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DYNAMIC CHANGES IN POLYPHENOLS, FREE CARBOHYDRATES, AND ANTIOXIDANT ACTIVITY DURING GERMINATION OF WHEAT AND BARLEY FOR PREPARATION OF MALT FLOUR

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Abstract: The study aimed to determine the free sugar and total phenolic contents (TPC), and in vitro antioxidant activity in different wheat and barley malt flour. Thirty malt flour samples were prepared from wheat cultivars Lorena and Snaša, and malting barley Tristan, feed barley Zlatko, hull-less barley Osvit, and Mandatar, during different periods of germination from 1 to 4 days. The identified sugars in the samples were maltose, glucose (Glc), and sucrose, but fructose (Fru) and isomaltose were found in trace amounts. The maltose content was the highest, reaching up to 8.6 % among the detected sugars. The Glc content ranged between 1.0 % and 4.4 %, and sucrose was from 0.6 to 4.3 %. The samples Tristan (14.9 %) and Osvit (13.5 %) were characterized by the highest total free sugar content. Snaša flour contained high comparable amounts of soluble sugars (11.6 - 8.1 %) at 0 - 96 h of germination. The TPC and in vitro DPPH radical scavenging activity increased during germination, as they were higher in barley varieties than in wheat ones. Wheat and barley malt flour can be combined to increase the amounts of fermentable sugars and antioxidants to create bakery products, sourdoughs, craft beers, functional and dietary foods, and fermented drinks.

Keywords: *Antioxidant activity, Barley, Malt flour, Polyphenols, Sugars, Wheat*

INTRODUCTION

Wheat (*Triticum* spp.) and barley (*Hordeum vulgare* L.) are two important cereal crops, as wheat worldwide production reached more than 779 million metric tons in 2021/2022, and barley ones come to about 146 million metric tons in the same period [1]. Species of both cereals are sources of dietary fibers, proteins, vitamins of B complex, phenolic acids, flavonoids, lignans, carotenoids, tocopherols, phytosterols and their esters with fatty acids and phenolics, etc. [2 – 6]. They are used for production of different foods and drinks, such as bread, other bakery goods and baking mixes, pasta, beer, spirits, cereals, instant soups, sauces, breaded foods, and feed products. Nowadays, the scientific and consumers' interest in the production of whole grain flour functional foods is gradually increasing, as a result of hectic everyday life and unhealthy lifestyle. Combining wheat and barley flour has demonstrated an enhanced nutritional value of prepared bakery products by increasing their fiber and ash contents, and antioxidant activity *in vitro* [7]. Some of the health-promoting effects of barley grains can be explained by their high content of *β*-glucan, resistant starches, and tocopherols, responsible for cholesterol-lowering effects, better blood Glc metabolism, and gastrointestinal health [8].

Interestingly, malting of wheat and barley grains improves the technological properties of the derived flour, and the nutritional values, taste, and aroma of the final products [9 – 10]. Particularly, malt flour is a rich source of fermentable sugars, vitamins, minerals (e.g. Fe, Zn, Mg), enzymes and other nutrients, and it is used for the preparation of malt extracts, beer, high-alcoholic beverages, vinegar, volatile organic chemicals, and feeds [11 – 14]. Different wheat and barley cultivars are developed and studied in order to prepare malt flour with improved qualities for production of traditional and novel bakery and other products. It has been revealed that the addition of wheat (0.7 %, w/w) and barley (0.5 %, w/w) malt flour to wheat flour from different varieties improved their baking properties, demonstrated by a higher bread volume with reduced hardness, gumminess, and chewiness of the bread [15]. There is enough evidence that partly replacement of wheat flour with malted barley flour has increased the nutritional value and improved technological and organoleptic properties of fortified bread [16, 17]. Apart from that, malting of composite flour has decreased the content of antimetabolites like phytate, tannin and oxalate with 60 %, 60 % and 49 %, respectively [14].

During the malting process, macromolecules, such as proteins, starch, and non-starch polysaccharides are partly degraded by specific hydrolases resulting in more free amino acids and reducing sugars in malt flour. The released amino acids and sugars can interact through the malt drying and the final baking of food products generating new derivative compounds. These compounds and sugars alone are essential for the flavour and taste of bakery products because of the Maillard and Caramelization reactions, respectively. Furthermore, malted wheat and barley grains can have a higher TPC in comparison with unmalted grains, which can be explained by the released antioxidants, resulting from elevated enzyme activities during germination and Maillard reaction during kilning. For example, Vingrys et *al.* [18] have determined that the TPC of wheat and barley grains increased during malting from 0.36 to 1.01, and from 0.86 to 1.45 μg gallic acid equivalents·mL^{-1}. This potentially increases the intake of antioxidants during consumption of malted grain-derived products. In general, sugars in wheat and barley

flour are fermented by bakery yeasts during the process of bread making. Additionally, carbohydrates strongly influence the physicochemical properties of doughs and final products. Li et *al.* [19] have shown that starch and especially that of a B-type, and its granule size distribution have a vital role in rheological and mixing properties of wheat doughs. Park et *al.* [20] have determined that fermentable sugar content of Heugho and Hopum barley increased from 5 to 41.0 %, and from 4.7 to 33.9 %, respectively. This revealed that both cultivars can be used in beer production, where the level of fermentable sugars is a critical parameter to obtain a high quality drink. Therefore, high and low molecular weight carbohydrates, and phenolics are essential techno-functional ingredients of malted grains and their products. That is why, the present study aimed to determine the free sugar and TPCs, and in vitro antioxidant activity in different wheat and barley malt flour. The prepared malt flour can find application not only in fermented and spirit drinks but also in the production of bakery products and functional foods.

