ANTIRADICAL ACTIVITY OF DIFFERENT BARLEY VARIETIES AND MALT TYPES

Ilona Dabina – Bicka, Daina Karklina, Zanda Kruma
Latvia University of Agriculture
ilona.dabina@inbox.lv

Abstract

Cereal grains have long been thought to be less important sources of antioxidants than fruits and vegetables although they contain many antioxidants and are major dietary components worldwide. The aim of the current research was to study and compare an antioxidant activity (AOA) and total phenolic content (TPC) of different barley varieties and malt types as well as to evaluate possible interconnection between TPC and AOA of barley and malt samples.

The research was carried out on four lines of hull-less barley '3528'; 'L-400'; '3475'; '3537' and one variety of flaky barley 'Klass' grains, which were cultivated in Latvia in 2010, and their corresponding malt. Commercial sorts of malt - Pilsener, Munich, Caramel and Dark were used in the research to compare with the malt produced in the laboratory scale.

The antioxidant potential of barley and their products is analyzed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. Total phenolic content of barley and malt samples was determined according to the Folin-Ciocalteu spectrophotometric methods.

The values of DPPH radical scavenging activity for 5 barley samples ranged from 4.57 to 5.89 μmol TE g^{-1} DW. The total amount of phenols ranged from 1.96 to 2.43 mg GAE g^{-1} DW for unprocessed barley samples and from 2.5 to 3.4 mg GAE g^{-1} DW for their corresponding malt. TPC of malt commercial sort ranged from 3.5 to 6.7 GAE g^{-1} DW. The increase of TPC for commercial malts is strongly related with Maillard reaction products.

Key words: barley; hull-less barley; malt; phenols; antioxidants

Introduction

A broad definition of an antioxidant is: a substance that, present at low concentration compared with those of an oxidizable, substrate significantly delays or prevents oxidation of that substrate. However, the way antioxidants can exert their antioxidant activity differs among compounds. Phenolic compounds are known to quench superoxide radicals and to inhibit lipid peroxidation. Melanoidins (brown, nitrogenous polymers and co-polymers) are the final products of the Maillard reaction and are known to scavenge free and active oxygen, act as reducing and chelate metals. However, pro-oxidant activity has been reported as well (Preedy, 2009).

Interest in barley (*Hordeum vulgare* L.) as a food grain is emerging. Over the past decade an increasing interest in barley for human consumption has been observed, mainly due to its content of health-related bioactive components. The health benefits normally associated with barley are attributed to high amounts of dietary fibre. However, antioxidants or phenolic structured antioxidant compounds are also detected in barley (Holtekjolen et al., 2006), and recent studies have shown that cereals contain more phytochemicals than previously considered.

Barley is grown in many parts of the world both for food and for feed uses. In many countries it is mostly used as a feed crop as well as for brewing and production of ethanol but in small quantities barley is used in the production of soups, dressings, baby foods and speciality items. Whole-grain cereals are a major source of polyphenols, especially phenolic acids such as ferulic, vanillic, caffeic, syringic, sinapic and p-coumarin acids. All of them have potentially antioxidant properties due to the presence of an aromatic phenolic ring that can stabilize and delocalize the unpaired electron within its aromatic ring. However, their mechanisms of action are not fully elucidated (Fardet et al., 2008), but antioxidant properties of phenols works as a food preservation (to inhibit lipid oxidation), or for disease prevention (Liu and Yao, 2007).

Phenolic acids are the major phenilpropanoid components in barley and different kind of these phenolics are found in different fraction of cereals (Antoine et al., 2004). In different barley varieties, the starchy endosperm contains low level of poliphenols, whereas the outer layers of the grain (pericarp, aleurone layer, and germ) contain the highest level of poliphenols (Holtekjolen et al., 2006). Lately more and more new barley varieties are selected and one of those is hull-less barley.

In the food industry, hull-less barley (*Hordeum vulgaris* L.) is acknowledged as more valuable and more economical, compared with covered barley. Selected hull-less barley varieties are able to pass flaky barley criteria; moreover, the amount of extract substances in hull-less barley is higher by 4-5% compared to malting barley (Dabina – Bicka et al., 2010). However hull-less barley is hull free which contains most of the existing phenol components.

Malting barley and the malting process can have impact on beer instability owing to the presence of pro-oxidant and anti-oxidant activities. Polyphenols and phenolic acids present in malt are natural antioxidants, capable of delaying; retarding or preventing oxidation processes, and therefore thought to have a significant effect in malting and brewing as inhibitors of oxidative damage. About 80% of beer polyphenols originate from malt and the remaining 20% come from hop (Dvorakova et al., 2008).

