Overall Antioxidant Properties of Malt and How They Are Influenced by the Individual Constituents of Barley and the Malting Process

Daniel O. Carvalho, Luís M. Gonçalves, and Luís F. Guido

Abstract: In the past several years researchers have focused on the study of the antioxidant properties of barley and barley malt as well as their influence on beer quality. Some malt constituents have been reported as potent antioxidants due to their radical-scavenging and reducing properties, with a positive effect on beer oxidative stability. However, barley and malt can suffer some serious modifications during malting and roasting, namely on the levels of phenolic compounds and the development of Maillard reaction products, which may have a great impact on the overall antioxidant properties of malt. Although some studies have reported an increase of the antioxidant capacity during malting, others have mentioned an opposite effect. Recently, researchers have shown that compounds developed in malt during heat treatment at high temperature and long periods of time, as result of the Maillard reaction, can also exhibit pro-oxidant properties involving the metal-catalyzed Fenton reaction due to its reductive properties. This paper reviews important information and recent data regarding the chemical changes malting and roasting undergo along with their influence on the different anti- and pro-oxidant properties described for barley and malt. The contribution of individual components to the overall antioxidant capacity of malt is also discussed.

Keywords: antioxidant, barley, malt, polyphenols, pro-oxidant

Introduction

It is impressive to learn that beer has been produced and consumed for thousands of years; there is evidence that barley was used to produce beer as long as 5500 y ago (Michel and others 1992). Beer can be defined as a fermented and flavored alcoholic beverage derived from starch, encompassing the use of barley (malt), hop and yeast, not forgetting water that is usually more than 90% of the composition of beer (Bamforth 2002; Cortacero-Ramírez and others 2003).

Malt is produced from barley grains in a process called malting and is used in beer production as a source of starch, contributing to beer’s color and organoleptic characteristics. Malt also plays an important role in the oxidative stability of beer and as a natural source of antioxidants that can limit reactions caused by reactive oxidizing species (ROS). Antioxidants from malt are able to scavenge oxygen-free radicals and prevent oxidative reactions, avoiding the addition of exogenous antioxidant compounds that could be used to maintain the oxidative stability of beer (Vanderhaegen and others 2006). Moreover, malt antioxidants may bring important benefits to a consumer’s health, preventing and neutralizing ROS known to be associated with numerous diseases (Landete 2013).

The malting process and roasting are responsible for changes in the composition of barley and malt grains, involving modifications and degradation of endogenous phenolic compounds (Goupy and others 1999; Samaras and others 2005; Inns and others 2007, 2011; Lu and others 2007; Dvoříková and others 2008b; Leitao and others 2012) and the generation of Maillard reaction products (MRPs) (Coghe and others 2003, 2004, 2006; Samaras and others 2005; Magalhães and others 2011; Carvalho and others 2014; Yahya and others 2014), with a great impact on the overall antioxidant capacity of malt. In fact, the development of MRPs during thermal processing has been associated with a pro-oxidative effect and with a negative effect on the oxidative stability of malt (Coghe and others 2006; Papetti and others 2006; Hoff and others 2012; Carvalho and others 2014) and beer (Nøddekær and Anders 2007; Furukawa Suárez and others 2011; Kunz and others 2012; Kunz and others 2013). The mechanisms behind anti/pro-oxidant effects of MRPs are still unclear since their structures are still unknown. The mechanisms are assumed to be based on their ability to trap positively charged electrophilic species, to scavenge oxygen radicals, to have reducing power, and to chelate metals to form inactive complexes (Echavarria and others 2012).

This review presents recent research and important studies concerning the influence of malting and roasting on the overall antioxidant capacity of malt. In addition, an overview of the modifications occurring in barley and malt grains during malting and their association with anti- and pro-oxidant properties is presented.
Overall antioxidant properties of malt...
Overall antioxidant properties of malt...  

Over roasted barley product

The malting process leads to a large decrease of the content of catechin, prodelphinidin B3, procyanidin B3, and ferulic acid from barley (Goupy and others 1999; Samaras and others 2005; Lu and others 2007; Dvořáková and others 2008c; Dvořáková and others 2008a; Magalhães and others 2011).

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Overall antioxidant properties of malt...

