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

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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).

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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; Dvořáková 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 Andersen 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 (Echavarría 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.

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Malting: When Barley becomes Malt

The malting process aims at the transformation of insoluble starch and proteins of barley grains into a substrate capable of dissolution and extraction by hot water. Barley is the primary cereal used in malt production, barley contains high levels of β-glucans and phenolic compounds (Sharma and Gujral 2010). The German beer purity law requires that only barley can be used (vice is a common mixture in other countries). In turn, malt is mostly used in beer production as a source of starch and contributing to its color and organoleptic characteristics. Malting is responsible for an increase of some hydrolytic enzymes, a degradation of the cell wall, proteins, and starch, and also for a reduction in the structural strength of the grain tissue (Briggs and others 1981, 1982, 2004).

The malting process is comprised of 3 stages (Figure 1): (1) steeping (beginning of germination and early growth of the embryo); (2) germination (formation of green malt); and (3) kilning or roasting of green malt (final heat treatment).

Steeping

During steeping, water is absorbed by the raw barley grains and germination begins, resulting in an increase of the moisture content from approximately 12% up to 42% to 46%, along with higher concentrations of reducing sugars and amino acids. These compounds are important precursors of thermally generated flavors during heat treatment and contribute to the development of some thermally preformed volatile compounds and their precursors.

A typical steeping process consists of an initial water stage for 6 to 16 h (under water period) with a consequent rise of the moisture content to 33% to 37%. Air is then removed trough the grain bed in order to remove moisture films in grains and carbon dioxide produced during respiration. Grains are allowed to rest for 12 to 24 h (air rest period) and embryos are exposed to oxygen. Afterwards, grains are immersed in water for more 10 to 20 h and reach the required moisture content (Briggs and others 1982).

Germination

The germination stage leads to the production of green malt, which is characterized by high moisture content and high enzyme activity. Enzymes are activated through germination and inactivated in the last stage of thermal processing (Channell and others 2010).

Enzymes are responsible for the hydrolysis of the cell walls, proteins and starch. The steeped grains are kept 4 to 6 d under humid and ventilated conditions in a controlled temperature between 14 and 20 °C by a flow of air through the bed. This can lead to some loss of moisture that is compensated by spraying water onto the green malt during the first days of germination. The germination step is controlled by regulating the growth of rootlets that are expected to grow to a length of between 1.5 and 2 times the original length of the grain. The formation of α- and β-amylase, and degradation of proteins and β-glucans are also essential to control and regulate the germination process. The breakdown of β-glucans in cell walls, mediated by β-glucan solubilase and endo-β-glucanase, is very important to achieve fast lautering and to improve filterability of beer. Since it is an enzymatic step the degradation of β-glucans to smaller water-soluble molecules, such as glucose, is favored by high moisture contents and a temperature of around 19 °C (Briggs and others 1981, 1982, 2004).

Kilning and roasting

The final step is a heat treatment of grains, also called kilning or roasting. The thermal processing steps have the greatest impact upon color and flavor of malt, depending on the time course, temperature, and moisture content. The steps aim at the reduction of the moisture content of green malt and to a condition that ensures stability during transportation and storage (approximately 5%). The removal of water prevents further growth and modification of the grains. Moreover, enzymes are inactivated and preserved. During the first phase of kilning, malt is exposed to air at 25 °C and moisture is removed from the grain, from approximately 44% to 12%. This phase is referred to as the "whitering" or "free-drying" phase. During the second phase of drying, malt is dried from 12% to 4%, and it is a much slower process, commonly referred to as the "falling rate" phase. At the end of the drying process the temperature is increased ("curing" stage). This is followed by a cooling period to ensure an optimum temperature for discharge and storage (Briggs and others 1982). The thermal processing steps (kilning or roasting) have the greatest impact upon final beer color and flavor, depending on the time course, temperature, and moisture content (Yahya and others 2014).