MATERIAL AND METHODS

Materials

Two winter wheat cultivars Lorena and Snaša were provided by the Bc Institute (Zagreb, Croatia). Four winter barley cultivars: feed barley Zlatko, malting barley Tristan, hull-less barley Osvit, and Mandatar were provided by the Agricultural Institute Osijek (Osijek, Croatia). All grain varieties were produced in 2021. D-(+)-Glc (≥ 99.5 %), D-(-)- Fru (≥ 99 %), D-(+)-xylose (Xyl) (≥ 99 %), D-(+)-galactose (Gal) (≥ 99 %), D-(+)-sucrose (≥ 99.5 %), and D-(+)-maltose (≥ 99 %) were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-(+)-arabinose (Ara) (99 %) was obtained from Alfa Aesar (Haverhill, MA, USA), and D-isomaltose (98 %) from Extrasynthese (Genay, France). All other chemicals were of analytical grade and were obtained from Sigma-Aldrich.

Methods

Preparation of malt flour samples

Malt flour from two wheat and four barley cultivars were obtained experimentally using a micro malting system (Joe White Malting Systems, Perth, Australia), according to Jukić et *al.* [21]. The steeping parameters were kept constant for 37 hours in total. Firstly, the cereals were immersed at 16 °C for 5 hours, then left to rest at 17 °C for 12 hours at 100 % air flow. After that, the grain mass was immersed at 17 °C for 6 hours and rested at 18 °C for 12 hours at 100 % air flow. The immersion of the grains continued at 17 °C for 2 hours. The same constant parameters were applied for the kilning operation that was planned to last 25 hours in total. The regime applied by hours was as follows: 5 hours at 60 °C; 17 hours at 65 °C; 2 hours at 70 °C, and 1 hour at 80 °C. The germination period of different cereals varied between 1 and 4 days.

Determination of dry matter content

At least 0.5 g of each flour sample was weighed into aluminum plates and the samples were dried at 105 °C to a constant weight in a moisture analyzer Kern DLB (Kern & Sohn GmbH, Balingen-Frommern, Germany). The balance was calibrated periodically before operation. Samples were stored in a desiccator for at least 24 h before analysis and were analyzed in duplicate.

Extraction of free sugars

About 1 g of each flour sample was weighed into Falcon-type conical plastic tubes (50 mL) and 28 mL of ultrapure distilled water, with conductivity of 0.055 $\mu S \cdot cm^{-1}$ (Adrona Crystal B6.1, Riga, Latvia), was added. The samples were carefully suspended on a vortex mixer and stirred for 1 h on a magnetic stirrer, ensuring a stable homogenization, at room temperature. Then, 2 mL of Carez II reagent was added to precipitate proteins and other undesirable assay-interfering components, and the samples were stirred for an additional 10 min, as modified from Pico et *al.* [22]. Finally, the samples were centrifuged at 4428 g for 10 min at room temperature. Two milliliters of the obtained extracts were filtered through syringe filters (0.45 µm) and 1 mL of each sample was transferred to glass vials for the chromatographic analysis of free sugars.

Quantification of free sugars

The content of various free sugars in the malt flour samples was examined on a Nexera-i LC2040C Plus ultra chromatographic system (Shimadzu Corporation, Kyoto, Japan), equipped with a Zorbax Carbohydrate column (4.6 × 150 mm, 5 µm, Santa Clara, CA, USA), a Zorbax Reliance Cartridge guard column (4.6 × 12.5 mm, 5 µm, Santa Clara, CA, USA), and a RID-20A refractive index detector (Shimadzu Corporation, Kyoto, Japan). The mobile phase was 80 % aqueous acetonitrile, in an isocratic mode, at a flow rate of 0.6 $mL \cdot min^{-1}$. The samples were injected using an auto-injector in a volume of 10 µL. The column chamber was maintained at 35 °C and the detector temperature was 40 °C. The data obtained were analyzed using LabSolutions DB version 5.98 software (Shimadzu Corporation, Kyoto, Japan). Individual sugars were determined by comparing the retention times of the unknown analytes with those of the corresponding standards of analytical purity ≥ 98 % (Xyl, Ara, Fru, Gal, sucrose, maltose, and isomaltose). The quantification of each detected carbohydrate was carried out by constructing standard curves using the linear relationship between the peak area (dependent variable) and the corresponding concentration (independent variable) of the standard (0.5 - 10 $mg \cdot mL^{-1}$) by a least squares method. R^2 values for all standard curves were ≥ 0.998. Samples were analyzed in duplicate, and the results were presented as mean percentages on a dry matter basis (w/w) ± standard deviation.

