracting phenolic and other polar substances from cereals (LAHOUAR et al. 2014).

2.2.3 Total phenolic content determination

Total phenols (TP) were determined using a modification of the Folin–Ciocalteu method method described by SINGLETON & ROSSI (1965). Briefly, the assay was conducted by mixing 4 mL of deionized water, 0.25 mL of extracts (see 'Extract preparations' in this section), 0.25 mL of Folin–Ciocalteu reagent, and 0.5 mL of Na_2CO_3 . After 30 min at room temperature, the absorbance of the mixture was measured at 725 nm. A standard curve was prepared with gallic acid. The final results were expressed as mg of gallic acid equivalents (GAE) per g of dry weight (DW). All of the analyses were conducted in triplicate and the results report the sum of bound and soluble phenolic compounds.

2.2.4 Determination of ferric reducing antioxidant power (FRAP) activity

A ferric reducing antioxidant power (FRAP) assay was performed according to the method described by BENZIE & STRAIN (1999), which was adapted for 96-well plates and an automatic reader (Infinite 2000, Tecan, Salzburg, Austria). The method is based on the reduction of the Fe^{3+} -2,4,6-tripyridyl-s-triazine (TPTZ) complex to its ferrous form at a low pH. Briefly, 160 mL of FRAP assay solution (consisting of 20 mM ferric chloride solution, 10 mM TPTZ solution, and 0.3 M acetate buffer at pH 3.6) was prepared daily, mixed with 10 mL of the sample, standard, or blank, and dispensed into each well of a 96-well plate. The absorbance was measured at 595 nm at 37 °C after 30 min of incubation. All of the analyses were conducted in triplicate. The final results are expressed as $\mu\text{mol Fe}^{2+}$ equivalents per g of the DW of the samples, and the results were obtained using a standard curve with different concentrations of ferrous sulfate heptahydrate ($\text{FeSO}_4 \times 7\text{H}_2\text{O}$).

2.2.5 The oxygen radical absorbance capacity (ORAC) assay

The oxygen radical absorbance capacity (ORAC) was determined using the hydroxyl radical antioxidant capacity (HORAC) assay kit (Oxford Biomedical Re-

search, Oxford USA) according to the manufacturer's instructions. The final ORAC values were expressed as gallic acid equivalents and determined according to the standard curve. All of the analyses were conducted in triplicate.

3. RESULTS AND DISCUSSION

Slovenian barley samples were analysed and compared with the foreign ones. Results of the ash (i.e. total con-

tent of mineral elements), protein and β -glucans concentration are presented in Table 2.

Table 2: Concentration of ash, protein and β -glucans in barley samples, mean values and standard deviations (DW=dry weight).

Preglednica 2: Koncentracija pepela, beljakovin in β -glukanov v vzorcih ječmena, povprečne vrednosti in standardni odkloni (DW=sušina).

Sample	Moisture %	Ash % DW	Protein % DW	β -glucans % DW
A	10.9 \pm 0.3	1.83 \pm 0.08	8.7 \pm 0.1	4.56 \pm 0.09
B	11.0 \pm 0.5	1.67 \pm 0.10	8.8 \pm 0.2	4.06 \pm 0.07
C	11.0 \pm 0.3	1.30 \pm 0.11	8.2 \pm 0.6	5.73 \pm 0.11
D	11.3 \pm 0.2	1.66 \pm 0.04	8.2 \pm 0.5	4.19 \pm 0.06
E	10.9 \pm 0.4	1.72 \pm 0.07	9.7 \pm 0.5	5.36 \pm 0.08
F	10.7 \pm 0.3	1.95 \pm 0.09	9.8 \pm 0.2	4.76 \pm 0.04
G	10.3 \pm 0.5	2.25 \pm 0.06	11.1 \pm 0.7	5.19 \pm 0.06
H	11.4 \pm 0.3	2.42 \pm 0.03	9.6 \pm 0.4	3.73 \pm 0.10

All analyzed samples show a high protein content (average 9.3% DW), with values ranging from 8.2% DW to 11.1% DW. The sample G had a highest value. From the point of view of β -glucans the sample D (Šalovci, Slovenia) is the most interesting because it had highest β -glucans concentration.

