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Evaluation of Total Phenolic Content and Antioxidant Activity of Malting and Hulless Barley Grain and Malt Extracts

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Abstract

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Nine malting and six hulless barley varieties were evaluated for their grain characteristics, β-glucans, total phenolic contents, and corresponding antioxidant activities determined by DPPH radical scavenging method. Samples were malted to compare malt quality properties between hulled and hulless types of barley and to estimate the influence of malting process on total phenolics. The highest levels of total phenolics and antioxidant activity were observed in the hulless barley lines GZ-191 and GZ-186. Malts were found to have higher antioxidant activity and contents of total phenolics than their corresponding barley. Results from the present study showed that hulless barley lines had on average higher protein, starch, β-glucan, malt extract content, and wort viscosity, but lower Kolbach Index and malt friability when compared to hulled malting varieties. The analysis of fifteen different barley samples shows that the barley type has an influence on both the total phenolic content and the antioxidant activity, as well as on the malting performance of hulless and hulled barley.

Keywords: cereal crop; quality; bioactive; DPPH; β-glucan

Barley is a widely grown cereal crop, mostly used as feed for animals, raw material in the production of malt for the brewing industry, and in the bakery industry (Newton *et al*. 2011). Historically, it was one of the earliest cereals to feature in the human diet in many parts of the world. At present, only 2% of barley is used for human food (Baik & Ullrich 2008). The malting of barley grain results in an increase in enzyme activity, soluble protein, and breakdown of starch into simple sugars, along with the development of typical colour and flavour (Gupta *et al*. 2010). From the different quality parameters reported in the literature, malt extract content, kernel size fractions, kernel weight, β-glucan, and protein contents, malting losses, friability, α-amylase activity, viscosity, and soluble nitrogen ratio are common assays used to test the quality of malting barley (Fox *et al*. 2003).

The renewed interest in barley for food uses centres around the discovery of a positive physiological role of β-glucan, a cell-wall polysaccharide found both in oats and barley (Newton *et al*. 2011). The health benefits of barley β-glucans include reduction of blood cholesterol and glucose and weight loss by increased satiety, and therefore, the control of heart disease and type-2 diabetes (Baik & Ullrich 2008). However, new findings revealed that cereal grains also contain many health-promoting components such as vitamins, minerals, essential fatty acids, phytochemicals and other bioactive food components, which include phenolic compounds (Dykes & Rooney 2007). In recent years, there has been

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a growing interest in the use of barley and malt in processed foods as natural antioxidants because of their high content of phenolic compounds. During malting, the natural germination process leads to an increase of bioactive compounds (Madhujith & Shahidi 2007). Malted barley grain contains various compounds from barley (endogenous phenolic compounds) or from the malting process (Maillard reaction products) that can play a significant role in malting and brewing through their antioxidative properties (Qingming *et al*. 2010).

The majority of barley production in Croatia is for animal feeds and malt. However, on a smaller scale, barley can be processed for human consumption as a good source of functional components such as β-glucan and phenolic antioxidants. The aim of this study was to examine the range of variation in total phenolic content and antioxidant activity of barley varieties of diverse genetic origin. Malting was performed to compare the barley grain and its corresponding malt samples for their total phenolic content (TPC) and antioxidant activity (AOA).

MATERIAL AND METHODS

Material. Nine malting barley varieties and six experimental hulless barley lines grown in field trials of Agricultural Institute Osijek located in the region of eastern Croatia and corresponding malts were studied. An overview of the varieties, their characteristics, and breeding companies is shown in Table 1. In a sieve analysis, the samples of barley were differentiated on kernel size in a shaking machine provided with three sieves with slotted holes of 2.8, 2.5, and 2.2 mm in width. The amounts of grain remaining on 2.8 mm and 2.5 mm sieves are reported as fractions I and II, respectively, and expressed as a percentage of the total barley grain sample used for the sieve analysis. Only grains of the size larger than 2.5 mm were used for a micro-malting procedure.