Determination of total phenolics and in vitro antioxidant activity

For the determination of the TPC and antioxidant activity, the Folin–Ciocalteu method and the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method, presented by Nakov et *al.* [23], were used, respectively. The absorbance for the total phenolic determination was measured at λ = 765 nm, while the absorbance for the antioxidant activity analysis was measured at λ = 515 nm.

Statistical analysis

Statistical analysis of the results for the soluble carbohydrate composition of malt wheat and barley flour was performed employing Statview software version 5.0 (SAS Institute, Cary, NC, USA) by one-way analysis of variance (ANOVA) and unpaired t-test to compare mean data (± SD). The results were considered significant when $p < 0.05$. Analysis of variance (ANOVA) and Fisher's Least Significant test (LSD) at $p < 0.05$ were performed with the software XLSTAT 2019 and Microsoft Office Excel 2016 for the TPC and antioxidant activity analyses.

RESULTS AND DISCUSSION

Wheat and barley are among the first cereal crops to be planted in the world, because of their use as a food source, since ancient times. The detected low-molecular-weight carbohydrates in wheat and barley are Glc, Fru, sucrose, maltose, and raffinose, and those of polymeric nature are starch, cellulose, *β*-glucan, arabinoxylans and (gluco) fructans [8, 24]. The effectiveness of the malting process is strongly dependent on the activity of hydrolytic enzymes in the grains or the so-called "diastatic power". Important enzymes in the liberation of fermentable sugars from fibers are *α*-amylase isoforms, *β*-amylase, dextrinase, *α*-glucosidase, *β*-glucanase, xylanase, and many others [25]. Determination of the α-amylase in most of the studied malt flour was a subject of another study [21]. Table 1 presents the results from the determination of dry weight and free sugars in barley and wheat malt flour samples.

The dry weight content in all 30 malt flour samples varied in a narrow range between 94.2 % and 96.3 %. The identified sugars in the different malt flour samples were maltose, Glc and sucrose, while Fru, isomaltose, Xyl, and Ara were mainly present in trace amounts. The quantitative content of free sugars in the raw and malt flour was in agreement with different other studies [22, 26 – 28]. In general, maltose was found in representative amounts in all initial samples and after different germination periods. This was in agreement with the results of Huang et *al.* [28] who also identified Glc, Fru, sucrose and maltose in 100 malt barley samples, using attenuated total reflectance mid-infrared spectroscopy. For the Snaša wheat cultivar, the maltose content was increased from 4.6 ± 0.1 % to 7.1 ± 0.3 %, and 8.6 ± 0.1 % after 48 and 72 h of germination, respectively ($p < 0.05$). Similarly, for the Mandatar barley variety, it was raised from 4.8 ± 0.1 % to 5.8 ± 0.1 %, and 6.6 ± 0.1 % after 72 and 96 h of germination, respectively ($p < 0.05$). The increase in maltose level was expected as a result of partly starch depolymerization during germination. This disaccharide can be simply hydrolyzed under the catalytic action of the bakery yeast lysosomal enzyme glucoamylase into 2 Glc units. Interestingly, for the Lorena wheat sample and Osvit barley sample maltose content was mainly increased after 24 h of germination with 95 % ($p < 0.05$) and 39 %, respectively. This fact suggested that both grain cultivars were easier to germinate and that their starch macromolecular structures were more susceptible to enzyme depolymerization. These statements need to be further investigated because shorter germination periods with an effective saccharification process are in high demand for the development of efficient and competitive industrial fermentation technologies.

Table 1. *Content of free sugars and dry matter in wheat and barley malt flour samples*