Specialty malts are produced not for their enzyme content but to provide extra color and flavor to beer (Table 1). They are used in relatively small quantities (usually <5%), compared to pale malts (>95%), because specialty malts results in lower levels of fermentable sugars and amino acids in wort obtained by the mashing process. During mashing milled malted barley grains are steeped in hot water to induce enzymatic hydrolysis of starch and create a malty liquid called wort, rich in fermentable sugars and amino acids. In fact, the heat treatment of malts is responsible for a nonenzymatic browning, with consumption of sugars and amino acids, contributing to malt color. Malts are not all equal and their chemical composition largely depends on the time and temperatures of the process (kilning and roasting) (Briggs and others 1982, 2004).

According to its color, a barley malt can be classified as a pale (lager) or a dark (specialty) malt. Pale malts are the main ingredients used for beer production and are mildly heated at temperatures from 70 to 95 °C. Usually, these malts are dried in conventional kilns at temperatures less than 100 °C to reach moisture contents around 4% to 5% and to ensure stability during storage and transportation. Dark malts can be classified into color brew malts, caramel malts, and roasted malts. Color brew malts are obtained using temperatures up to 105 °C, while caramel malts and roasted malts are produced by roasting green malt (germinated but not kilned) or pale malt (kilned) up to 160 °C and 220 to 250 °C, respectively.

Modifications through Malting and Roasting

Malt not only contributes to the final beer color and the organoleptic characteristics, but also to its oxidative stability, which means maintaining physical and chemical stability of beer due to its content in antioxidants (Vanderhaegen and others 2006). In fact, a correlation between oxidative stability of wort and corresponding beers was found by electron spin resonance (ESR), indicating that malt antioxidants largely contribute to the oxidative stability of beer (Cortes and others 2010). Around 80% of the phenolic compounds identified in beer is derived from malt, while the remaining 20% comes from hops (De Keukeleire 2000; Quifer-Rada and others 2015). Moreover, malt can contribute to about 95% and 86% of the antioxidant capacity of dark and pale beers, respectively (Čechovská and others 2012), while hopping did not significantly affect the phenolic content or the antioxidant activity of beer (Leitao and others 2012). Accordingly, malt can be described as the principal source of antioxidants in beer.