The aim of the current research was to study and

compare an antioxidant activity and total phenolic content of different barley varieties and malt types as well as to evaluate possible interconnection between TPC and AOA of barley and malt samples.

Materials and Methods

Barley and malt samples

The research was carried out on hull-less barley (four lines '3528'; 'L-400'; '3475'; '3537', further in text abbreviated: A; C; D; B, respectively) and flaky barley (one line 'Klass') grains, which were harvested in Latvia in 2010. The following technology was used for malt production: washing and steeping of grains (H_2O t = 17 ± 2 °C) until moisture content is reached 38 – 40%. Afterwards the grains were placed for germination from four to six days at a temperature of 19 ± 1° C. The kilning of the germinated grains was completed in eight hours in a laboratory kiln. Grains in a thin layer were spread on sieves in a chamber-type drier with hot air circulation at a temperature of 50 °C to 80 °C till in the grains a constant moisture content was achieved (5 ± 1%).

In this study experimentally produced malt from hull-less barley was compared to commercial sorts of malt. Four kinds of malt, which are produced in "Viking Malt" (Lithuania) – Pilsner, and "Slodownia Strzegom" (Poland) - Munich, Light caramel and Black. Kilning and roasting temperatures were the following: Pilsner – 75 °C; Munich –100 °C; Caramel – 150 °C and Black – 230 °C.

Chemicals

Gallic acid, Folin-Ciocalteus phenol reagent, 2,2-diphenyl-1-picrylhydraziyl (DPPH) and (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Switzerland). All other chemicals and solvents (Na_2CO_3, ethanol, acetone) are used in the research were with the highest commercial grade and are obtained from BARTA a CIHLAR spol.s.r.o. (Czech Republic).

Preparation of extracts from barley and malt

Barley and malt was finely ground in the laboratory mill CIATRONIC KSW 2669. Four grams of grounded samples were extracted for 10 minutes in the ultrasound bath (ULTRASONS, SELECTA P) with 40 ml of solvent. To reach a compromise between alcoholic and acetone extractions, a 7/7/6 ethanol/acetone/water (v/v/v) mixture was tested (Bonoli et al., 2004). After centrifugation at 3000 min^{-1} for 10 min using a centrifuge MEDITRONIC BL-C, the supernatant once more was removed and the extraction was repeated. The supernatant was collected in a 50 ml volumetric flask and refilled by solvent till the marked line (Jakobsone, 2008).

Determination of total phenolic content (TPC)

The TPC of the barley and malt extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999) with some modifications. First, 0.25 ml of sample was transferred to a 25.0-ml volumetric flask containing 6 ml of H_2O, to which was subsequently added 1.25 ml of undiluted Folin-Ciocalteu reagent. After 1 min, 3.75 ml of 20% aqueous Na_2CO_3 was added, and the volume was made up to 25.0 ml with H_2O. The control sample contained all the reaction reagents except the extract. After 2 h of incubation at 25 °C, the absorbance was measured at 760 nm using a spectrophotometer JENWAY 6300. Total phenols were expressed as gallic acid equivalents (Damien Dorman et al., 2004).

Determination of DPPH radical scavenging activity

Antioxidant activity of the barley and malt extract were measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydraziyl (DPPH) radical as outlined by Yu et al., (2003). Briefly, barley or malt extracts diluted in ethanol/acetone/water (v/v/v) (1:1). The antioxidant reaction was initiated by transferring 0.5 ml of grain extract into a sample cavity containing 3.5 ml of freshly prepared $DPPH^{\cdot}$ methanol solution (0.004 g $DPPH^{\cdot}$ to 100 ml methanol). After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm using a spectrophotometer JENWAY 6300. For determination of dilution ratio the inhibition of DPPH radical was calculated as a percentage (%) using the formula:

$$\text{Percentage inhibition (\%)} = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

where: $A_{control}$ is the absorbance of the control reaction (containing all reagents except test compounds),

A_{sample} is the absorbance of the test compound.

Percentage inhibition of DPPH radical is acceptable within range of 45 – 90%.

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The Trolox calibration curve was plotted as a function of the absorbance to concentration of Trolox. The final results were expressed as micromoles of Trolox equivalents (TE) per gram of dry weight (μmol TE g^{-1} DW) (Zhao et al., 2008).

Statistical analysis

The differences in the antioxidant activity and total phenol amount of samples are presented as a mean ± standard deviation (SD). The differences between independent groups were specified by two way analysis of variance (ANOVA), and value of $P<0.05$ was regarded as statistically significant. In case of establishing statistically significant differences, homogeneous groups were determined by Tukey's multiple comparison test at the level of confidence $\alpha=0.05$.