Samples	Days of germination	Carbohydrates [% dry weight]							
		Xyl	Ara	Fru	Glc	Gal	Suc	Mal	Isomal
LORENA	0	-	-	4.1±0.1	traces*	-	traces	2.1±0.02c	traces
	1	-	-	traces	1.0±0.004c**	-	1.4±0.03b	4.1±0.1a	traces
	2	traces	traces	traces	1.4±0.1ab	-	1.0±0.01c	3.3±0.2b	traces
	3	-	traces	traces	1.3±0.04b	-	1.4±0.1b	4.0±0.4ab	traces
	4	-	-	-	1.5±0.1a	-	2.0±0.03a	3.7±0.1b	traces
SNAŠA	0	5.7±0.2	-	-	traces	-	1.3±0.03c	4.6±0.1cde	traces
	1	traces	-	3.7±0.1	traces	-	1.5±0.1bc	4.7±0.03e	traces
	2	-	-	traces	1.3±0.1a	1.9±0.04	1.2±0.1c	7.1±0.3b	-
	3	-	-	traces	1.5±0.03a	-	1.3±0.1c	8.6±0.1a	-
	4	-	-	traces	1.4±0.1a	-	1.9±0.1ab	4.9±0.02cd	-
ZLATKO	0	traces	traces	traces	1.3±0.02d	-	1.1±0.1c	4.8±0.3abcd	traces
	1	-	-	traces	3.2±0.03b	-	0.8±0.01c	3.1±0.03e	0.3±0.004
	2	-	-	traces	2.0±0.1c	-	1.8±0.01b	4.0±0.004d	traces
	3	-	-	traces	2.0±0.01c	-	1.8±0.1b	4.4±0.01b	-
	4	traces	-	-	4.4±0.1a	-	2.6±0.04a	4.0±0.1cd	traces
TRISTAN	0	-	traces	-	1.3±0.1e	-	-	3.9±0.02ab	traces
	1	-	-	traces	1.7±0.01d	-	1.0±0.1b	4.1±0.6abd	traces
	2	-	-	traces	2.4±0.01b	-	1.2±0.1ab	3.3±0.1cd	traces
	3	6.5±0.3	-	-	4.2±0.1a	-	1.3±0.02a	3.0±0.02d	traces
	4	-	-	traces	1.9±0.04c	-	1.4±0.1a	3.8±0.1bc	traces
OSVIT	0	traces	4.7±0.1	-	1.2±0.03b	-	0.9±0.01ad	3.1±0.02ac	-
	1	traces	2.8±0.1	-	1.1±0.04b	-	4.1±0.01a	4.3±0.03a	1.3±0.04
	2	traces	-	traces	1.5±0.03a	-	1.3±0.03c	3.1±0.06c	traces
	3	-	-	traces	1.5±0.004a	-	1.6±0.1b	3.5±0.1b	-
	4	-	-	-	1.6±0.1a	-	1.2±0.1c	2.1±0.04d	-
MANDATAR	0	-	-	traces	1.7±0.01b	-	0.6±0.004c	4.8±0.1c	-
	1	-	-	traces	1.5±0.02c	-	1.2±0.1b	4.7±0.04c	-
	2	-	-	traces	1.9±0.03a	-	1.3±0.1b	4.1±0.02d	-
	3	-	-	traces	1.4±0.03cd	1.9±0.01	1.3±0.02b	5.8±0.1b	-
	4	-	-	-	1.3±0.03d	2.0±0.01	1.5±0.2ab	6.6±0.1a	-

**Amounts below the detectable minimum;*
***Values with the different letters in the same column and within the same cultivar indicate statistical significance ($p<0.05$). Xyl (Xylose), Ara (Arabinose), Fru (Fructose), Glc (Glucose), Gal (Galactose), Suc (Sucrose), Mal (Maltose), Isomal (Isomaltose)*

The Glc content in the flour samples was mainly lower than that of maltose, which was expected. Both wheat samples, Lorena and Snaša even contained Glc under the detection limit, which was marked as a trace amount. After 24 h of germination for the Lorena sample and only after a longer treatment of 48 h for the Snaša one were detected

1.0 ± 0.004 % and 1.3 ± 0.1 % glucose, respectively. However, all initial barley samples contained small amounts of Glc between 1.2 ± 0.03 % Tristan sample and 1.7 ± 0.01 % Mandatar sample.

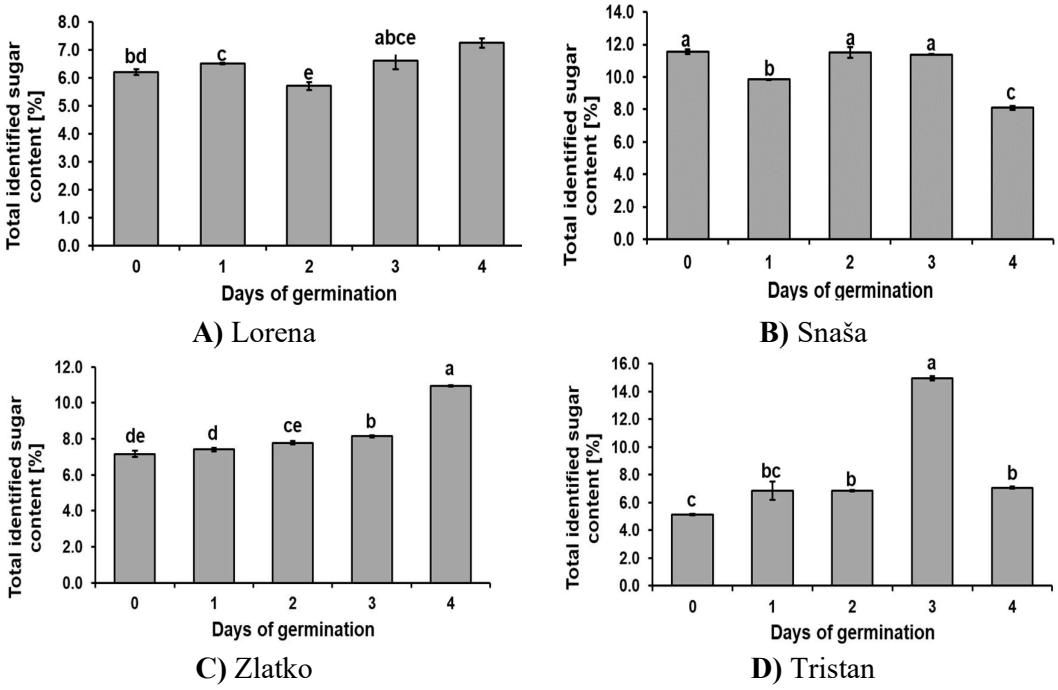
The highest elevation of Glc levels after 24 h of incubation, which was statistically significant, was determined for the Zlatko and Tristan flour samples, and the increment was 146 % and 31 %, respectively. Normally, Glc can be released from polymers, such as starch and β-glucans, and smaller molecules like sucrose or other saccharides.

