Effect of Maillard Reaction on Reducing Power of Malts and Beers

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Abstract

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HPLC with amperometric detection was used to evaluate the reducing power of 23 beers and aqueous extracts of 17 barley malts. While brew pale malts were only slightly higher in electrochemical reducing capacity than natural barleys (about 1.3 g BHAE/kg), caramel malts with the colour of 60–450°EBC showed 7.5–17.2 g BHAE/kg. The superior reducing power of the darker caramel malts was partly due to the presence of a Maillard-derived 2,3-dihydro-3,5-dihydroxy-6-methyl-(4*H*)-pyran-4-one (DDMP), which was responsible for 21–55% of their electrochemical capacity. Among common brew malts, only the Munich type showed a significantly increased electrochemical capacity – 6.8 ± 0.8 g BHAE/kg. In addition to the malts, the brewing can also affect the electrochemical capacity of beers, which ranged from 0.4 ± 0.1 to 1.9 ± 0.3 g BHAE/l. Simple indigenous malt-derived phenolics were responsible for 48–57% of capacity in pale lagers and for 33–45% of it in dark and other specialty lagers. DDMP was not detected in most pale lagers, while it was responsible for up to 11% of the electrochemical capacity in dark and special beers. High-molecular-weight fraction (> 1kDa) of beers comprised 19–39% (pale lagers) and 14–21% (dark and special beers) of the capacity. The reducing power of malts and beers determined by the amperometric method was confirmed by a good correlation with the results of DPPH$^{\bullet}$ scavenging assay.

Keywords: malting; brewing; electrochemical detection; electrochemical capacity; non-enzymatic browning; 2,3-dihydro-3,5-dihydroxy-6-methyl-(*4H*)-pyran-4-one

Barley (*Hordeum vulgare* L.) is the primary cereal used in the production of malt, which is used mostly for the production of beer. The dietary intake of beer is associated with a decreased risk of numerous chronic diseases. Up to date, numerous epidemiological and interventional studies have addressed the health effects of beer (Bamforth 2002). The beneficial effects of beer constituents on human health are usually related to phenolic compounds, particularly to their antioxidant activity (AOA) and bioavailability (Rivero *et al*. 2005).

Apart from the physiological role, the presence of antioxidants in beer has also technological connotations. In recent years, they had been reported to be responsible for the suppression of undesirable carbonyl compounds' formation in the course of beer staling. Thus, antioxidants are involved in maintaining physical and chemical stability of beer (Vanderhaegen *et al*. 2006).

Phenolic compounds in barley and malt – mostly flavan-3-ols, proanthocyanidin oligomers, hydroxycinnamic acid derivatives and low amounts of flavonols – are classified as free, soluble esters and glycosides, and insoluble-bound forms. There are numerous data on the antioxidant capacity (AOC) values and phenolic content of barley in literature (Rivero *et al*. 2005; Piazzon *et al*. 2010). In barley, the majority of free phenolics

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are flavan-3-ols, whereas the bound phenolics are mainly phenolic acids. The authors demonstrated that the phenolic profiles and AOC of barley varied considerably across varieties and vintage. In analysing ethyl acetate extracts using $ABTS^{\bullet+}$ method, (+)-catechin, prodelphinidin B3, and procyanidin B3 were identified as three major contributors to the AOC of barley (Leitao *et al*. 2012).

Malting, brewing, and roasting may induce changes in the AOC of natural barley. During malting, a significant decrease of bound phenolics takes place with a corresponding increase of soluble esterified fraction (Dvořáková *et al*. 2008b). This can be explained by the enzymatic release of the bound antioxidants of barley as well as glycosylation reactions during malting and non-enzymatic changes during kilning. Therefore, the levels of extractable phenolic compounds are usually reported to be higher in malt than in barley. During kilning, the level of flavan-3-ols is decreasing, whereas the content of extractable phenolic acids increases. In addition to the naturally occurring, released and transformed phenolic species, numerous process-induced Maillard reaction products (MRPs) with significant AOA can be formed.