Results and Discussion

Relatively stable organic radical DPPH has been used widely for the determination of antioxidant activity of pure antioxidant compounds of barley and malt extracts. For evaluation of antiradical activity of barley and malt, different malting barley varieties and malt types were measured and compared with their DPPH radical scavenging activities. Results are expressed as micromoles of Trolox equivalent per gram of dry weight of samples (μmol TE g^{-1} DW) and are shown in Fig. 1. All malting barley varieties exhibited strong DPPH radical scavenging activity at the test concentration. The values of DPPH

radical scavenging activity for 5 barley samples ranged from 4.57 to 5.89 µmol TE g^{-1} DW. Obtained results are lower compared to those reported by Zhao et al., 2008. The significant differences in DPPH radical scavenging activity for different barley varieties suggested that variety might have significant influences on the antioxidant activity of malting barley. According to Ragaee et al., (2006) the antioxidant activity in scavenging DPPH radical of barley was higher than other cereals, like wheat, corn and rye, and it is important for nutrition because it comprises significant part of our daily intake.

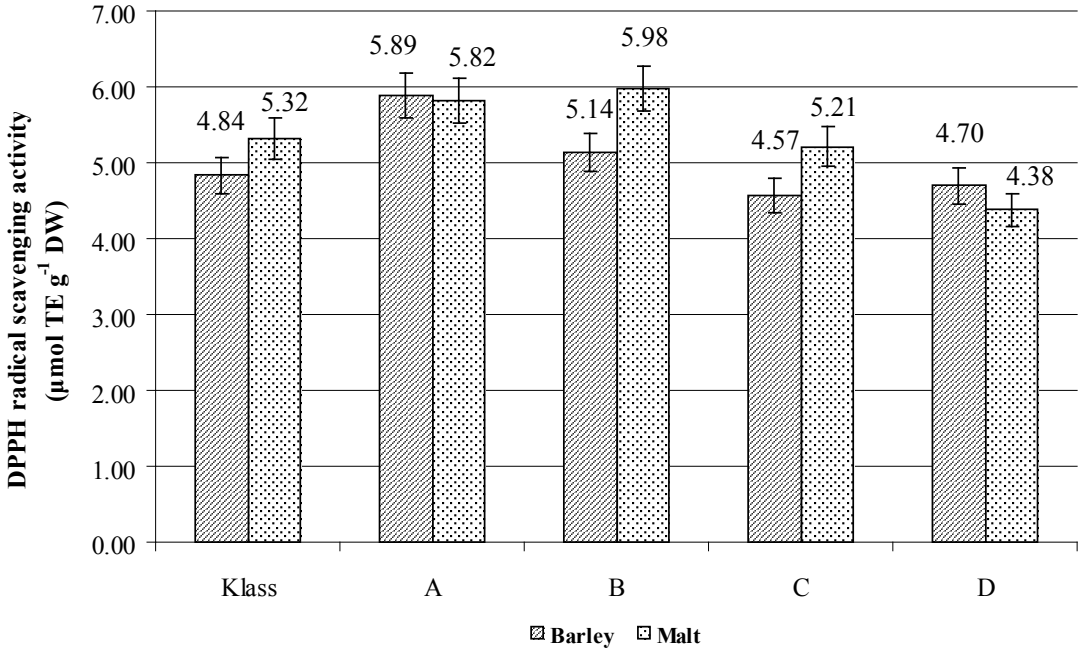

Figure 1. DPPH radical scavenging activities in barley grains and malt.

The TPC of flaky and hull-less barley varieties and their corresponding malt are presented in Table 1. A close linear correlation in the phenolic content (r=0.90) was found between the barley and malt (Palmer, 2006). Similar trend was observed on barley and their corresponding malt antiradical activity, respectively Klass – 0.91 and a line of hull-less barley B – 0.86, and C – 0.89.

Table 1

TPC of flaky barley 'Klass' and hull-less barley lines and their corresponding malt

Varieties or line	TPC, mg GAE g^{-1} DW	
	Barley	Malt
Klass	2.06	3.40
3528 (A)	2.27	3.21
3537 (B)	2.27	2.98
L-400 (C)	1.96	2.53
3475 (D)	2.43	3.29

During malting change of DPPH radical scavenging activity for different barley varieties were not disparity, with the exception of line B and C. The majority of malts have higher antioxidant activities than their corresponding barley, but no qualitative differences were found as Goupy et al., (1999) reported. The cereals with higher TPC values were not necessarily better in DPPH inhibition. According to Dordevic et al., (2010), ferulic acid, the main phenolic acid in cereal grain, showed a weak antiradical effect in experiments with the DPPH radical, which may explain the discrepancies. In addition, although the Folin-Ciocalteu method is widely used to determine total phenolic contents in botanical and biological samples, it has its own limitations.