For both wheat cultivars and barley cultivar Zlatko the highest sucrose accumulation was observed after 96 h of germination, which is really a long period of time. Osvit and Mandatar samples did not change considerably their sucrose content between 24 h and 96 h of germination. Moreover, the malting procedure within 24 h resulted into more sucrose released in all flour samples, except for that of Zlatko one. The highest increase of 356 % was determined for the Osvit sample after 24 h of incubation. It is well-known that sucrose can be split into Fru and Glc by the catalytic action of β-fructofuranosidase from the non-reducing Fru side. Langenaeken et *al.* [29] have demonstrated that Glc and sucrose contents in malt barley (var. Sebastian) reached 2.2 % and 4.7 %, respectively after 4 days of germination. For example, the barley Zlatko sample had 4.4 % and 2.6 % of Glc and sucrose. Moreover, in general such a high sucrose content after 96 h of germination was not registered in the current study. However, not only the varieties, their composition and application purposes, but also the conditions of germination are vital for preparation of good malts. It should be noted that the precise change of the detected soluble carbohydrates is better to be calculated on the basis of the yield of the respective flour after each step of germination. Additionally, a decrease, instead of an increase of a specific low-molecular-weight sugar between different steps of germination cannot be considered a phenomenon. For instance, from biochemical and physiological point of view, part of Glc released from the polymers is consumed by the developing cells in the grain embryos, as well as sucrose is transported as a preferable energy source in the embryos, because of its non-reducing nature [29].

Isomaltose was found mainly in trace amounts in most of the samples, as it was mentioned Isomaltose was found mainly in trace amounts in most of the samples, as it was mentioned above. It was not detected in the Mandatar ones. Isomaltose like maltose was recognized as a degradation product of starch in the flour. Surprisingly, Lorena and Snaša contained 4.1 ± 0.1 % and 3.7 ± 0.1 % Fru, respectively. It is known that Fru can be released from fructans and sucrose. Interestingly, Xyl, Ara and Gal were found in a limited number of samples from both grain cultures. Xyl, Ara, and Gal can originate from depolymerization of hemicelluloses in the malt flour. Meaningful amounts of Xyl were detected in Snaša (5.7 ± 0.2 %) and Tristan (6.5 ± 0.3 %) samples and Ara was detected only in Osvit sample 4.7 ± 0.1 % and 2.8 ± 0.1 % in 0 and 24 h after germinated period. Contrarily, free Gal was not found in the initial samples, and it was released after 48 h of germination in the Snaša flour and after 72 and 96 h in the Mandatar flour. Perhaps, arabinoxylan- and galactan-degrading enzymes need more time to act, because their activities are considerably lower in the grains than those of glucanases, as it has been reported for xylanases [30]. Interestingly, Zheng et *al.* [31] have studied the monosaccharide composition of hull-less barley bran, shorts and flour, after acid hydrolysis, and they found that the flour contained mainly Glc, followed by Xyl, Ara, and Gal. The flour contained the lowest amount of arabinoxylan (2.1 %) in

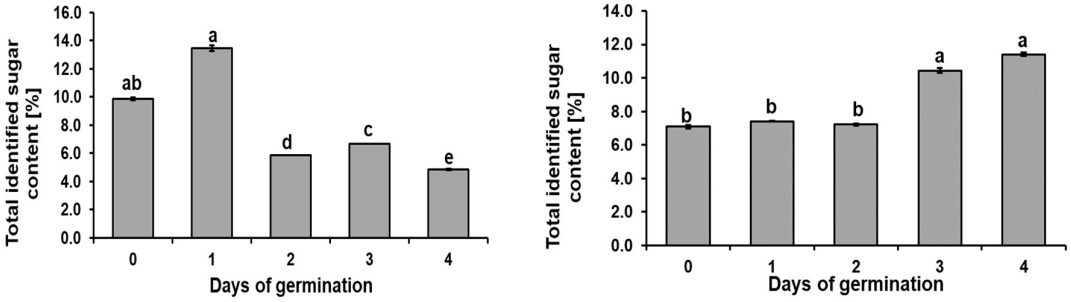
comparison with the bran and shorts [31]. In general, the starch content in barley grains is much higher than that of *β*-glucan or arabinoxylan [29, 32].