The Folin-Ciocalteu reagent (Merck, Germany) and sodium carbonate with gallic acid (Sigma-Aldrich, Germany) were employed for the measurement of the Folin-Ciocalteu TPC. For AOA measurement, DPPH was sourced from Sigma-Aldrich. The mixed linked β-glucan content in barley grain samples was determined using a Megazyme mixed-linkage β-glucan assay kit (Megazyme Ltd., Ireland). The whole grain barley flour was prepared by grinding in a hammer type cyclone mill (Laboratory Mill 3100; Perten Instruments AB, Sweden) to pass through a 0.8 mm sieve, and then the powder was stored at 4°C until used. Whole grain and malt meals were analysed for moisture content using a halogen drying moisture analyser (Mettler Toledo Balance HR83).

Barley samples were malted in an Automated Joe White Malting Systems Micro-malting Unit (Australia). Prior to micro-malting the barley grain protein and starch content were determined by a non-destructive near infrared transmission method (Infratec 1241 Grain Analyser; FOSS, Denmark). Malt quality attributes were determined according to European Brewery Convention methods (EBC Analysis Committee 1998).

Total phenolic content. The total phenolics in barley and malt extracts were determined with Folin-Ciocalteu reagent using the method described by Singleton and Rossi (1965) with some modifications. Extracts were prepared by weighing 2 g of ground barley grains (or malt) and mixed with 5 ml of acidified methanol (HCl/methanol, 1 : 100, v/v). The mixture was homogenised by vortexing for 2 min, placed in a Sonorex Digitec ultrasonic bath (RK 510 H Model; Germany) at room temperature and sonicated for 1 hour. It was then centrifuged at 9000 rpm for 5 min at 4°C on a centrifuge (Universal 320R; Hettich, Germany). TPC was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

Antioxidant activity. AOA was measured using a modified version of the method explained by Brand-Williams *et al*. (1995). This involved the use of a free radical DPPH solution in methanol. Every sample extract (0.2 ml) was reacted with 1 ml of a 0.5 mmol/l methanol solution of DPPH and 2 ml of methanol. The reaction mixture was shaken, and incubated in the dark for 30 minutes. The absorbance (A) of the solution was measured against a methanol blank at 517 nm. AOA was calculated as the inhibition of free radical DPPH in percent (%) by using the following equation:

$$\text{Antioxidant activity (\%)} = \left[1 - (\text{A of sample}_{t=30}/\text{A of control}_{t=0})\right] \times 100$$

Statistical analysis. Analyses of total phenolic content and antioxidant activity were carried out in triplicate and the data were reported as means ± standard deviations. Differences in means were evaluated using one-way analysis of variance (ANOVA) and Tukey's test; P values < 0.05 were regarded as significant.

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RESULTS AND DISCUSSION

In this study, malting and hulless barley varieties were characterised with focus on total phenolics and AOA of barley grains and malted barley grains. Prior to micro-malting the contents of the whole grain protein, starch and β-glucan were determined (Table 1). The difference in β-glucan content between the hulled and hulless samples shows a tendency towards higher β-glucan content in hulless barley varieties. This is in agreement with results reported by Holtekjølen *et al*. (2006) and Ehrenbergerová *et al*. (2008). Hulless barleys often have high β-glucan contents, and are mainly used as human food because of ease in processing and their edibility (Ferrari *et al*. 2009). The malt quality parameters are presented in Table 2. The most important feature of malt is its behaviour in the mashing process and its potential for producing a wort soluble extract. The hulless varieties averaged the higher malt extract content when compared with malting varieties. There were also differences between malting and hulless varieties in Kolbach index, wort viscosity, and friability, and a difference in extract yield between finely and coarsely ground malt. Differences in malting behaviour revealed that malting varieties achieved better cytolytic and proteolytic modification than hulless barley lines.