Barley malts can be classified into pale (lager, brew) malts and dark (specialty) malts. The main ingredients for the production of beer, pale malts, are mildly heated (to 70–95°C). Dark malts can be further categorised into colour brew malts, caramel (crystal) malts and roasted malts. Colour brew malts (Munich type) are produced in a kiln which uses elevated curing temperatures (up to 105°C), while caramel malts and roasted malts are obtained by roasting green malt and pilsner malt (up to 220–250°C). For caramel malts, a stewing step is included in the process prior to roasting to allow extensive carbohydrate and protein hydrolysis inside the malt kernel.

Generally, about 80% of phenolic compounds in beer originate from malt, and the remaining 20% come from hops. Leitao *et al*. (2012) concluded that hopping did not significantly affect either the phenolic content or the AOC. The contribution of malt to the AOC of beer was estimated at about 95% in dark and about 86% in pale lagers (Racek *et al*. 2001). Therefore, malts and brewing itself are the principal sources of antioxidants in beer.

The effects of mashing and brewing on the contents of phenolic compounds as well as the AOC of wort and beer have been investigated by Woffenden *et al*. (2002) and Pascoe *et al*. (2003). The elevated temperatures during mashing may contribute to the release and solubilisation of phenolic compounds from malt. On the other hand, the overall brewing process reduces the initial content of total phenolic compounds by 50% when quantified by Folin-Ciocalteu method (Fumi *et al*. 2011), particularly due to the decrease in tannins and flavonoids contents. This finding is in accordance with the results of Zhao *et al*. (2010), who reported gallic and ferulic acids as dominant phenolic compounds accounting for > 50% of total phenolics in beers.

Regarding the changes in AOC during the process from wort to the final beer, Gorjanovic *et al*. (2010) reported a 37.6% decrease of AOC. On the other hand, Leitao *et al*. (2011) documented statistically non-significant changes in AOC throughout beer processing (i.e., brewing, boiling, and fermentation), using an $ABTS^{\bullet+}$ assay. Zhao and Zhao (2012) showed 10–17% increase in AOC during mashing. The data are in agreement with the report of Pascoe *et al*. (2003), who showed that mashing together with boiling, fermentation, chill-lagering, and pasteurisation resulted in a significant increase of AOC measured by $ABTS^{\bullet+}$ and FRAP assays. A significant decrease in the AOC level was observed on milling and beer filtration (Pascoe *et al*. 2003). After the removal of 20–40% of phenolic compounds by colloidal stabilisation with a polyvinyl polypyrrolidone clarifier, the AOC of beer decreased by only 5–15% employing a $DPPH^{\bullet}$ scavenging assay. Mikyška *et al*. (2010) concluded that phenolic compounds possessing a low AOA are preferably removed from beer. However, another explanation is the major role of MRPs as antioxidants in beer.

Over the past years, the AOC of malt and beer has been investigated using numerous assays (Gorjanovic *et al*. 2010). The methods for the determination of the AOC in wort and beer have been reviewed by Karabín *et al*. (2006). The researches have focused mainly on the relationship between AOC and total phenolic content, whereas limited data are available on the phenolic profiles and their contribution to the AOC of malts and beers. Moreover, it is difficult to compare the literature data due to the lack of agreement on the appropriate method for the assessment of AOC. As a consequence, AOC and the levels and species of phenolic compounds and other constituents with AOA in malts and beers are unsatisfactory and often contradictory.

A recent study on the AOC of malts and beer has shown that the known antioxidants account for only a part of the AOC and many active compounds are still unknown. To determine the potential AOA of the active constituents, Leitao *et al*. (2011) used $ABTS^{\bullet+}$ assay after previous HPLC separation. This on-line assessment of AOC allowed complex mixtures to be separated by HPLC and the antioxidant contribution of the individual components to be evaluated. Using this method, phenolic compounds show the actual contributions to the overall AOC. Gallic acid and flavan-3-ols possess much higher AOA than ferulic acid or *p*-coumaric acid, the least active compounds. In addition to the set of the known phenolics, Leitao *et al*. (2011) found three unknown antioxidant compounds possessing 64% of the total AOC in ethyl acetate extracts of beer. Unfortunately, no detailed characteristics of these unknown compounds, including UV-Vis spectra, were introduced. They may be either components extracted during brewing or process-induced MRPs. To date, the nature of the MRPs in beer and malts and their impact on AOC has not been sufficiently described.