Figure 2—Structure of some of the compounds found in barley and malt. The following concentration ranges can be found in the literature (all in $\mu$g/g, except when mentioned otherwise). (A) Gallic acid, in barley 0.3 to 0.9 (Dvořáková and others 2008b), 0.2 to 0.9 (Dvořáková and others 2008a), 1.7 to 2.5 (Lu and others 2007), 1.2 to 14.2 (Zhao and others 2006), in malt 0.1 to 0.3 (Dvořáková and others 2008b), 3.4 to 6.7 (Dvořáková and others 2008a), 2.0 to 3.4 (Lu and others 2007), 8.4 to 28.6 (Magalhães and others 2011); (B) protocatechuic acid in barley 0.1 to 0.8 (Dvořáková and others 2008b), 1.6 to 1.7 (Lu and others 2007), 1.6 to 2.8 (Zhao and others 2006), 12 $\mu$mol/L (Leitao and others 2012), in malt 0.2 to 0.9 (Dvořáková and others 2008b), 1.7 to 1.9 (Lu and others 2007), 3 $\mu$mol/L (Leitao and others 2012); (C) p-hydroxybenzoic acid in barley 7 $\mu$mol/L (Leitao and others 2012), in malt 5 $\mu$mol/L (Leitao and others 2012); (D) caffeic acid in barley 0.3 to 2.1 (Dvořáková and others 2008b), 0.1 to 0.6 (Dvořáková and others 2008a), 5.5 to 7.3 (Lu and others 2007), 1.0 to 7.3 (Zhao and others 2006), 3 $\mu$mol/L (Leitao and others 2012), 0.3 to 3.9 (Madhujith and others 2006), in malt 0.3 to 1.9 (Dvořáková and others 2008b), 0.2 to 4.7 (Dvořáková and others 2008a), 6.9 to 8.9 (Lu and others 2007), 0.2 to 2.0
killing (Samaras and others 2005; Inns and others 2007, 2011; Dvoráková and others 2008a). The thermal processing steps also induce important changes in the individual phenolic content of malt. For example, high temperatures can induce the degradation of phenolic compounds (including the phenolic acids) or their polymerization (as the proanthocyanidins). In fact, hot kilning regimens were shown to be responsible for a decrease of the levels of ferulic acid (Inns and others 2011). This can also be attributed to the formation of melanoids during kilning. They are mainly present in dark malts and can trap polyphenols within its structure and decrease the content of these phenolic compounds (Maillard and Berset 1995). Also, the thermal degradation of ferulic acid esterase and related enzymes, which are responsible for the release of bound phenolics from cell walls, may promote an overall decrease of phenolic acids with increasing the kilning temperature (Inns and Ames, 2011).

Thermal treatment of malt may result in nonenzymatic brown-

ning also known as Maillard reaction (Coghe and others 2004, 2005, 2006; Yahya and others 2014). As illustrated in Figure 3, MRPs can result from the reaction of reducing sugars with amino acids and amino groups of peptides or proteins, involving a cascade of consecutive and parallel reactions, resulting in a complex mixture of compounds (Morales and others 2005; Wang and others 2011). Formation of MRPs largely depends on time and temperature applied during kilning and roasting. It has been shown that conditions of intermediate moisture content and moderate temperatures favored aqueous-phase Maillard reactions, while conditions of intermediate moisture content and moderate temperatures can induce the degradation of phenolic compounds (Magalhães and others 2011; Carvalho and others 2014; Cammerer and others 2008b; Madhujith and others 2006). It has been reported that the occurrence of sugar–sugar caramelization, involving aldolization/dehydration products of sugars, may be linked to proteins or other sources of amino nitrogen (Rizzi 1997). Cammerer and others (2002) have found that intact carbohydrate structure can be part of the MLD skeleton in model systems obtained under water-free reaction conditions, similar to roasting conditions. It seems that the Maillard reaction under water-free conditions can induce the incorporation of a relevant amount of dimer- and oligomer carbohydrates with intact glycosidic bond into the MLD skeleton with consequent side chains formation (Cammerer and others 2002). MLD with a carbohydrate-based skeleton (Figure 4A) can also be formed in water-free conditions due to aldol condensations of α-dicarbonyl compounds and from transglycosylation reactions of saccharides (Cammerer and others 2002; Wang and others 2011). Amino acids may well react with

HMW brown compounds formed in the late stages of the Maill-

lard reaction are often referred to as melanoids (MLDs). MLDs can be defined as polymeric nitrogenous compounds of HMW with high reducing potential and an intense brown color, responsible for the brown color development in roasted malts (Morales and others 2005; Echavarria and others 2012). However, it has also been reported that the occurrence of sugar–sugar caramelization, involving aldolization/dehydration products of sugars, may be linked to proteins or other sources of amino nitrogen (Rizzi 1997).