Correlation coefficient between ash and proteins in grain among all samples analysed was 0.74; correlation coefficient between ash and β -glucans was -0.46; correlation coefficient between proteins and β -glucans was 0.15. We used Pearson correlation coefficient. Thus the correlation between ash and proteins is significant ($p < 0.5$) and positive, correlation between ash and β -glucans was significant ($p < 0.5$) and negative. There was no significant correlation between the amount of proteins and β -glucans in studied barley samples. Barley has a distinct advantage over some other grains in that β -glucan soluble fiber is found throughout the entire barley kernel. In some other grains, the fiber is only found in the outer bran layer. If these grains are processed, the fiber can be easily lost (WIEGE et al. 2016).

The all samples analysed showed average values of β -glucans 4.69% DW, with a maximum value of 5.73% DW and a minimum value of 3.73% DW.

The ash values were with a minimum of 1.3% DW, and a maximum of 2.42% DW.

To better understand the relationship between the antioxidant activity and total phenolic content, the TPC of the sample extracts were determined using the Folin-Ciocalteu phenol reagent. The results are expressed as mg of gallic acid equivalents per gram of dry mater, and are presented in Table 3. Barley samples with high amount of phenolics also showed high antioxidant activity (FRAP and ORAC assay).

The total antioxidant capacity was in linear regression with the total phenolic content in barley samples ($R^2 = 0.9948$ and 0.8192 for the results of FRAP and ORAC assays, respectively) (Fig. 1).

There were differences in the antioxidant activities by ORAC and FRAP, and in content of total polyphenols of investigated barley samples.

Table 3: The total phenolic contents (TP) and antioxidant capacity (FRAP and ORAC method) of barley samples. Data are reported to mean ($n=3$) \pm standard error. Values in the same column not sharing a common letter differ significantly at $P \leq 0.05$. Test ANOVA. X mg GAE/g dried weight (DW). y $\mu\text{mol Fe}^{2+}$ equivalent/g DW. z GAE mM/g DW. GAE: gallic acid equivalent; DW: dry weight

Preglednica 3: Vsebnost skupnih fenolov (TP) in antioksidativna zmogljivost (metodi FRAP in ORAC) pri različnih vzorcih ječmena. Podatki so povprečje ($n = 3$) \pm standardna napaka. Vrednosti v istem stolpcu, ki nimajo iste črke se med seboj značilno razlikujejo $P \leq 0.05$. Test ANOVA. X mg GAE/g suhe teže (DW). y $\mu\text{mol Fe}^{2+}$ ekvivalent/g DW. z GAE mM/g DW. GAE: ekvivalent galne kisline; DW: suha teža

Sample	TPCx	FRAPy	ORACz
A	$2.8 \pm 0.2\text{b}$	$227.0 \pm 1.5\text{b}$	$21.0 \pm 0.1\text{b}$
B	$2.0 \pm 0.3\text{b}$	$180.2 \pm 2.9\text{c}$	$21.0 \pm 0.3\text{b}$
C	$1.5 \pm 0.2\text{c}$	$149.2 \pm 1.6\text{d}$	$17.1 \pm 0.2\text{c}$
D	$2.4 \pm 0.3\text{b}$	$202.7 \pm 0.2\text{b}$	$20.6 \pm 0.4\text{b}$
E	$3.5 \pm 0.5\text{a}$	$273.0 \pm 0.9\text{a}$	$23.2 \pm 0.9\text{a}$
F	$2.1 \pm 0.2\text{b}$	$184.2 \pm 1.3\text{c}$	$21.1 \pm 0.2\text{b}$
G	$3.8 \pm 0.4\text{a}$	$286.6 \pm 2.1\text{a}$	$25.7 \pm 0.2\text{a}$
H	$3.0 \pm 0.1\text{ a}$	$231.0 \pm 2.2\text{ b}$	$24.1 \pm 0.1\text{ a}$