In our study, the potential antioxidant capacity (AOC) of a range of beers and malts produced by different procedures was evaluated using HPLC with amperometric detection (as electrochemical capacity, EC) and $DPPH^{\bullet}$ scavenging assay (as antiradical power, ARP). The main objectives were to assess the participation of the process-induced MRPs on the reducing power of malts and beers, and to make an attempt at the characterisation of MRPs possessing a significant electrochemical activity (EA).

MATERIAL AND METHODS

Chemicals and reagents. Butylated hydroxyanisole (BHA), (–)-epicatechin, protocatechuic acid and syringic acid were obtained from Sigma (St. Louis, USA). 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), 4-hydroxy-5-methyl-3-(2*H*)-furanone (norfuraneol), salicylic, caffeic, ferulic, sinapic, 4-hydroxybenzoic, and chlorogenic acids were purchased from Sigma-Aldrich (Steinheim, Germany). (+)-Catechin, (–)-epicatechin gallate, (–)-epigallocatechin gallate, vanillic and *p*-coumaric acids, and 5-hydroxymethylfuran-2-carbaldehyde (HMF) were obtained from Fluka (Buchs, Switzerland). Sodium chloride and methanol were products of Penta (Prague, Czech Republic). Gallic acid, potassium dihydrogen phosphate, and sodium hydrogen phosphate were purchased from Merck (Darmstadt, Germany). All the reagents were of analytical grade. Acetonitrile (HPLC grade) was purchased from Merck (Darmstadt, Germany). High-purity water (18 MΩ) produced in-house using a Millipore Ultrapure purification system unit (Millipore, Bedford, USA) was used for all chemical analyses.

Materials. Two typical malting barley varieties (Tolar and Malz), the corresponding brew pale Czech malts – Tolar (2–3 European Brewery Convention (EBC) colour units) and Malz (2–3°EBC), caramel malts Karapils (5°EBC), Caramel I (105°EBC) and Caramel II (212°EBC), and coloured malts I (1350°EBC) and II (1550°EBC) were produced by the malting plant Litovel (Sladovny Soufflet ČR, Prostějov, Czech Republic). The other samples of malts were supplied by Mich. Weyermann GmbH & Co. KG (Bamberg, Germany), viz. brew Munich malt (20–25°EBC), crystal (caramel) malts Carapils (3–5°EBC), Carahell (20–30 °EBC), Carared (40–50°EBC), Caraamber (60–80°EBC), CaraMunich (110–130°EBC) and Caraaroma (350–450°EBC), chocolate roasted malt Carafa I (800–1000°EBC), special Melanoidin malt (60–80°EBC) and special Smoked malt (4–8°EBC).

The sets of three bottles each of 23 beers of different brands from five breweries localised in the Czech Republic were supplied or purchased from local outlets. They comprised pale lagers (No. 1–7), amber (semi-dark) lagers (8–10), dark lagers (11–17), stouts (top fermented specials; 18, 19), wheat beers (20, 21), a special lager with the addition of honey (22), and a beer aperitif with herbs and honey added (23). The extract of original (hopped) wort in the analysed beers ranged from 9% to14%. In addition, unfiltered amber lager (12% of the extract) with the corresponding wort was supplied by a Prague microbrewery.

Sample treatment. The samples of malt and barley were finely ground in a common mill. The aliquots (about 10 g) were extracted with three volumes of water or methanol for 3 min each. Beers and wort were degassed by sonication for 15 minutes. The samples (extracts) were then filtered (Nylon, 0.22 mm) and, alternatively, fractionated by ultrafiltration (regenerated cellulose membrane, nominal cut-off 1000 Da; Millipore, Bedford, USA). The extracts purified in this way were ready for HPLC analysis and $DPPH^{\bullet}$ scavenging assay.

Amperometric HPLC method with preceding UV-Vis detection (HPLC-PDA-ECD). The determination of electrochemical capacity (EC) was carried out according to the method optimised by Čechovská *et al.* (2011).