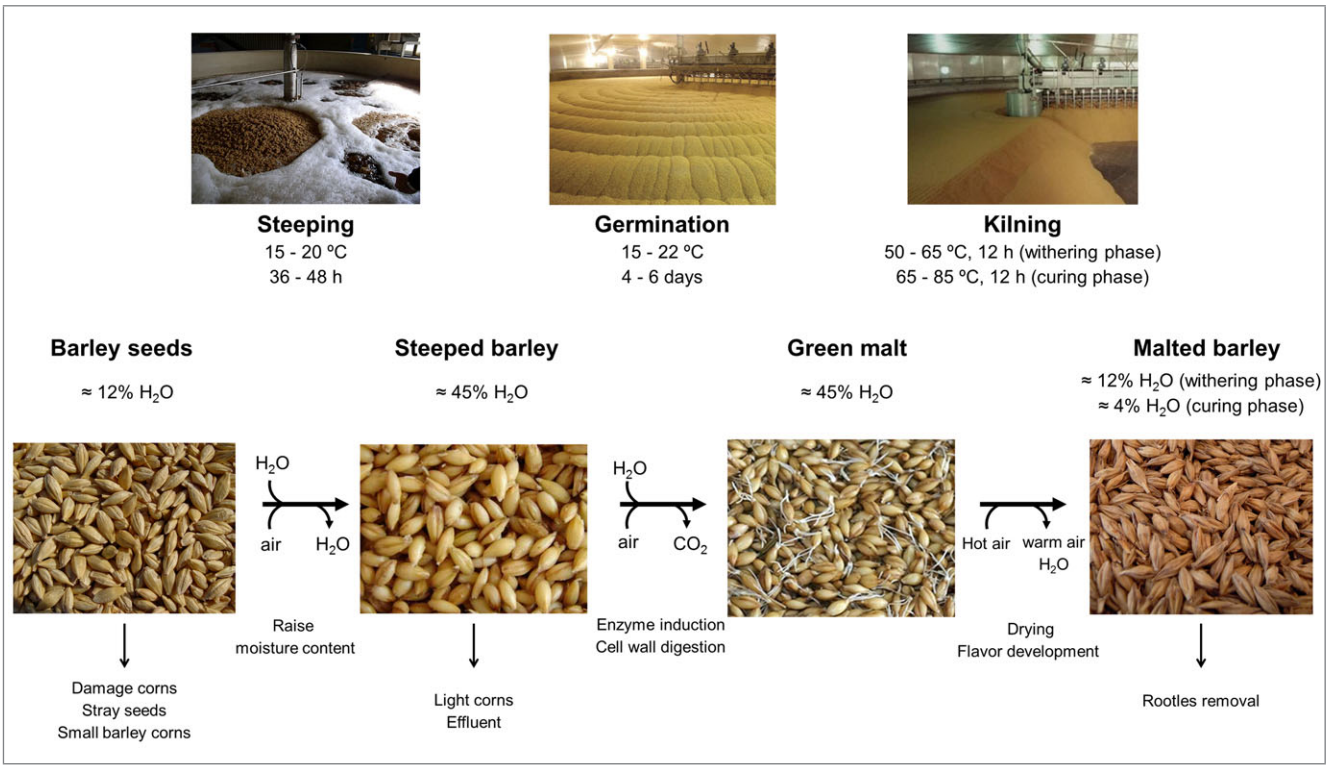

Figure 1–Malting scheme.

Table 1–Specialty malt products (adapted from Brissart and others (2000) and Guido and Moreira 2013)).

Malt type	Color range/EBC units	Moisture (%)	Flavor	
			descriptive	Chemistry
Roasted barley product	1440 to 1800	<3.5	Astringent, burnt, smoky	Maillard, heterocyclics, pyrazines
Pale malt products				
Amber malt	48 to 96	<3.5	Dry, baked, nutty, biscuit	Maillard
Chocolate malt	1200 to 1440	<3.0	Mocha, treacle, chocolate	Heterocyclics
Black malt	1440 to 1680	<3.0	Smoky, coffee	Pyrroles pyrazines
Green malt products				
Cara malt	25 to 40	<7.5	Sweet, caramel, nutty, toffee	Maillard
Crystal malt	90 to 360	~3.5	Malty, caramel, toffee	Furan pyran
Dark crystal malt	120 to 150	<3.5	Burnt coffee, caramel	
Caramel malt	260 to 320	<3.5	Burnt coffee, caramel	
Colored kilned malts				
Munich malt	10 to 15	~3.8	Grainy, malty (marked)	
Vienna malt	7 to 10	~4.5	Grainy, malty (subtle)	

However, barley and green malt can experience different chemical alterations during thermal treatment with a great impact on the composition of individual components. Kilning and roasting lead to changes in the phenolic content and enzymatic activity, and induce a nonenzymatic browning with a significant impact on the overall antioxidant properties of malt.

As summarized in Figure 2, different phenolic compounds have been identified in barley and malt, including flavan-3-ols, proanthocyanidin oligomers, hydroxycinnamic acid derivatives, and low amounts of flavonols. They can be found as free, soluble esters, and glycosides, as well as insoluble-bound forms. The bound form exhibit the main contribution to the total phenolic content compared to the free and esterified fractions (Dvořáková and others 2008a). The majority of free phenolics identified in barley and malt are flavan-3-ols, whereas the bound phenolics mainly include phenolic acids. Catechin and ferulic acid are the most abundant phenolics identified in the free and bound fractions (Holtekjolen and others 2006; Madhujith and others 2006; Lu and others 2007; Dvorakova and others 2008c; Dvořáková and others 2008a; Magalhães and others 2011).