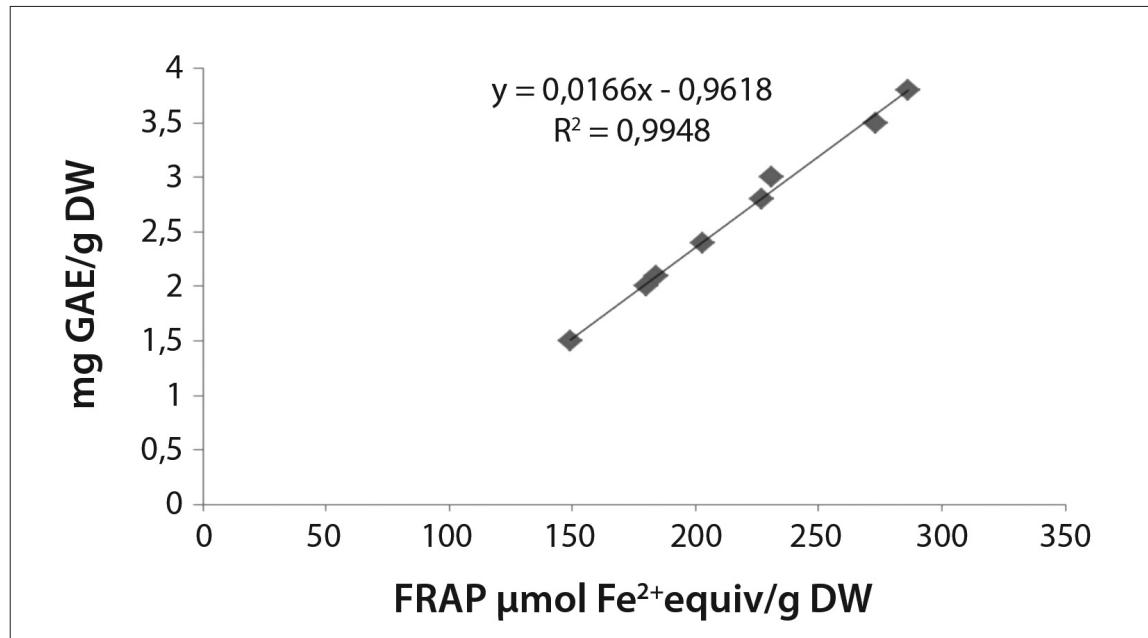


Figure 1: Linear regression between total phenolic content and the total antioxidant capacity (FRAP method - a ferric reducing antioxidant power) of barley samples. GAE: gallic acid equivalent; DW: dry weight

Slika 1: Linearna regresija med skupno vsebnostjo fenolov in skupno antioksidativno kapaciteto vzorcev ječmena (metoda FRAP - zmanjšanje antioksidativne moči železa). GAE: ekvivalent galne kisline; DW: suha teža

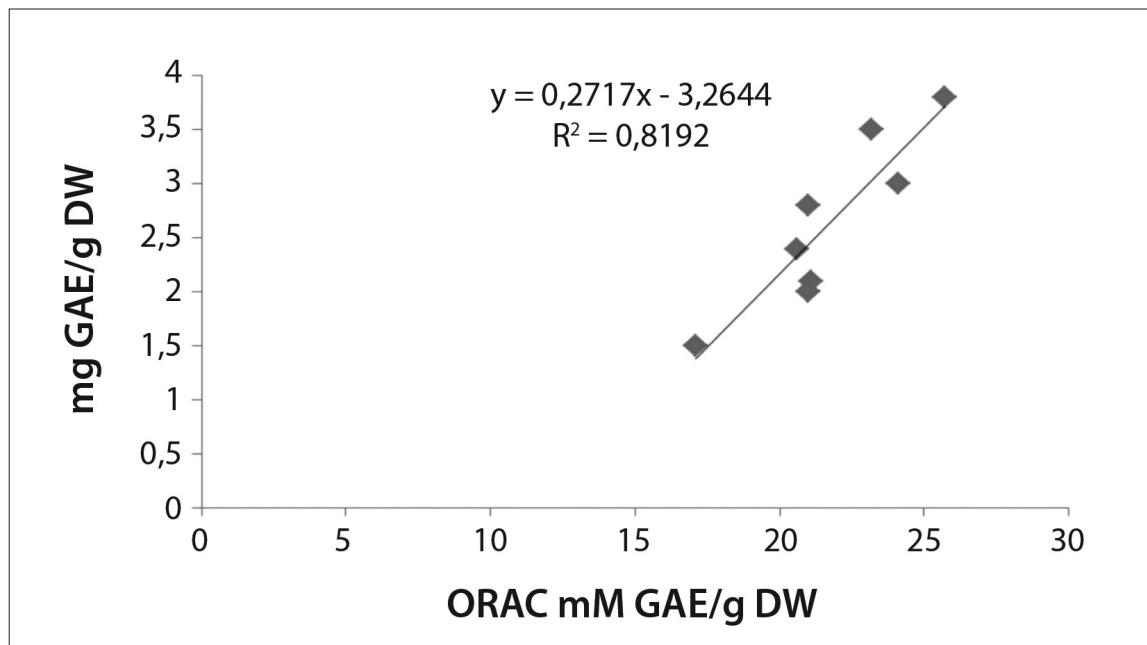


Figure 2: Linear regression between total phenolic content and the total antioxidant capacity (ORAC method - the oxygen radical absorbance capacity) of barley samples. GAE: gallic acid equivalent; DW: dry weight