DPPH• scavenging assay. Antiradical power (ARP) of 11 malt and 7 beer samples was assessed using a slightly modified method described by Čechovská *et al.* (2011).

Data processing. Reducing power of the samples as determined by the HPLC-ECD method (EC values) as well as DPPH• scavenging assay (ARP values) was expressed on a fresh weight basis as g BHAE/kg or g BHAE/l. Due to the low purity of the synthesised DDMP (Kim & Baltes 1996), norfuraneol was used for the quantitation of DDMP. Electrochemically inactive (at +0.8 V) HMF was quantified at 283 nm using the HPLC-PDA method. The data are reported as mean ± standard deviation (SD) for triplicate determinations, based on fresh weights of malts and barley used for the extractions. The comparisons of the means for EC values between different groups and sub-groups of the samples analysed were carried out by the least significance difference test at $P < 0.05$.

RESULTS AND DISCUSSION

Malts

Using reverse-phase HPLC with electrochemical detection (HPLC-ECD), aqueous and methanol extracts of 17 different malt samples were analysed for the values of electrochemical capacity (EC). Pale pilsner malts (Tolar and Malz) as well as smoked and caramel malts of low colour, which are subjected to kilning regimens similar to pale malts, were generally lower in the extractable electrochemically active compounds than the caramel malts with colour > 60°EBC (Figure 1). The aqueous extracts of two pale pilsner malts were 1.2 (Tolar) and 1.5-fold (Malz) higher in EC than the corresponding barleys (both about 1.3 g BHA equivalents (BHAE)/kg). From the brew malts, only the Munich type (20–25°EBC), used widely for dark beer production, showed a significantly higher EC (6.8 ± 0.8 g BHAE/kg). The lowest EC values were found in the aqueous extracts of two samples of the most roasted malts (Colored I and II; Figure 1); the values of 1.2 ± 0.2 and 0.9 ± 0.2 g BHAE/kg, respectively, did not exceed even the EC of barleys (1.3 g BHAE/kg). The AOC of the highly roasted malts was diminished due to the reduction of antioxidants from barley as well as soluble MRPs with AOA. Just the Colored malts were the only malts whose methanol extracts were higher in EC than the aqueous ones – by a factor of 1.3 and 1.7, respectively. The other malt samples consisted of more polar extractable EA compounds since the methanol extracts amounted to only 32–64% of the aqueous ones. The highest EC values were found in the aqueous extracts of the darker (60–1000°EBC) caramel and other malts, which amounted to 7.5–17.2 g BHAE/kg, with special Melanoidin malt being at the top.

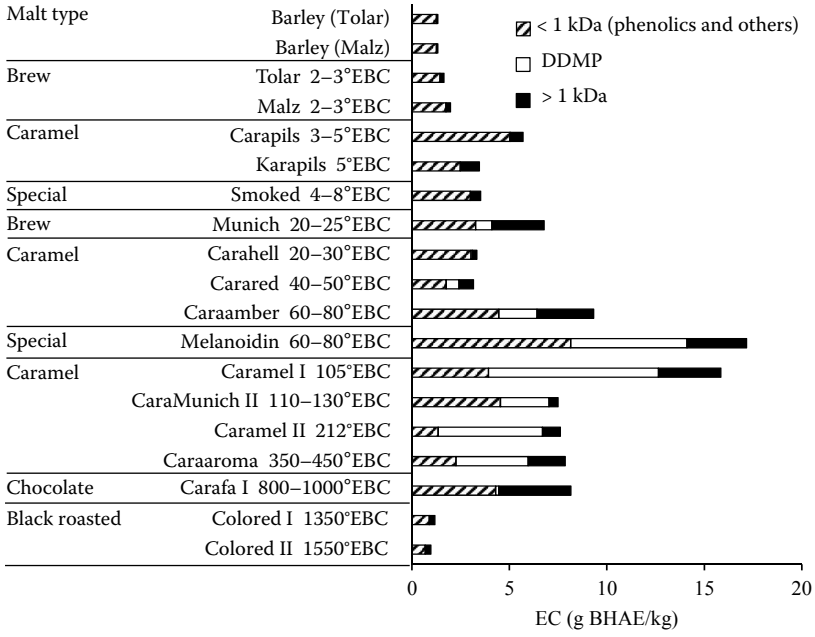

Figure 1. Contribution of DDMP, the other low-molecular active compounds and melanoidins to the overall electrochemical capacity (EC; E_a = +0.8V) of aqueous extracts from malts and barley

In comparison to most of pale malts, the darker caramel malts were also rich in soluble EA melanoidins (> 1 kDa); in chocolate Carafa malt, they were responsible for 45% of the overall EC.