LMW colorants while roasted malts are characterized by intense brown HMW (Faist and others 2002; Magalhães and others 2011; Carvalho and others 2014). HMW compounds isolated from roasted malts have been characterized by molecular weights higher than 60 kDa (Faist and others 2002), 100 kDa (Coghe and others 2004), and 300 kDa (Magalhães and others 2011; Carvalho and others 2014). Recently, a water-soluble MLD-derived radical (10<sup>5</sup> to 10<sup>7</sup> g/mol) was detected by ESR spectroscopy in dark malt (Jehle and others 2011).

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Figure 3—Formation of HMW advanced MRPs, during the Maillard reaction, according to (Wang and others 2011; Echavarría and others 2005).
the unsaturated carbonyl structure to form MLDs with amino compounds (Figure 4B). The sudden formation of HMW MLDs also coincides with the abrupt decrease of the level of vicinal diketones and aldehydes, indicating a possible involvement of these compounds in the polymerization reactions and formation of advanced HMW MLDs (Coghe and others 2006). Additionally, phenolic compounds, ascorbic acid, and other carbonyl compounds may also take part in the Maillard reaction itself, a reason why their contents can decrease during heat treatments (Rizzi 1997).

**Antioxidant Potential of Malt**

The sensory properties of beer are altered during storage and aging, as a result of various chemical, physical, and sensory transformations which can affect beer quality (Guido and others 2003; Vanderhaegen and others 2006). Oxygen plays an important role in aging reactions causing a rapid deterioration of beer flavor (Vanderhaegen and others 2006). Oxygen reacts with ferrous iron (Fe²⁺), through the Fenton and the Haber–Weiss reactions (Figure 5), leading to the production of ROS, such as hydroxyl radicals. Formed radicals are very reactive with ethanol, the second most abundant component in beer, leading to the formation of off-flavors and consequent beer deterioration (Andersen and Skibsted 1998; Andersen and others 2000; Vanderhaegen and others 2006). Generally, the sensory profile of beer is affected by the reaction of ROS (O₂⁻, HO₂, H₂O₂, and HO) with organic molecules in beer, such as polyphenols, isohumulones and alcohols (Vanderhaegen and others 2006).

Malt antioxidants play an important role in the preservation of the oxidative stability of beer, but are also important to the consumer’s health, namely by the prevention and neutralization of ROS associated with numerous diseases: cancer, and cardiovascular and neuronal diseases (Landete 2013). According to the data presented in Table 2, antioxidant compounds identified in barley and malt are mainly polyphenols, such as catechin and ferulic acid, as well as other compounds generated during malting and roasting, such as MRPs.

The antioxidant properties of malt and beer are usually associated with phenolic compounds (Rivero and others 2005). In fact, phenolic acids have been reported as strong antioxidants due to their ability to donate hydrogen and electrons, and also due to the formation of stable radical intermediates which prevent oxidation of other compounds (Maillard and Berset 1995; Subba Rao and Muralikrishna 2002). Still, compounds with a flavonoid structure generally have shown higher antioxidant activity than nonflavonoid compounds (Zhao and others 2010), mainly determined by their hydroxyl groups (Fukumoto and Mazza 2000; Qingming and others 2010).

However, phenolic compounds in malt account for only a part of the overall antioxidant capacity (Leitao and others 2011). It was reported that the antioxidant capacity of malt can increase during kilning and roasting, not only because modification or release of phenolic compounds, but also due to the development of reducing sugars and MRPs through the Maillard reaction (Maillard and others 1996; Woffenden and others 2002; Samaras and others 2005; Vanderhaegen and others 2006; Inns and others 2011). Herein, MRPs have been identified as the major contributors to the antioxidant activity of roasted malts (Coghe and others 2003, 2006; Samaras and others 2005), with a positive influence on the maintenance and development of malt reducing properties (Cechovská and others 2012).