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 2008a; Leitao and others 2012). However, ferulic acid showed a better ability to withstand the malting process and was reported as the most abundant phenolic compound in malt (Samaras and others 2005; Dvořáková and others 2008a, 2008b; Leitao and others 2012). Besides, bound phenolics decrease during malting, while the content of soluble esterified fraction is increased (Dvořáková and others 2008ba). These changes were attributed to the enzymatic release of bound phenolic compounds of barley as well as to the glycosylation reactions, leading to higher levels of free phenolic acids and their easier extractability due to changes in the matrix in the early

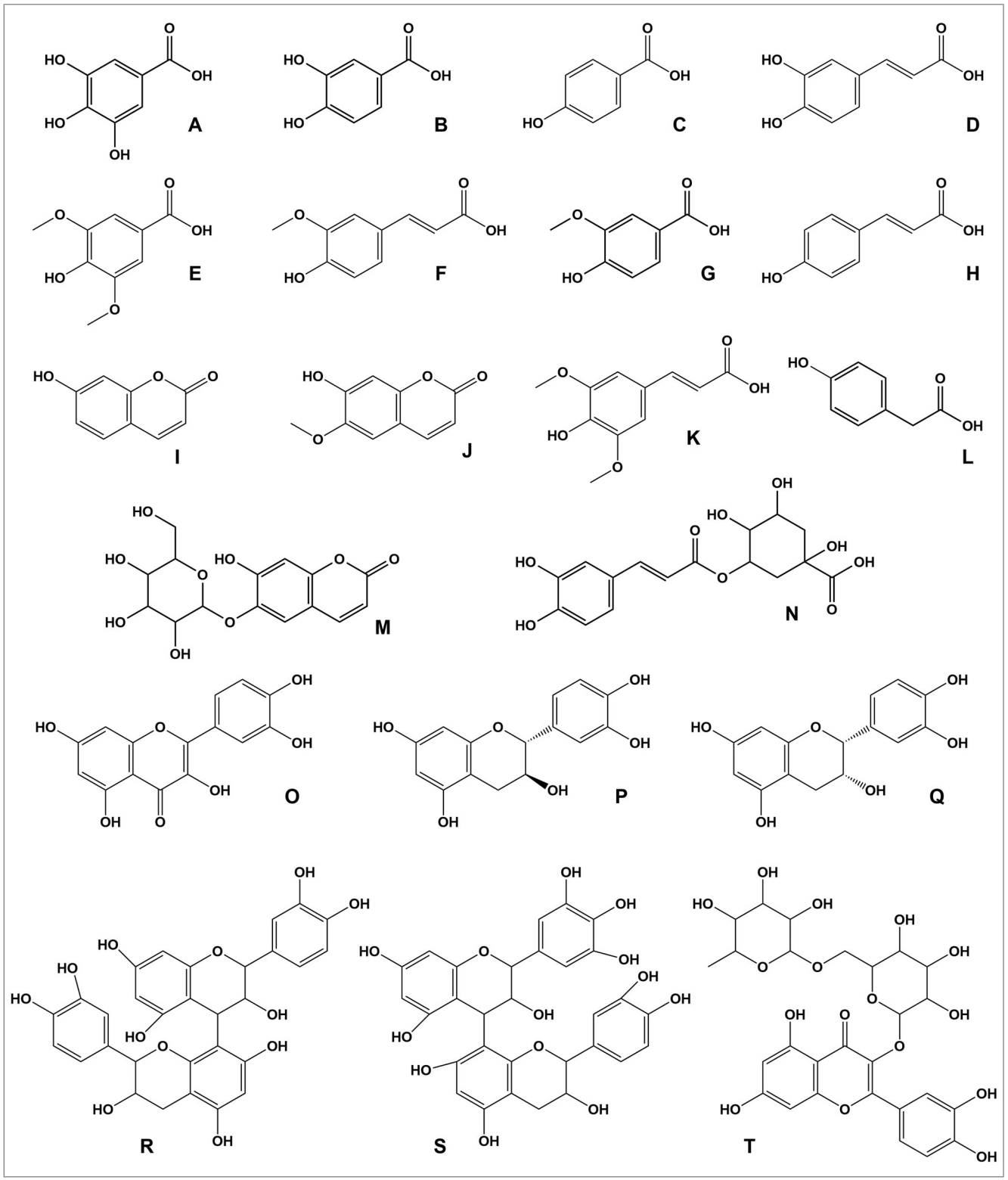

Figure 2–Structure of some of the compounds found in barley and malt. The following concentration ranges can be found in the literature (all in μg/g, except when mentioned otherwise). (A) Gallic acid, in barley 0.3 to 0.9 (Dvořáková and others 2008b), 0.2 to 0.9 (Dvořáková and others 2008a), 1.7 to 2.5 (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 μmol/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 μmol/L (Leitao and others 2012); (C) p-hydroxybenzoic acid in barley 7 μmol/L (Leitao and others 2012), in malt 5 μmol/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 μmol/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