TPC in whole barley flour varied among the analysed malting varieties (Table 3). Grains such as oat, barley, buckwheat, sorghum, rye, or wheat are valuable sources of bioactive compounds with significant antioxidant activities, and much attention has been paid to their potential role in the prevention of human diseases as well as possible food enrichment. Zieliński and Kozlowska (2000) reported that TPC in barley and oat whole grains was found to be higher than that of wheat and rye and lower than that of buckwheat. Sharma and Gujral (2010) reported in their study that TPC in whole barley flour varied significantly among cultivars, from 3.07 to 4.48 mg GAE/g. Zhao *et al*. (2008) reported significant amounts of total phenolics in 14 different malting barley varieties ranging from 2.17 to 2.56 mg GAE/g DW. The TPC values of malt extracts are higher in all cases in kilned malt than in

Table 1. Different barley varieties and their characteristics

Variety	Hulled/ hull-less	Thousand corn weight (g)	Fractions I + II (%)[a]	Hectolitre weight (kg)	Protein (%)[b]	Starch (%)[b]	β-glucan (%)[b]	Breeding company/country
Barun	H	51.0	75.3	62.0	11.00	62.50	3.70	AIO/CR
Bravo	H	51.3	77.9	63.7	11.55	62.34	4.25	AIO/CR
Bingo	H	48.7	82.0	67.4	11.67	62.43	4.24	AIO/CR
Premium	H	50.9	91.2	67.5	11.38	62.81	3.33	AIO/CR
Vanessa	H	47.8	83.2	61.2	11.50	62.51	2.63	SJB/GE
Tiffany	H	51.8	87.4	64.8	11.13	62.16	3.34	SJB/GE
Maxim	H	53.5	88.8	68.0	11.08	62.37	3.41	AIO/CR
Gazda	H	47.4	72.0	67.3	11.38	62.45	3.13	AIO/CR
Rex	H	48.7	79.1	65.3	11.45	62.42	4.30	AIO/CR
Average		50.1	81.9	65.2	11.35	62.44	3.59	
CV (%)		4.10	7.86	3.87	2.02	0.28	16.08	
GZ-179	H-L	42.4	31.4	63.1	12.65	63.04	4.12	AIO/CR
GZ-184	H-L	46.6	39.1	71.2	13.85	63.10	4.29	AIO/CR
GZ-186	H-L	46.4	31.9	70.0	13.45	63.38	5.27	AIO/CR
GZ-189	H-L	48.5	63.7	68.6	13.70	63.15	5.42	AIO/CR
GZ-190	H-L	40.8	6.5	64.5	12.45	62.10	4.65	AIO/CR
GZ-191	H-L	43.1	28.9	72.3	12.43	64.83	4.97	AIO/CR
Average		44.63	33.58	68.28	13.09	63.27	4.79	
CV (%)		6.64	54.91	5.44	4.97	1.40	10.97	

[a]Total of barley grain fractions > 2.5 and > 2.8 after sieving test expressed as percentage; [b]dry weight basis; AIO – Agricultural Institute Osijek; CR – Croatia; SJB – Saatzucht Josef Breún GdbR; GE – Germany; CV – coefficient of variation

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Table 2. Malt quality parameters

Variety	Content of malt extract (%)	Kolbach index (%)	Wort viscosity (mPas)	Fine/coarse difference (%)	Malt friability (%)
Barun	80.58	42.57	1.595	3.71	58.90
Bravo	80.43	41.89	1.482	5.16	68.18
Bingo	80.42	36.73	1.791	5.68	46.56
Premium	80.89	40.02	1.647	3.31	55.30
Vanessa	81.15	39.02	1.498	2.29	72.94
Tiffany	82.55	41.31	1.564	3.12	67.92
Maxim	80.75	39.61	1.805	5.06	45.80
Gazda	81.38	39.89	1.799	4.54	51.16
Rex	80.80	40.44	1.684	6.08	51.18
Average	80.90	40.16	1.652	4.33	57.55
CV (%)	0.82	4.28	7.690	29.68	17.43
GZ-179	86.30	38.66	1.657	4.22	58.32
GZ-184	84.91	34.13	1.822	6.15	45.80
GZ-186	84.51	35.43	1.731	5.11	54.34
GZ-189	83.62	35.16	1.863	6.63	38.38
GZ-190	83.40	37.26	1.649	4.63	56.92
GZ-191	85.73	37.72	1.990	6.86	52.14
Average	84.75	36.39	1.785	5.60	50.98
CV (%)	1.35	4.79	7.40	19.62	14.87