Slika 2: Linearna regresija med skupno vsebnostjo fenolov in skupno antioksidativno kapaciteto vzorcev ječmena (ORAC metoda - absorbanca kisikovega radikala). GAE: ekvivalent galne kisline; DW: suha teža

4 CONCLUSIONS

The Slovenian barley sample from Šalovci, Prekmurje had the highest content of β -glucans among the studied Slovenian and foreign samples, namely 5.73%

in dry weight. The results obtained by both methods of the determination of antioxidant capacity were in linear relation with the total phenols content.

5 POVZETEK

Zanimanje za pridelovanje ječmena, stare poljščine, se je zadnje čase povečalo v Sloveniji, Evropi in drugod, predvsem zaradi njegove hranične vrednosti. Ječmen gojijo za prehrano ljudi že stoletja, vendar so se območja pridelave zmanjšala zlasti v sredini 20. stoletja. V prehrani ljudi so zadnje čase zelo zaželeni živila, kot je ječmen, z visoko vsebnostjo vlaknin. Žlahtenje in pridelovanje ječmena sta bili vrsto let osredotočeni predvsem na doseganje visokih pridelkov sušine, veliko škroba in nizko vsebnost prehranskih vlaknin, ker so ga večinoma uporabljali bodisi za živalsko krmo ali za proizvodnjo ječmenovega sladu. Sorte, ki vsebujejo več prehranskih vlaknin, še posebej β -glukanov, so v novejšem času vedno bolj pomembne za uporabo v prehrani ljudi.

V zadnjih mesecih je bil hiter razvoj raziskav in objavljanja člankov o ječmenovih β -glukanih. SHAIK

in sod. (2016) so poročali, da je genotip ječmena z amiloznim škrobo pokazal pomembno zmanjšanje kopiranja škroba s preusmerjanjem sinteze na beljakovine in akumulacijo β -glukanov.

Štiri vzorce ječmena iz Slovenije smo analizirali na vsebnost pepela, beljakovin, β -glukanov in skupnih fenolov ter za antioksidativno kapaciteto z dvema metodama. Antioksidativna zmogljivost je bila določena z zmanjšanjem antioksidativne moči železa (metoda FRAP) in s sposobnostjo absorbcije kisikovega radikala (metoda ORAC). Za primerjavo smo analizirali štiri tujte komercialne vzorce ječmena, tri nemškega izvora in enega iz Italije. Slovenski vzorci so imeli enako ali nižjo vsebnost pepela, beljakovin in β -glukanov v primerjavi s tujimi. Vendar je slovenski vzorec ječmena iz Šalovcev (Prekmurje) imel najvišjo vsebnost β -glukanov

in sicer 5,73% suhe teže (DW). Vzorci imajo povprečne vrednosti β -glukanov 4,69% suhe teže (DW), z najvišjo vrednostjo 5,73% suhe teže (DW) in minimalno vrednostjo 3,73% suhe teže (DW). Rezultati obeh metod določanja antioksidativne kapacitete so pokazali pomembno korelacijo s celotno vsebnostjo polifenolov.

Za boljše razumevanje razmerja med antioksidativnim delovanjem in skupno vsebnostjo fenolov so bili analizirani ekstrakti vzorcev za TPC z uporabo Folin-Ciocalteu-jevega fenolnega reagenta. Rezultati so bili izraženi kot mg ekvivalentov galne kisline na gram

suhe teže in so predstavljeni v razpredelnici 2. Raziskani vzorci ječmena E, G, H vsebujejo najvišjo vrednost TPC, medtem ko je bila najnižja vrednost v vzorcu C (razpredelnica 2, prvi stolpec). Na splošno je ječmen z veliko količino fenolov pokazal tudi visoko antioksidativno aktivnost (po metodah FRAP in ORAC). Skupna antioksidativna zmogljivost je bila tudi linearno povezana s skupno vsebnostjo fenolov v vzorcih ječmena ($R^2 = 0,9948$ in $0,8192$ za rezultate iz FRAP in ORAC metod) (sl. 1). Rezultati so pokazali, da so različni vzorci imeli različne antioksidativne sposobnosti.

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