We found that the superior EC of darker caramel malts was partly due to the presence of 2,3-dihydro-3,5-dihydroxy-6-methyl-(4*H*)-pyran-4-one (DDMP), which was responsible for up to 55% of EC (in Caramel I malt, 105°EBC; Figure 1). DDMP is a well-known sugar degradation product arising from 1-deoxy-D-*erythro*-hexo-2,3-diulose under neutral pH conditions. DDMP was proved in various thermally processed foods such as heated vegetables, meat, caramel sweets, and bread crust (Ledl *et al*. 1976). Recently, we reported on the pronounced role of DDMP in the reducing power of several types of prunes (Čechovská *et al*. 2011). Similar pre-conditions suitable for the formation of DDMP – heat treatment, low a_w, and the presence of reducing sugars – are applied also during the production of Munich and darker caramel malts. However, no general data on the kinetics and activation energy of the DDMP formation are available. The EA of DDMP in Caramel I, II and Melanoidin malts corresponded to 5.3–8.7 g BHAE/kg (Figure 1). On the other hand, in the roasted chocolate malt Carafa I (800–1000°EBC), only traces of DDMP were found amounting to less than 2% EC. From the common brew malts, only the Munich type of brew malt (20–25°EBC) formed at higher kilning temperatures contained significant levels of DDMP, which were responsible for 12.4% EC. The levels of both DDMP and overall EC did not follow the development of 5-hydroxymethylfuran-2-carbaldehyde (HMF), the common marker of the Maillard reaction. While the peak value for both DDMP and overall EC was observed in malts with 80–105°EBC (Melanoidin and Caramel I), HMF rose with the malt colour and reached the maximum in Caraaroma malt (350–450°EBC). Relatively low levels of HMF in comparison to DDMP were found in Munich malt.

The amperometric detection used is a method for the direct measurement of the content of all potential antioxidants (reductants) in a sample. The reducing power of a particular compound obtained by using the HPLC-ECD method is based on redox potential. It is a thermodynamic variable, and therefore the activity of even slow-acting antioxidants, not working well in real food systems, is taken into account. Thus, the EC values make only the prediction of the overall reducing power in foods. To assess adequately the AOC in malts and beers, we used a DPPH• assay, which gives a kinetic measure of the ability of antioxidants to scavenge free radicals. In a group of 11 malts consisting of Tolar pale malt and all the malts from Weyermann, the values of antiradical power (ARP) ranged from 1.3 g BHAE/kg (Tolar) to 23 g BHAE/kg (Melanoidin). This is in a good agreement with the values of the DPPH• assay for pale malt found by Zhao and Zhao (2012), which ranged from 9.0 to 13.4 mmol Trolox equivalents (TE)/kg. Nevertheless, the methods used for the assessment of AOC can affect the investigation of extractable antioxidants in malts strongly, as shown in the comparison of a series of 10 barleys and corresponding pale malts (Dvořáková *et al*. 2008a). While no significant differences were observed using DPPH• assay, the AOC values higher by factors of 1.2–1.6, 1.4–2.9 and 1.7–2.4, respectively, were found in malts using Folin-Ciocalteu, ABTS•+, and FRAP methods. Using FRAP and ABTS•+ methods, the AOC of pale malts ranged from 0.23 to 0.45 g gallic acid equivalents (GAE)/kg d.w.

Comparing the measured values of EC and ARP in the group of 11 malts, a fairly close correlation between HPLC-ECD and DPPH• assays was found (R^2 = 0.815; Figure 2). A much better correlation was observed within the reduced group consisting of paler malts (< 40°EBC; R^2 = 0.932). The significantly higher ARP as compared to the EC values in darker caramel malts may have been caused by the higher portion and a rather different character of melanoidins (Coghe *et al*. 2006). The data reflect the differences in the nature of the compounds responding to the different antioxidant assays.