kilning (Samaras and others 2005; Inns and others 2007, 2011; Dvořáková 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 melanoidins 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 browning 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 lower moisture contents (less than 2%) and high temperatures (200 °C) led to extensive pyrolysis reactions and generations of compounds such as maltol and methylpyrazine (Yahya and others 2014). Color development was faster when using intensive roasting comparing to mild roasting, whereas long and intensive roasting (using temperatures around 160 to 170 °C) led to a fast development of high-molecular-weight (HMW) brown-colored compounds (Coghe and others 2006). The formation of HMW compounds occurs during the final stages of the Maillard reaction by polymerization of highly reactive intermediates (Figure 2) (Cammerer and others 2002; Coghe and others 2006; Wang and others 2011). As demonstrated, malt roasting induces the polymerization of early-formed low-molecular-weight compounds (LMW) (<10 kDa) into HMW brown compounds (>300 kDa), reason why the content of LMW in roasted malts is lower than in pale malts (Coghe and others 2004; Carvalho and others 2014). Thus, pale and caramel malts are characterized by light brown 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 (10^6 to 10^8 g/mol) was detected by ESR spectroscopy in dark malt (Jehle and others 2011).

HMW brown compounds formed in the late stages of the Maillard reaction are often referred to as melanoidins (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; Echavarría 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). 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