Antioxidant capacity and reducing power of barley extracts were found to be positively correlated with the phenolic content (Madhujith and others 2006; Zhao and others 2006, 2008; Guido and others 2007). Pearled barley fractions with higher levels of phenolic compounds, with reference to vanillic, caffeic, coumaric, ferulic, and sinapic acids, exhibited higher antioxidant capacity compared to fractions with lower phenolic contents (Madhujith and others 2006). In other works, flavan-3-ols such as (+)-catechin, prodelphinidin B3, and procyanidin B3 were identified as the most abundant in barley and the major contributors to the antioxidant capacity of barley ethyl acetate extracts (Goupy and others 1999; Leitao and others 2012).

The majority of barley phenolic compounds have also been identified in malt (Figure 2), which implies that natural antioxidants present in barley make a large contribution to the antioxidant activity of malt (Chandra and others 2001). In fact, the antiradical power has been found to be very similar for malt and barley and well correlated with the polyphenolic content, emphasizing the key role of barley endogenous polyphenols (Guido and others 2007).

Malt extracts, obtained with 80% acetone, exhibited a strong *in vitro* and *in vivo* antioxidant activity, demonstrated by their ability to scavenge hydroxyl and superoxide radicals, and also by their high reducing power and protection against biological macromolecular...
Overall antioxidant properties of malt...
Table 2—Antioxidant compared with pro-oxidant potential of barley and malt.

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proline (the most abundant free amino acid in malt) at kilning temperatures, leading to higher antioxidant activity. However, LMW compounds bound to MDL have exhibited higher antioxidant activity than pure MLD to which they are linked. Nevertheless, no correlation between color and antioxidant activity was found, except for ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay, supporting the idea that MLD chromophores are not responsible for these actions (Rufán-Henares and Morales 2007).

Some enzymes contained in barley or synthesized during germination can have antioxidant activity. Superoxide dismutase (SOD, EC 1.15.1.1) catalyzes the dismutation of superoxide radical to hydrogen peroxide which is then decomposed into H₂O and oxygen by means of catalase (CAT, EC 1.11.1.6). By their sequential action, both enzymes act to maintain oxygen in the groundstate which is much less reactive than the superoxide and hydroperoxide. These enzymes are present in barley, and their activities increase not only during germination but also survive mild kilning, being destroyed at mashing temperatures above 65 °C (Bamforth 1991). Peroxidase (POD, EC 1.11.1.7) is able to protect against oxidation by removing hydrogen peroxide. In this context it constitutes an endogenous primary antioxidant, but malt POD is also capable to oxidize endogenous barley phenolic compounds, such as ferulic acid, (+)-catechin and (+)-epicatechin (Bovin 2001). Residual enzyme activities in malt will depend on both the barley cultivar and the malting process.

Natural antioxidant compounds in malt may play a significant role in malting and brewing as inhibitors of oxidative processes. They can inhibit lipoxygenase action during malting and mashing, and decrease the autoxidation reaction during the brewing process and beer storage. Enzyme antioxidants can only act during malting and at the beginning of mashing. Phenolic compounds and MRPs, in contrast, can act throughout the process and even after beer has entered storage.

**Pro-Oxidant Potential of Malt**

Flavonoids, procyanidins, and certain MRPs can also contribute to the pro-oxidant activity of malt extracts (Ames 2001; Woffenden and others 2002). In fact, it was reported that phenolic compounds can act as antioxidants and also as pro-oxidants due to autoxidation (Yen and others 1997), thus leading to the formation of ROS in the presence of oxygen and transition metals (Galati and O’Brien 2004). Many phenolic compounds are antioxidants at high concentrations, but act as pro-oxidant at lower levels, as shown in tea (Yen and others 1997) and berry extracts (Fukumoto and Mazza 2000). In accordance, van der Woude and others (2003) have described that dietary polyphenols can act as pro-oxidants and enhance ROS production depending on the concentration.

Malt kilning has been associated with pro-oxidant activity, especially when using a rapid kilning regimen, whereas MLDS has been reported to exert both antioxidant and pro-oxidant activity (Woffenden and others 2002; Carvalho and others 2014). In fact, some studies suggest that MRPs exhibit antioxidant properties with a positive influence on the oxidative stability of wort due to its reducing properties (Coghe and others 2003, 2006), while other studies suggest a negative influence of these compounds on malt and beer stability (Nøddede and Andersen 2007). Malt roasting, radical intensity and iron content are closely linked, whereas compounds formed during the Maillard reaction can induce the formation of radicals by the Fenton reaction (Hoff and others 2012). In agreement, Nøddede and Andersen (2007) have described that roasted malts are able to induce radical formation by metal-catalyzed oxidation reactions and not by direct reaction with other antioxidants present in beer.