As shown in the research of Dvorakova et al. (2008), the highest antioxidant activities were detected for the hull-less barley lin, than those for hulled varieties. But obtained results did not match with the given statement. DPPH scavenging activity of hull-less barley lines A and B are higher than flaky barley 'Klass', but hull-les barley lines of C and D are lower than varieties of flaky barley.

Four kinds of commercial malt, which are produced in different kilning and roasting temperatures, were analyzed of DPPH radical scavenging activity and total phenolic content.

The influence of kilning temperature on antioxidant activity is shown in Fig. 2 and on total phenolic content – Fig. 3. The increase of antioxidant activity could come from development of such non-enzymatic browning products as Maillard products; which can also act as antioxidants, particularly melanoidins (Goupy et al., 1999). Moreover, kilning leads to more friable tissues and probably allows better extraction of phenolic acids, mostly present in the outer layers of the grain (Dvorakova et al., 2008). This result highlights the hypothesis that after kilning there is possible better extraction of flavonoids and phenolic acids. .

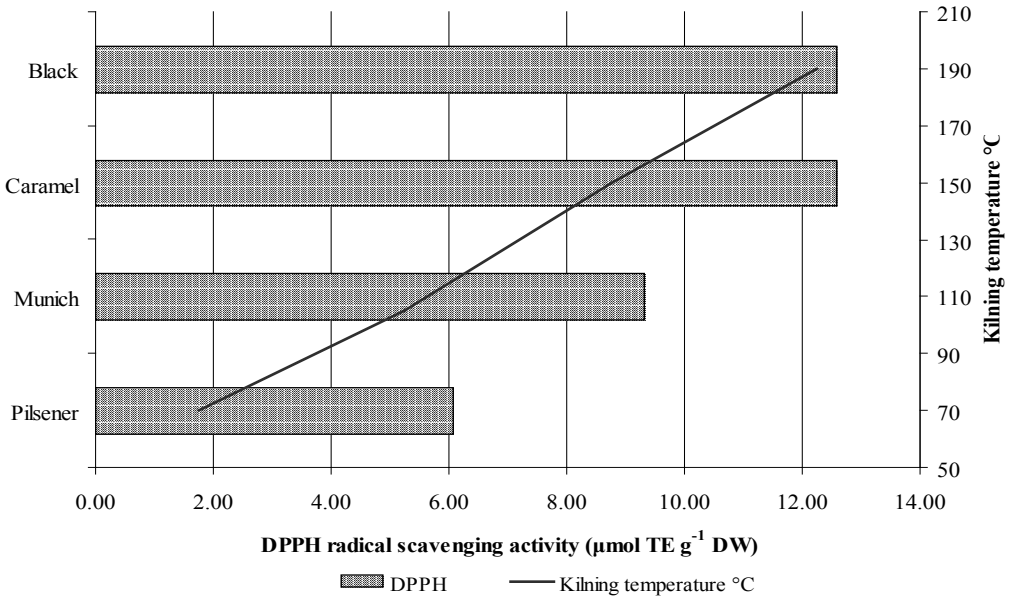

Figure 2. The influence of kilning on DPPH radical scavenging activity of commercial malts.

Stronger scavenging effects on the DPPH radical were found for Caramel and Black malt 12.58 µmol TE g^{-1} DW. The DPPH radical scavenging effect observed in this work is in agreement with literature data reported by Randhir et al., (2007), where a substantial increase in antioxidant activity due to the thermal processing was observed in all samples. Results showed that higher malt kilning temperature resulted in higher antiradical activity, respectively, (Fig.2.) with increase of kilning temperature per 80 – 150 °C, antiradical activity increased for 50% (from 6.06 till 12.58 58 µmol TE g^{-1} DW). Studies indicate that the reactions mechanism of the antioxidant and DPPH depends on structural conformation of the antioxidants, hence perhaps thermal processing alters the phenolic structure resulting in improved antioxidant function. Other factors for improved antioxidant activity could be due to the additive and synergistic effects between other phytochemicals and thermally altered phenolics (Randhir et al., (2007).