CV – coefficient of variation

barley (Tables 3 and 4). These results are in accordance with literature data (Dvořáková *et al*. 2008; Qingming *et al*. 2010; Leitao *et al*. 2012), confirming that better extraction of phenolic compounds is possible after kilning. Malting is a complex process responsible for modifications in the composition of barley. These structural changes involve many enzyme degradations, including enzymatic release of phenolic compounds bound to cellular structures of barley and with glycosylation reactions during malting, leading to easier extraction of free phenolic acids due to changes in the matrix in the early kilning (Carvalho *et al*. 2016). Leitao *et al*. (2012) found in their study that malting allowed a better release and/or extraction of phenolic compounds, while the first brewing step caused the most significant decrease in the total polyphenols extracted. When compared ($P < 0.05$) to hulled barley varieties, hulless barley lines had higher TPC (Table 4). These results are in accordance with those reported for the TPC of experimental hulless varieties in comparison with malting barley varieties (Dvořáková *et al*. 2008). Žilić *et al*. (2011) reported that among the small grain species included in their study, hulless barley had the highest reducing power, contained the most active scavengers of free radicals and the highest content of total phenolics and flavonoids.

The DPPH assay has been widely used in an assessment of the radical scavenging activity of different cereal extracts because of its ease and convenience (Liu & Yao 2007; Zhao *et al*. 2008; Sharma & Gujral 2010). Malts were found to have a higher AOA than their corresponding barleys (Tables 3 and 4), but the increase of AOA varied greatly from one variety to another. Furthermore, varieties which were characterised by the highest percentage increase in total phenolic content after malting also showed the highest increase in antioxidant activity. As far as malting is concerned, several studies investigated its effect on AOA (Lu *et al*. 2007; Dvořáková *et al*. 2008; Fogarasi *et al*. 2015), confirming that the malting process contributes to an increase of scavenging activity. Hulless barley lines were found to have (at the significance level $P < 0.05$) an average content of phenolics and antioxidant activity higher than malting varieties (Table 4). Among the hulless barley lines studied, hulless barley varieties with the highest contents of total phenolics (GZ-191

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Table 3. Total phenolic contents (TPC) and antioxidant activity of malting (hulled) barley grain and malt extracts

Variety	TPC (mg GAE/g DW)		TPC increase (%)	DPPH (% inhibition)		DPPH (% inhibition) increase (%)
	barley	malt		barley	malt	
Barun	1.57 ± 0.06^{a}	1.72 ± 0.12^{ab}	9.33	62.20 ± 0.50^{bc}	68.18 ± 1.46^{a}	9.62
Bravo	1.46 ± 0.03^{b}	1.78 ± 0.10^{ab}	21.97	58.19 ± 2.15^{d}	64.22 ± 3.34^{ab}	10.37
Bingo	1.58 ± 0.07^{a}	1.66 ± 0.11^{bc}	4.99	61.54 ± 0.36^{c}	63.73 ± 3.39^{ab}	3.56
Premium	1.57 ± 0.05^{a}	1.63 ± 0.05^{bc}	3.69	62.83 ± 0.43^{bc}	63.06 ± 1.88^{b}	0.36
Vanessa	1.67 ± 0.09^{a}	1.73 ± 0.05^{ab}	3.77	65.19 ± 1.49^{a}	68.37 ± 2.01^{a}	4.89
Tiffany	1.30 ± 0.07^{c}	1.82 ± 0.00^{a}	40.18	58.48 ± 0.39^{d}	64.71 ± 3.09^{ab}	10.66
Maxim	1.37 ± 0.04^{bc}	1.55 ± 0.03^{c}	12.81	59.20 ± 0.82^{d}	64.63 ± 0.53^{ab}	9.18
Gazda	1.27 ± 0.03^{c}	1.53 ± 0.09^{c}	20.84	59.72 ± 0.43^{d}	63.64 ± 0.55^{ab}	6.58
Rex	1.56 ± 0.06^{a}	1.64 ± 0.09^{bc}	5.05	63.82 ± 0.51^{ab}	65.13 ± 3.49^{ab}	2.05
Average	1.48 ± 0.06	1.67 ± 0.07	13.63	61.24 ± 0.79	65.08 ± 2.19	6.36