In Figures 1A to F are shown the calculated total amounts of identified free sugars in all flour samples. It was calculated that the initial flour with the highest total identified free sugars was Snaša (0 days germination) - 11.6 %, followed by Osvit - 9.9 %. Zlatko - 7.2 %, Mandatar - 7.1 %, Lorena - 6.2 % and Tristan - 5.1 % (0 days germination) were characterized by a lower total free sugar content. Only the Osvit cultivar achieved a significant increase in the total free sugar content of 37 % after 24 h of germination. It is evident from Figures 1A, C, and F that the Lorena, Zlatko, and Mandatar flour samples released the highest amount of free identified sugars after 96 h of treatment ($p < 0.05$). For a comparison, Bhatty [32] has reported that the mean total soluble carbohydrate content in four hull-less barley varieties increased from 4.0 % to 10.4 % in the malt samples (76 h). However, the elevation of the mean soluble sugar content in two soft white spring kinds of wheat was much lower from 3.1% to 5.8 % in malt samples after 76 h [32]. Determination of the total carbohydrate content of the flour studied, as well as the amounts of starch, arabinoxylans and β-glucan, was also necessary to more fully follow the changes in the carbohydrate composition during malting. Arif et *al.* [11] have determined that the total carbohydrate and crude fiber contents of raw barley flour and malted barley flour, after 3 days of treatment, were changed from 67.1 % to 77.3 %, and from 6.1 % to 4.8 %. Although the cultivar was not mentioned in the cited study, it is evident that more sugars will be available for further fermentation after malting.



A) Lorena **B)** Snaša

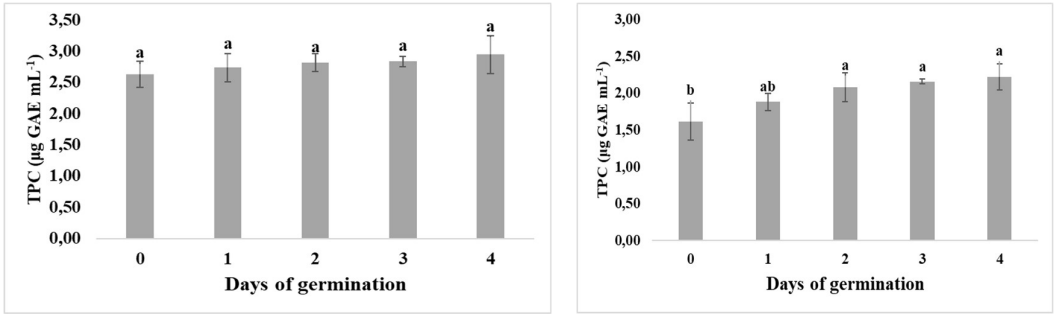
C) Zlatko **D)** Tristan



E) Osvit **F)** Mandatar

Figure 1. *Calculated total identified free sugars in wheat and barley malt flour*
Values with the different letters within the same cultivar indicate statistical significance ($p < 0.05$)

It is worth mentioning that the released Glc, Fru and other monosaccharides from hemicelluloses can be fermented during the production of bread, beer, or other foods. For example, Langenaeken et *al.* [29] have calculated that 41 % of the barley (var. Sebastian) malt was converted into fermentable sugars in an industrial scale, which is a good value for beer production. Similarly, Park et *al.* [20] have reported that the total sugar content of Heugho, Hopum and Kwangmaeg barley increased in the respective malt, prepared on a pilot-scale level, from 10.9, 10.4, and 9.3 to 47.9, 40.8 and 50.8 %, respectively. Codină et *al.* [33] have shown that there is a positive correlation between the gas production and Glc content after 60 min of wheat dough fermentation, and a negative correlation with the Fru content after 120 min of fermentation. Aside from the recognized fermented bakery products that are produced by malt flour, considerable efforts are put into the development of wheat malt types to prepare a new generation of craft beers, sourdoughs, or other healthy functional bakery foods [34, 35]. Interestingly, Pan et *al.* [36] have found that the levels of fasting blood Glc, HbA1c, total glycerides, as well as fat mass, fat rate, and visceral fat ameliorated in patients with metabolic syndrome after consumption of wheat noodles, prepared with fermented by lactobacilli barley flour. Therefore, malt barley can become a regular useful ingredient of many functional bakery products [37].
Figure 2 shows the TPC, determined at different times of germination of the two wheat and four barley cultivars used in the study.