Recent studies demonstrated a direct correlation between the content of MRPs and higher reducing power of roasted malts, as well as higher levels of radicals measured by ESR spectroscopy. Furukawa Suárez and others (2011) showed that specialty malt leads to a decrease of endogenous anti-oxidative potential of beer, related to an increase of the reducing power and the reductone/endio structure of MRPs. However, MRPs rapidly reduce oxidized metallic ions, such as Fe³⁺ to Fe²⁺, leading to oxygen activation and intensification of the Fenton–Haber–Weiss reaction system. Consequently, the oxidative processes are accelerated and the formation of reactive radicals is increased (Kunz and others 2013). Wunderlich and others (2013) have also shown that the development of radical formation and reducing power are linked during roasting. More recently, it was also demonstrated that MLD can induce a pro-oxidant effect in a Fenton system, leading to a decrease of the oxidative stability of malt worts, due to the catalytic formation of hydroxyl radicals in the presence of ferric ions in a Fenton reaction-based system (Carvalho and others 2014).

Actually, transition metals have a significant effect on the oxidative stability of malt and beer since they can act as catalysts in radical generation and oxidation reactions. Wort samples during the early stages of the mashing process induce higher rates of spin adduct formation than wort samples collected during the later stages. The addition of Fe(II) to the wort samples increased the rate of spin adduct formation determined by ESR (Frederiksen and others 2008). However, the authors also stated that this effect did not involve iron-catalyzed formation of radicals since stout beer led to a reduction of the radicals. The decreased lag phase for radical formation and reduction of the oxidative stability of beer after the addition of MRPs can be caused by reactions that are able to induce the formation of radicals by means other than iron-catalyzed reactions. Other study suggested a mechanism of auto-oxidation of MRPs. MLD are able to quench hydroxyl radicals, but are not able to reduce Fe³⁺, proving there is no
effect in the reducing of iron in Fenton-type reactions (Morales 2005). The pro-oxidative effect of MRPs probably involves other mechanism than the Fenton catalysis, since stout beer was able to decrease the levels of radicals and the lag phase for formation of radicals in a beer model system based in a Fenton chemistry and measured by spin trapping and ESR spectroscopy, but not as much as lager beer (Nøddekær and Andersen 2007). It was also proposed that, since polymerization process can involve different groups with radical-scavenging properties, it can lead to a decrease in the overall radical-scavenging capacity due to the involvement of antiradical compounds in the formation of MLD (Coghe and others 2006).

Melanoids are also responsible for the oxidation of higher alcohols to volatile aldehydes, as reported by Hashimoto, leading to beer oxidation and deterioration. The mechanism involves the transference of electrons or hydrogen from alcohols to carbonyl groups of melanoids in conditions of high temperature and low pH (Hashimoto 1972). The melanoidin-mediated oxidation of higher alcohols, associated with the oxidation of isohumolones and unsaturated fatty acids, is responsible for the formation of volatile aldehydes with a negative impact in beer flavor and storage stability (Hashimoto 1977).

Pro-oxidant malt compounds are mainly the enzymes involved in the degradation of lipids: lipase (EC 3.1.1.3), lipoxygenase (LOX, EC 1.13.11.12), and hydroperoxide-reactive enzyme system. Oxidation of malt phenolic compounds by the catalytic action of polyphenol oxidase (PPO, EC 1.14.18.1) also occurs during the malting process. All these enzymes are found in most cereals, including barley (Gardner 1988), but they may also be synthesized by the microflora during malting.