Means of three determinations ± standard deviation; means with different letters in the same column are significantly different at the level $P < 0.05$; the percentage increases of TPC and DPPH (% inhibition) between malts and barleys are shown in separate columns; GAE – gallic acid equivalent

and GZ-186) also showed the highest AOA. Žilić *et al*. (2011) reported a considerable variation in AOA observed between the small grain cereals and that hulless barley had the best antioxidant properties. Similarly, Abdel-Aal and Choo (2014) observed appreciable total phenols and AOA in hulless barley over 23 environments in eastern Canada. A significant positive correlation ($P < 0.05$) was found between TPC and antioxidant activity (hulled – $r = 0.83$; hulless – $r = 0.86$), which is in agreement with the data already published (Zhao *et al*. 2008; Sharma & Gujral 2010). These data indicate that phenolic compounds contribute to the DPPH radical scavenging activity.

Among cereal grains, barley is naturally high in phenolic compounds. The majority of the barley phenolic compounds have also been identified in malt, which implies that natural antioxidants present in barley make a large contribution to the antioxidant activity of malt (Carvalho *et al*. 2016). Therefore, the screening of different barley varieties for their antioxidant potential seems important from the aspect of malt and beer production. In this study, hulless barley showed higher TPC and radical scavenging activity than hulled varieties, which increased during malting. In addition, hulless barley varieties were found to be rich in β-glucan soluble fibre, which makes them a good choice to a healthy diet. Barley flour and whole-grain meal can also be incorporated into baked and extruded food products with intention to improve their nutritional value.

Table 4. Total phenolic content (TPC) expressed in gallic acid equivalents (GAE) and antioxidant activity analysed by DPPH method in hulless barley grain and malt extracts

Variety	TPC (mg GAE/g DW)		TPC increase (%)	DPPH (% inhibition)		DPPH (% inhibition) increase (%)
	barley	malt		barley	malt	
GZ-179	1.62 ± 0.07^{b}	2.14 ± 0.12^{b}	31.95	66.92 ± 1.92^{bc}	76.29 ± 1.72^{ab}	14.01
GZ-184	1.39 ± 0.11^{d}	1.73 ± 0.13^{d}	24.53	62.09 ± 0.81^{d}	73.28 ± 2.45^{bc}	18.03
GZ-186	1.75 ± 0.05^{a}	2.31 ± 0.03^{a}	32.08	73.98 ± 1.36^{a}	85.09 ± 1.22^{a}	15.01
GZ-189	1.47 ± 0.09^{cd}	1.89 ± 0.15^{c}	28.58	64.37 ± 0.65^{cd}	69.11 ± 3.05^{c}	7.37
GZ-190	1.53 ± 0.05^{bc}	1.93 ± 0.09^{c}	25.75	68.03 ± 2.23^{b}	74.43 ± 2.78^{b}	9.41
GZ-191	1.78 ± 0.04^{a}	2.19 ± 0.05^{b}	22.71	75.61 ± 1.03^{a}	84.72 ± 1.06^{a}	12.04
Average	1.59 ± 0.07	2.03 ± 0.09	27.60	68.50 ± 1.33	77.15 ± 2.05	12.64

Means of three determinations ± standard deviation; means with different letters in the same column are significantly different at the level $P < 0.05$

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