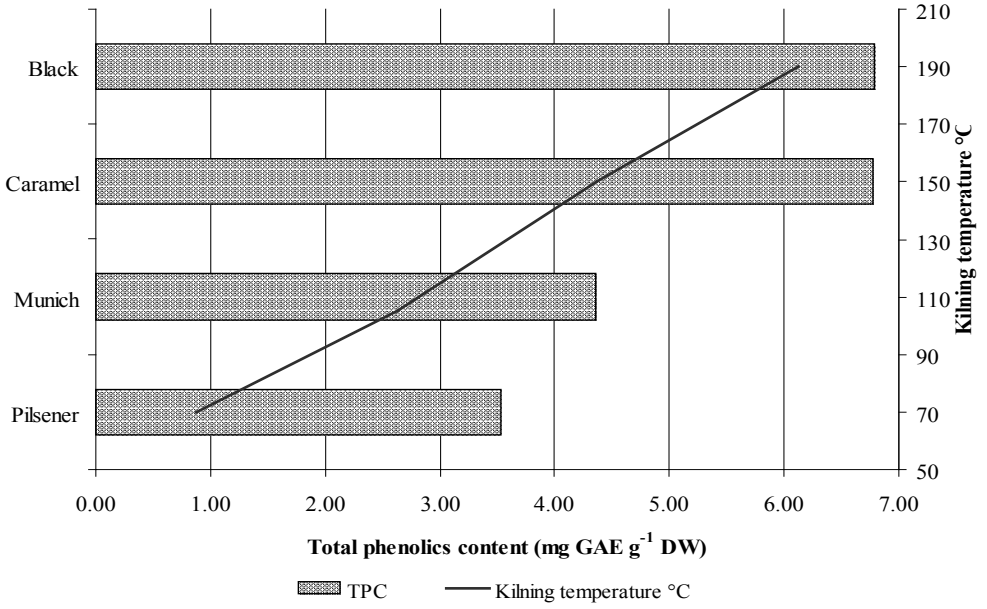

Figure 3. The influence of kilning on Total phenolic content of commercial sort of malts.

Polyphenols are known to have antioxidant activity and thus, possible health benefits, and barley is therefore claimed to be good sources of natural antioxidants (Holtekjolen et al., 2006) The current research showed that the malt kilned at the lower kilning temperature contains lower content of TPC comparing to those that have been kilned at the higher kilning temperature. There we can assume that TPC has an increase by increasing kilning temperature (Fig. 3). Positive correlation between kilning temperature and TPC of malt was observed. The values of TPC for four evaluated, commercial malt samples ranged from 3.53 to 6.78 mg GAE g^{-1} DW.

Dvarokova et al., (2008) reported TPC of barley ranged from 0.7 to 1.5 mg GAE g^{-1} DW, but their corresponding malt from 1.6 to 2.7 mg GAE g^{-1} DW. Differences could be explained by used variety, kilning temperature, phenolic compounds extraction method and solvent.

The nature of the phenolic material in wort is influenced strongly by how strongly the malt has been kilned (Briggs et al., 2004). Barley malt contributes phenolic and polyphenolic compounds to the early stages of the brewing process. The malt phenolics, upon processing, polymerize to give rise to beer polyphenolics that furnish colour, impart astringent taste, as well as a browning substrate, and participate in precipitation of poorly coagulable beer proteins (Shahidi and Naczk, 1995). Higher levels of antioxidant substances in the beer retard deterioration processes. On the other hand, proanthocyanidins from barley malt influence the development of haze in beer, because 80% of these phenolics present in regular beer are derived from barley malt (Shahidi and Naczk, 1995).

Conclusions

Even if the amount of phytochemicals is higher in the hulled barley compared to the hull-less varieties of barley, the industry should consider the hull-less varieties. The necessarily of pearling of the hulled varieties will decrease the amounts of antioxidants in these genotypes considerably compared to the hull-less samples. Furthermore, the variations observed in the amount of phytochemicals within the different types of barley should provide an advantage for breeders to produce barley varieties of high antioxidant levels for food uses.

The analysis of five different varieties of barley did not allow a clear correlation between the level of phenolic compounds and the antioxidant activity to be demonstrated because the differences between the samples were not significant. However, in the case of commercial type of malt, a great increase of total phenolics also led to an importance increase of antiradical activity. Thus, even if phenolic compounds are not the only components responsible for the antioxidant activity of barley and malt, they could play a major role.

Studies addressing the impact of thermal processing on total phenolics and antioxidant activity in foods are becoming more important due to its role in human health and disease management. It is essential to understand how to optimize levels of beneficial phenolics linked to health-relevant functionality in commercially processed grains. In general, thermal processing improved the total phenolic content and antioxidant activity in barley. Therefore, the antioxidant activity of cereal products is far from negligible.

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