Pro-oxidant enzymes are mainly involved in lipid degradation. Lipase is the first enzyme acting on the ester bond between the fatty acids and glycerol of triglycerides and diglycerides, releasing free fatty acids. Lipoxygenase catalyzes the oxidation of polyunsaturated free fatty acids, such as linoleic acid (C18:2), forming hydroperoxides. Lipoxygenase could also be involved in the creation of oxidative cross-linking between thiol-rich proteins via reactions, resulting in macromolecular reticulations, possibly altering filterability performance of wort and beer (Boivin 2001). The primary oxidation products of lipoxygenase activity, hydroperoxides, are decomposed to off-flavor compounds by hydroperoxide-reactive enzyme systems, namely hydroperoxide lyase and hydroperoxide isomerase (EC 4.2.1.92) (Boivin and others 1996). A study concerning the activity of lipoxygenase and lipase in malt suggest that both enzymes have a great impact in the production of linoleic and linolenic acid hydroperoxides during mashing. Both linoleic and linolenic acid hydroperoxides content are higher after mashing at 65 °C. This is associated with higher lipase activity, leading to higher levels of free linoleic and linolenic acids and consequent production of hydroperoxides by lipoxygenase (Kobayashi and others 1993).

Polyphenol oxidase is able to catalyze the oxidation of polyphenol compounds with oxygen into very reactive quinonic compounds (Figure 6). In the oxidized state, they can cross-link and polymerize with proteins or cell-wall polysaccharides, influencing directly the formation of nombiological haze in wort and beer. Polyphenol oxidase is the main responsible for the enzymatic browning in fruits and vegetables. Enzymatic or chemical oxidation of polyphenols is generally responsible for a loss in their antioxidant capacity; however, recent observations suggest that partially oxidized polyphenols can exhibit higher antioxidant activity than nonoxidized phenols (Manzocco and others 2000).

Overall antioxidant properties of malt . . .

Figure 7 illustrates some possible routes of the pro- and antioxidant enzymatic activity in the malting and brewing processes proposed by (Boivin 2001). By their sequential action, these enzymes can mostly act during the malting and mashing processes. Enzymatic activity is destroyed during the kilning and mashing steps, except for POD, which is a very heat-stable enzyme. However, POD, which can oxidize phenolic compounds, seems to have limited action in the finished product due to the extremely low hydrogen peroxide levels. Phenolic compounds and MRPs, in contrast, may play a significant role throughout the entire process and even during the storage of beer.

Influence of Extraction Solvents and Antioxidant Assays

The determination of antioxidant properties of foods and biological systems is very difficult due to their complexity involving a variety of mechanisms such as: free radical chain breaking, oxygen scavenging, singlet oxygen quenching, metal chelation, and inhibition of oxidative enzymes. Valid evaluation of antioxidant activity therefore requires the use of several different assay methods to include different mechanisms of inhibition of lipid oxidation (Frankel and Meyer 2000). Hindered phenols are the most common antioxidant compounds to readily scavenge lipid peroxy radicals by donating hydrogen atoms (Frankel and Meyer 2000). Metal chelators can be considered preventive antioxidants since metal-catalyzed initiation reactions and decomposition of lipid hydroperoxides can be inhibited due to the chelation of transition metal ions (Frankel 2005).

Particular problems arise from the use of rapid 1-dimensional methods to evaluate natural antioxidants, which are generally multifunctional. For this reason there cannot be a short-cut approach to the determination of antioxidant activity/capacity. For this reason, different methods for assessing the antioxidant activity/capacity have been published and applied in the study of antioxidants of interest in different samples.

The extraction solvent used in the extraction of antioxidants also plays an important role in the evaluation of the antioxidant activity/capacity. Some solvents are not compatible with some antioxidant assays and have different extraction selectivity (Zhao and others 2006). As demonstrated, barley and malt antioxidants are complex samples and their antioxidant activities and mechanisms depend on their composition and on the oxidative mechanism of the test system.