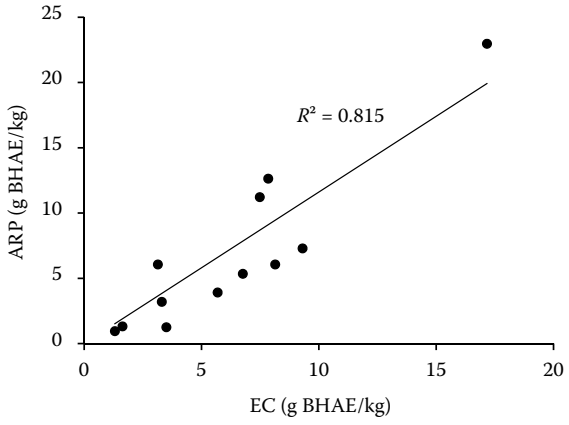

Figure 2. Correlation between electrochemical capacity (EC; HPLC with amperometric detection, E_a = +0.8V) and antiradical power (ARP; DPPH• scavenging assay) of aqueous extracts from 11 malts and a barley

Beers

To assess the reducing power of beers sold on the Czech market, a series of 23 pale, amber, and dark lagers, as well as other specialty beers including stouts and wheat beers, were analysed using the HPLC-ECD system. The total electrochemical capacity (EC) of filtered beers ranged from 0.4 ± 0.1 to 1.9 ± 0.3 g BHAE/l (Figure 3). Though the median of pale lagers (0.56 g BHAE/l) was lower by a factor 1.6–1.7 than those of amber and dark lagers, no significant statistical difference between the types of beer was found ($\alpha = 0.05$). In the pale lager group, the lowest and highest EC values differed by a factor of 3.5. Due to the broad range of BHAE values, the EC of beer cannot be referred to as a significant parameter determining the type of beer. In addition to the qualities of raw materials, the differences in the EC values could be caused by a great variability in the parameters of the brewing technology and storage conditions. Most of the recent studies, which employed various analytical methods and sets of beers, declared significant differences in AOC between diverse types of beer. Rivero *et al.* (2005), using $DMPD^{\bullet+}$ and superoxide radical assays, found the darkest beer most effective comparing the AOC of pale, dark, and alcohol-free lager beers. Using FRAP and $ABTS^{\bullet+}$ methods, Piazzon *et al.* (2010) showed that phenolic content and AOC increased in the order alcohol-free < lager < pilsner < wheat < ale < abbey < bock, so that bock beers were found to be about 3 times richer in relation to alcohol-free beers.

The amperometric determination of reductants after HPLC separation was used to evaluate the participation of particular compounds and groups in the potential reducing power of the selected beers. First, the participation of a group of 15 simple phenolic compounds (flavan-3-ols and phenolic acids) in total EC of the beer samples was assessed. These compounds, and two other EA compounds resembling UV-Vis spectra of (+)-catechin and ferulic acid, respectively, were responsible for about half EC in pale lagers (48–57%) and 33–45% in dark and other specialty lagers (Figure. 4). The distribution and contents of phenolic compounds in Czech beers were described elsewhere (Marova *et al.* 2011).

The higher participation of the known indigenous phenolic compounds in the EC of pale lagers takes place at the expense of high-molecular compounds (melanoidins, > 1 kDa), which comprise only 14–21% EC. For comparison, the melanoidins in specialty and dark beers represent 19-39% EC, using ultrafiltration as the separation step. However, comparing the patterns in beer and ultrafiltrate HPLC chromatograms, a partial decrease of low-molecular EA compounds in the ultrafiltrates was observed; this indicates the association (non-covalent binding) of the compounds with the core structure of melanoidins. The non-covalent nature of the interaction between phenolic compounds and melanoidins was demonstrated by Nunes and Coimbra (2010). Using the treatment with 1M NaCl before ultrafiltration of the beer samples, the low-molecular EA compounds retained in melanoidins by ionic interactions were released, comprising from 68% (dark lager) to > 95% of EA of high-molecular fractions separated from beer by ultrafiltration. Thus, the "pure" beer melanoidins do not seem to be the key EA components of beer.