A) Lorena **B)** Snaša

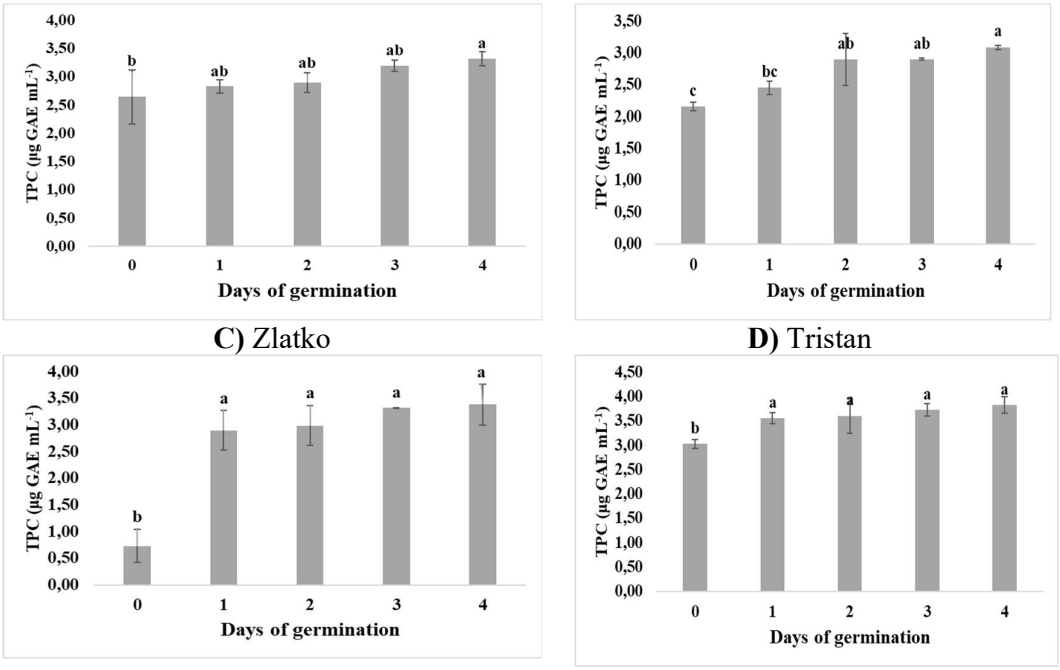


Figure 2. *Total phenolic content (TPC), expressed in gallic acid equivalents (GAE) in wheat (Lorena and Snaša) and barley (Zlatko, Tristan, Osvit and Mandatar) cultivars during four days of germination*

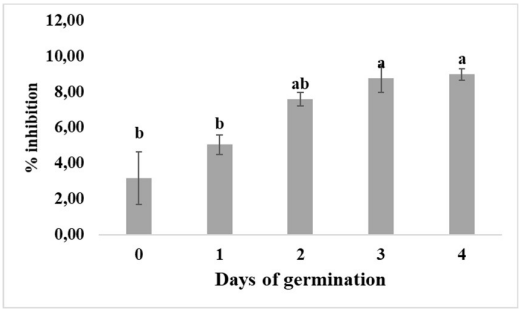
Same letters above the columns indicate that data are not significantly different ($p < 0.05$) following Fisher's LSD test

The changes of the TPC during the germination periods studied of wheat and barley grains were found to be statistically significant ($p<0.05$), based on an ANOVA analysis, although the details were not presented. The average amount of total phenolics was higher in barley (2.8 µg GAE·mL^{-1}), compared to wheat (2.4 µg GAE·mL^{-1}). Niroula et *al.* [38] have also determined that barley has a higher TPC, compared to wheat. The TPC was found to increase during the germination process, with values of 3.0, 2.2, 3.3, 3.1, 3.4 µg GAE·mL^{-1} on the fourth day of germination for wheat Lorena and Snaša, and barley Zlatko, Tristan, Osvit, and Mandatar samples, respectively (Figure 2). Similarly, Niroula [38] has confirmed the increase of total phenolics during the germination process in both barley and wheat samples. Apart from that, Bhinder et *al.* [39] have investigated the effect of germination on white and black quinoa seeds in the interval of 0 to 96 h, and found that the amount of TPC increased significantly during the process. It increased from 7.1 to 8.6 mg GAE·g^{-1} for white quinoa and from 7.7 to 9.2 mg GAE·g^{-1} for black quinoa over 96 hours of germination. Tarzi et *al.* [40] have also confirmed that the TPC in chickpea seeds increased during the germination process. The increase in the total phenolics during seed germination can be attributed to several factors, including enzymatic hydrolysis of bound polyphenolic compounds and therefore increase in free or extractive phenolics, biosynthesis of new phenolic compounds, and stimulation of phenyl-propanoid pathways [39, 41]. Furthermore, it is necessary to determine the phenolic profile of malt wheat and barley flour during different times of germination.

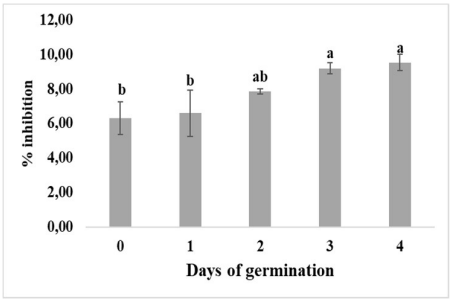
Phenolic compounds have the ability to donate electrons and hydrogen atoms to free radicals and thus contribute to a better antioxidant activity of the samples, which contain them [42]. The Figure 3 shows the results of DPPH free radical inhibition.

The antioxidant activity of the different wheat and barley cultivars was monitored (Figure 3) during a 4-day germination process, and an ANOVA analysis revealed that there was a statistical difference ($p < 0.05$) between the days of germination for each sample. The results showed that the antioxidant activity slightly increased during the germination process for each variety, and this increase could be explained by the elevation of released phenolic compounds (Figure 2).