The different phenolic composition and antioxidant properties of malt extracts described in the literature may in part be explained by the different solvents used for the extraction. As reported, the highest extraction capacity for (+)-catechin and ferulic, caffeic, vanillic, and p-coumaric acids was achieved with 80% acetone. Epicatechin and syringic acid were better extracted with 80% methanol, while protocatechuic and gallic acids present higher solubility in water (Zhao and others 2006). As shown, 80% acetone extracts yield the highest DPPH and ABTS radical-scavenging activities and reducing power, while 80% ethanol, 80% methanol, and water extracts demonstrated the strongest hydroxyl and superoxide radical-scavenging activity, and metal chelating activity, respectively. In other works, 70% acetone extract exhibited higher antioxidant activities comparing to 70% ethanol and methanol extracts, determined suing a linoleic acid system. These results were found to be associated with higher contents of phenolics and proanthocyanidins in 70% acetone extracts (Liu and Yao 2007). Moreover, extraction using aqueous methanolic solvents does not consider the contribution of bound phenolics, but instead
encompass free and water-soluble esterified fractions which could induce differences in the total phenolic and antioxidant activities (Dvořáková and others 2008ba). In fact, the contribution of phenolics to the total phenolic content was significantly higher than that of free and esterified fractions in malt and wheat, whereas the bound fraction demonstrated a significantly higher antioxidant capacity than the free and esterified phenolics (Liyana-Pathirana and Shahidi 2006; Dvořáková and others 2008a). Thus, bound phenolic compounds should be considered in the evaluation of antioxidant activity of grains and cereals, and extraction solvents should be selected according to the hydrophobicity of compounds in the study. The influence of the enzymatic activity, in particular feruloyl esterase, on the yields of free phenolic acids during the extraction with water may also be considered.

The antioxidant capacity of malt has been investigated using different methods for evaluating the antioxidant activity/capacity. DPPH radical-scavenging activity, ABTS radical-scavenging activity, superoxide anion radical, hydroxyl radical-scavenging activity, metal-chelating activity, and ESR spectroscopy have been used to evaluate antioxidant activity of malt, barley, and beer (Table 2 and 3).

Antioxidant activity of barley extracts using different assays has shown a positive correlation between polyphenol content, reducing power, DPPH, and ABTS-scavenging activities, but all were negatively correlated with metal chelating activity (Zhao and others 2006). The authors suggest that DPPH and ABTS assays or reducing power could be used to assess antioxidant capacity of barley, but they can be affected by the solvent used in the extraction, as previously discussed. The efficacies of flavonoids (myricetin, quercetin, apigenin, chrysin, kaempferol, morin, and taxifolin) showed either antioxidant or pro-oxidant activities depending on the concentration of iron and other metal catalysts used in a linolenic acid-containing hepatocyte test system (Sugihara and others 1999).

For this reason, Frankel and Meyer (2000) have pointed out the need to use more than 1 type of method to evaluate the antioxidant activity/capacity of samples. In fact, the activity of antioxidants largely depends on system composition and localization of...
antioxidants in the different phases. Moreover, the contradictions may result from the different mechanisms involved in the methods applied. While some assays are based on hydrogen atom transfer reactions (oxygen radical absorbance capacity, inhibition of linoleic acid, and low-density lipoprotein oxidation), others are based on electron transfer (ferric-reducing antioxidant power assay, DPPH and ABTS). So, antioxidant activity determination should be done under various conditions of oxidation in order to assess different oxidation mechanisms and products of oxidation (Frankel and Meyer 2000). For example, antiradical capacity assessed by the DPPH-scavenging assay is more specific for polyphenols than reducing power, which can be affected by the Maillard reducing products formed during malting (Dvořáková and others 2008ba). Recent methods are based on ESR experiments that consist of a complete sample system providing information about the competition between anti- and pro-oxidants, contrary to the antioxidant assay based on a simple model system. The antioxidant assays are used to measure the scavenging activity using a semi-stable radical that has high reactivity toward many types of compounds (Hoff and others 2012). Accordingly, several studies based on the radical-scavenging activity involving colorimetric assays result in antioxidant activity since the pro-oxidant activity is neglected.

Conclusions
Malt is an extremely complex mixture of components with different antioxidant properties. Therefore, the overall antioxidant potential of malt results from a synergy of effects induced by different components. Antioxidant properties of barley and malt are mainly influenced by the modification of individual components of grains induced by malting and thermal treatment. In general, recent reports indicate that the overall antioxidant capacity of malt decreases during malting in consequence of an increase of phenolic compounds. On the other hand, MRPds developed during roasting are mainly reported as responsible for a decrease of the oxidative stability of roasted malt involving iron-catalyzed radical formation by the Fenton reaction, due to their high reducing properties. Accordingly, the anti- and pro-oxidant capacity of malt should encompass the exploration of antioxidant activities of isolated malt components. Moreover, overall antioxidant potential of malt should be assessed using different antioxidant assays (multidimensional methods) in order to include different oxidation mechanisms.

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Conflict of Interest
The authors declare no conflicts of interest.

References

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phenolic compounds in different barley varieties and corresponding malts. J Inst Brew 114(1):27–33.