On the other hand, beer melanoidins were proved to exhibit non-site-specific hydroxyl scavenging activity in a Fenton-type reaction system, peroxyl radical scavenging activity and reducing properties (Morales 2005). However, only little information is available on the structure of the active sites of beer melanoidins. The investigations by Somoza *et al.* (2005) revealed that one of the key antioxidants in malt-derived melanoidins (melanoproteins) is pronyl-lysine moiety, earlier identified as the most

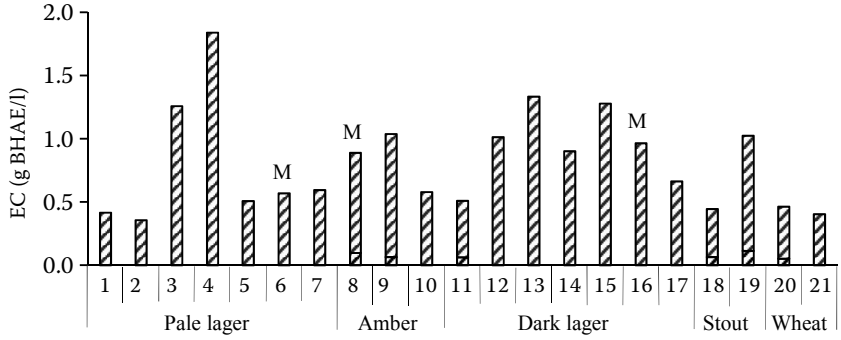

Figure 3. Electrochemical capacity (EC; E_a = +0.8V) of different types of beers analysed (M = median for a group of beers)

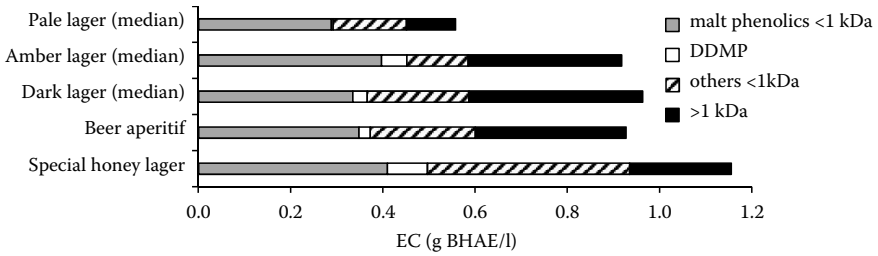

Figure 4. Contribution of DDMP and other fractions to the overall electrochemical capacity (EC) of selected beers

effective antioxidant formed in the bread crust. In addition to the melanoproteins, pure proteins in beer can also exhibit AOA. Wu *et al.* (2011) found the barley lipid transfer protein with a higher level of thiol groups maintaining the reducing power during the beer ageing process. The formation of EA melanoidins occurred also during boiling, fermentation, and chill-lagering, as documented by a lower proportion of high-molecular fraction in a wort sample (13% EC) in comparison to that of the corresponding lager (19% EC).

The rest of EC in the beer samples (14–46%) get a share of low-molecular MRPs and unidentified phenolic compounds, including prenylflavonoids from hop materials. Similarly to the malt samples, the only low-molecular MRP possessing EA identified in the beers was 2,3-dihydro-3,5-dihydroxy-6-methyl-(4*H*)-pyran-4-one (DDMP). It can come from malts, primarily Munich brew and caramel malts, though its formation during boiling cannot be excluded. On the other hand, the participation of DDMP in redox reactions during brewing is also expected. In most pale lagers, DDMP was below LOQ value; in two samples, DDMP was determined amounting up to 6% of the EC. In the group of amber and dark lagers, DDMP was found in seven samples of 10, possessing < 1.0–10.7% of the total EC value. Both analysed samples of stouts contained significant levels of DDMP comprising 6.6 and 11% of the total EC, respectively. In the special honey lager, a significant level of DDMP was also found, amounting to 7.8% of the EC (Figure 4). Despite the low addition of honey to the beer, high amounts of DDMP in heated honeys are expected and the available DDMP precursors – glucose and fructose – have to be also considered (Cejpek *et al.* 2009).