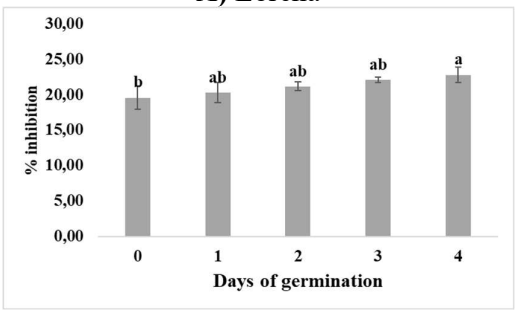
The lowest antioxidant activity (15.43 % inhibition) among the barley varieties was observed in Tristan sample, which was expected because it was characterized by the lowest TPC (2.15 GAE·mL^{-1}). The same trends have been observed in other studies. For example, Suryanti et *al.* [43] have found that the *in vitro* antioxidant activity of lead tree sprouts (Leucaena leucocephala (lmk.) De Wit increased to 49.7 % inhibition of the DPPH radical on the fourth day of the germination process from the initial inhibition of 17.9 % (0 day of germination). The antioxidant activity is dependent on the reaction system effect. It has been suggested that it is related to the formation of intramolecular hydrogen bonds between functional groups (4-OH and o-methoxy groups) [44]. The antioxidant activity of wheat and barley malt flour needs to be determined also by other methods to confirm the effects observed.



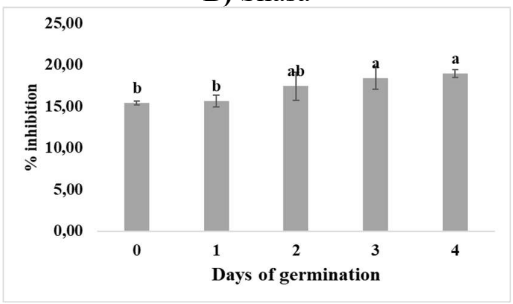
A) Lorena



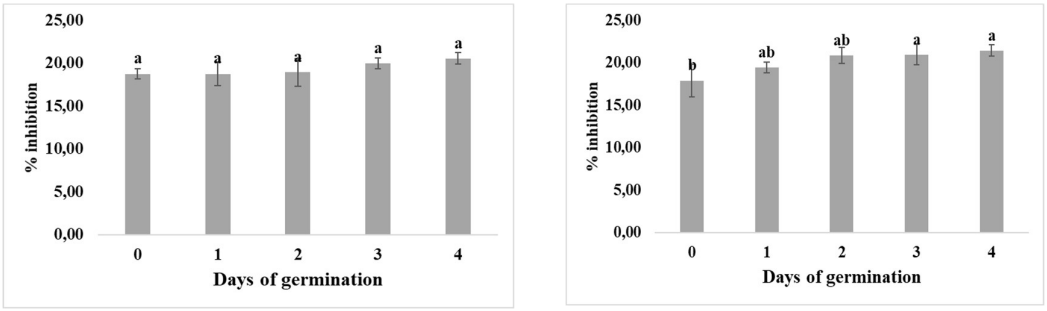
B) Snaša



C) Zlatko



D) Tristan



E) Osvit **F)** Mandatar

Figure 3. *In vitro antioxidant activity, expressed in % inhibition of DPPH radical in the presence of wheat (Lorena and Snaša) or barley (Zlatko, Tristan, Osvit and Mandatar) malt flour samples, during four days of germination*

Same letters above columns indicate that data are not significantly different ($p < 0.05$) following Fisher's LSD test

CONCLUSION

For the development and control of feasible fermentation processes and production of high-quality products for humans and animals, it is necessary to determine the content of total carbohydrates, individual polymeric fractions (starch, β-glucan, arabinoxylans and other hemicelluloses), and soluble sugars in the raw materials, as well as their changes during processing. The identified sugars in the different malt flour samples were maltose, Glc and sucrose, while Fru, isomaltose, Xyl and Ara were mainly present in trace amounts. The results obtained demonstrated that malt flour, prepared by the chosen cultivars of barley and wheat, contained representative amounts of soluble sugars that could be further fermented. Exceptionally, Snaša flour contained high comparable amounts of soluble sugars (11.6 - 8.1 %) at 0 - 96 h of germination that can be utilized when alcoholic drinks, bakery products, and functional foods are produced. Apart from that, the amount of total phenolics was found to be higher in the examined barley than in wheat varieties. Moreover, during the germination process, there was an increase in the content of total phenolics. Logically, the antioxidant activity of the examined samples was also found to increase with the continuation of the germination process. The fermented barley and wheat flour contain biologically active compounds and simple energy nutrients that can serve as food for commensal probiotic bacteria after consumption of synbiotic drinks or foods containing them. Therefore, the malt flour could be used for creation of a new generation of sourdoughs, craft beers, functional and dietary foods or added to traditional Balkan fermented drinks.

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