Generally, the proportion of DDMP in the total EC of beers is much lower than in the malts analysed, since the base malts for most of the beers analysed are pale brew malts, which are very low in DDMP. The other cause is the development of the Maillard reaction during mashing and boiling with the coincident involvement of DDMP in redox and other reactions. The stability of reductone-like DDMP in solutions is relatively low (Konečný *et al.* 2009); therefore a decrease of DDMP during beer staling is also expected. Thus, the level of DDMP may be substantially affected by the degree of beer ageing and redox status. Smuda and Glomb (2011) depicted the importance of the redox processes during Maillard degradation of maltose. They suggest 1-amino acid-1,4-dideoxyglucosulose as a highly potent player to undergo intermolecular redox reactions. In maltose systems, a maltose pyranone analogous to DDMP with bound 4-*O*-glucose is the other supposed reductone-like structure (Pischetsrieder & Severin 1994); however, no such structure with EA was identified in the beer and malt samples analysed.

In the subset of seven beers, representative for the different beer types, antiradical power (ARP) was measured using the DPPH• scavenging assay. The values of ARP ranged from 0.69–0.97 g BHAE/l and correlated quite closely with the EC data (R^2 = 0.79).

No correlation was observed between EC and colour intensity among a broad range of various beer types employing different malts. The high-molecular weight fraction (> 1 kDa, melanoidins) was responsible for 66–85% of the colour intensity

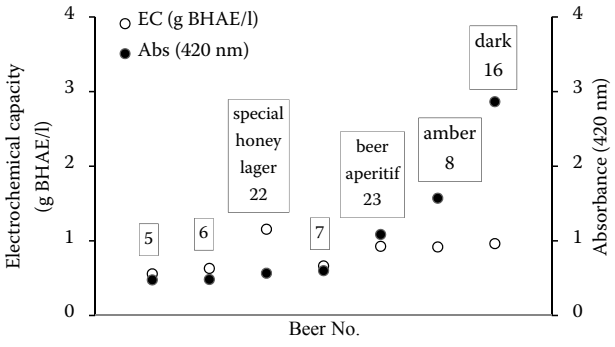

Figure 5. Melanoidin-related colour intensity (@ 420 nm) bears no relation to electrochemical capacity (EC) of beers (No. 5–7 = pale lagers; beers lined up in ascending order of colour)

of the beers. The inferior role of melanoidins in the reducing power was confirmed by the finding that melanoidin-related colour intensity bears no relation to the EC of beers. While the absorbance (420 nm) of dark beers was substantially higher than that of pale beers, the EC values did not differ significantly (Figure 5). Similarly, Morales (2005) demonstrated no correlation between the browning (absorbance at 420 nm) and efficiency for scavenging hydroxyl radicals in beers. Thus, chromophores in melanoidins of the dark and specialty beers brewed with the addition of coloured malts are certainly not superior in the reducing power (EC).

CONCLUSIONS

Despite the available reports on the phenolic composition and antioxidant capacity (AOC) of beers and malts, there is a scarcity of the published knowledge on the newly formed and/or released antioxidants in the literature. In summary, the Maillard reaction undoubtedly plays a major role in maintaining and developing the reducing power of malt and beer. The changes in the composition of reducing compounds during processing may be due to the degradation or other reactions of indigenous phenolic compounds, the generation of Maillard reaction products (MRPs), and the interaction of phenolics or their degradation products with the MRPs and other components. High-molecular MRPs, melanoidins (> 1 kDa), account for a minor part of electrochemical reducing capacity of both beers and aqueous extracts of malts. In addition to simple phenolic compounds, process-induced 2,3-dihydro-3,5-dihydroxy-6-methyl-(4*H*)-pyran-4-one (DDMP) is responsible for a substantial part of the reducing power in several types of malts and beers. Nevertheless, there is still a lack of the data on the stability of the process-induced reducing MRPs and their effects on the redox status and AOC maintaining throughout brewing and storage of beer.

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