(Magalhães and others 2011), 1 μmol/L (Leitao and others 2012); (E) syring acid in barley 0.3 to 0.9 (Dvořáková and others 2008b), 8.2 to 11.6 (Lu and others 2007), 1.23 to 12.01 (Zhao and others 2006), in malt 0.3 to 1.6 (Dvořáková and others 2008b), 7.2 to 11.8 (Lu and others 2007); (F) ferulic acid in barley 14.4 to 21.9 (Dvořáková and others 2008b), 1.6 to 216.4 (Dvořáková and others 2008a), 15.1 to 16.6 (Lu and others 2007), 2.5 to 12.1 (Zhao and others 2006), 20 μmol/L (Leitao and others 2012), 176 (Samaras and others 2005), 0.9 to 4.9 (Madhujith and others 2006), 408 to 723 (Holtekjolen and others 2006), in malt 7.3 to 56.1 (Dvořáková and others 2008b), 2.7 to 242.5 (Dvořáková and others 2008a), 16.6 to 19.9 (Lu and others 2007), 73 μmol/L (Leitao and others 2012), 123.6 to 257.8 (Samaras and others 2005), 1.21 to 167.6 (Magalhães and others 2011); (G) vanillic acid in barley 1.6 to 7.6 (Dvořáková and others 2008b), 1.0 to 3.6 (Dvořáková and others 2008a), 4.5 to 4.9 (Lu and others 2007), 1.6 to 4.5 (Zhao and others 2006), 17 μmol/L (Leitao and others 2012), 49 (Samaras and others 2005), 0.2 to 5.9 (Madhujith and others 2006), in malt 2.9 to 9.4 (Dvořáková and others 2008b), 0.4 to 0.9 (Dvořáková and others 2008a), 5.2 to 5.9 (Lu and others 2007), 10 μmol/L (Leitao and others 2012), 45.8 to 112.9 (Samaras and others 2005), 0.7 to 10.7 (Magalhães and others 2011); (H) p-coumaric acid in barley 0.1 to 2.4 (Dvořáková and others 2008b), 1.2 to 51.6 (Dvořáková and others 2008a), 1.9 to 2.0 (Lu and others 2007), 0.3 to 1.8 (Zhao and others 2006), 3 μmol/L (Leitao and others 2012), 19.9 (Samaras and others 2005), 0.2 to 1.7 (Madhujith and others 2006) 15 to 374 (Holtekjolen and others 2006), in malt 1.5 to 10.0 (Dvořáková and others 2008b), 0.9 to 74.0 (Dvořáková and others 2008a), 1.7 to 2.0 (Lu and others 2007), 30 μmol/L (Leitao and others 2012), 84.6 to 103.2 (Samaras and others 2005); (I) umbelliferone in barley 0.7 to 1.2 (Dvořáková and others 2008b), in malt 0.3 to 4.6 (Dvořáková and others 2008b); (J) scopoletin in barley 0.9 to 1.6 (Dvořáková and others 2008b), in malt 0.1 to 3.7 (Dvořáková and others 2008b); (K) sinapinic acid in barley 0.1 to 1.0 (Dvořáková and others 2008b), 0.1 to 8.6 (Dvořáková and others 2008a), 1 μmol/L (Leitao and others 2012), in malt 0.2 to 1.1 (Dvořáková and others 2008b), 0.8 to 14.8 (Dvořáková and others 2008a), 6 μmol/L (Leitao and others 2012) 0.1 to 9.8 (Magalhães and others 2011); (L) p-hydroxyphenylacetic in barley 1.1 to 5.9 (Dvořáková and others 2008b), 28.4 (Samaras and others 2005), in malt 1.9 to 9.0 (Dvořáková and others 2008b), 2.6 to 19.2 (Samaras and others 2005); (M) esculin in barley 0.1 to 3.8 (Dvořáková and others 2008b), in malt 1.2 to 6.8 (Dvořáková and others 2008b); (N) chlorogenic acid in barley 0.2 to 1.3 (Dvořáková and others 2008b), 5 μmol/L (Leitao and others 2012), in malt 0.1 to 0.4 (Dvořáková and others 2008b); (O) quercetin in barley 0.3 to 0.8 (Dvořáková and others 2008b), in malt 0.1 to 1.1 (Dvořáková and others 2008b); (P) catechin in barley 11.0 to 17.0 (Dvořáková and others 2008b), 15.0 to 21.4 (Dvořáková and others 2008a), 55.0 to 59.7 (Lu and others 2007), 20.9 to 59.1 (Zhao and others 2006), 15 μmol/L (Leitao and others 2012), 231.3 (Samaras and others 2005), 14 to 41 (Holtekjolen and others 2006), in malt 0.9 to 12.1 (Dvořáková and others 2008b), 1.1 to 5.3 (Dvořáková and others 2008a), 52.1 to 64.3 (Lu and others 2007), 350.3 to 688.4 (Samaras and others 2005), 0.8 to 21.8 (Magalhães and others 2011); (Q) epicatechin in barley 10.2 to 15.9 (Lu and others 2007), 1.8 to 15.1 (Zhao and others 2006), 24 μmol/L (Leitao and others 2012), in malt 0.1 to 0.6 (Dvořáková and others 2008b), 10.1 to 16.4 (Lu and others 2007), 15 μmol/L (Leitao and others 2012); (R) prodelphinidin B3 48 to 106 (Holtekjolen and others 2006); (S) procyanindin B3 in barley 63 to126 (Holtekjolen and others 2006); (T) rutin in barley 0.1 to 0.9 (Dvořáková and others 2008b), in malt 0.1 to 0.9 (Dvořáková and others 2008b).

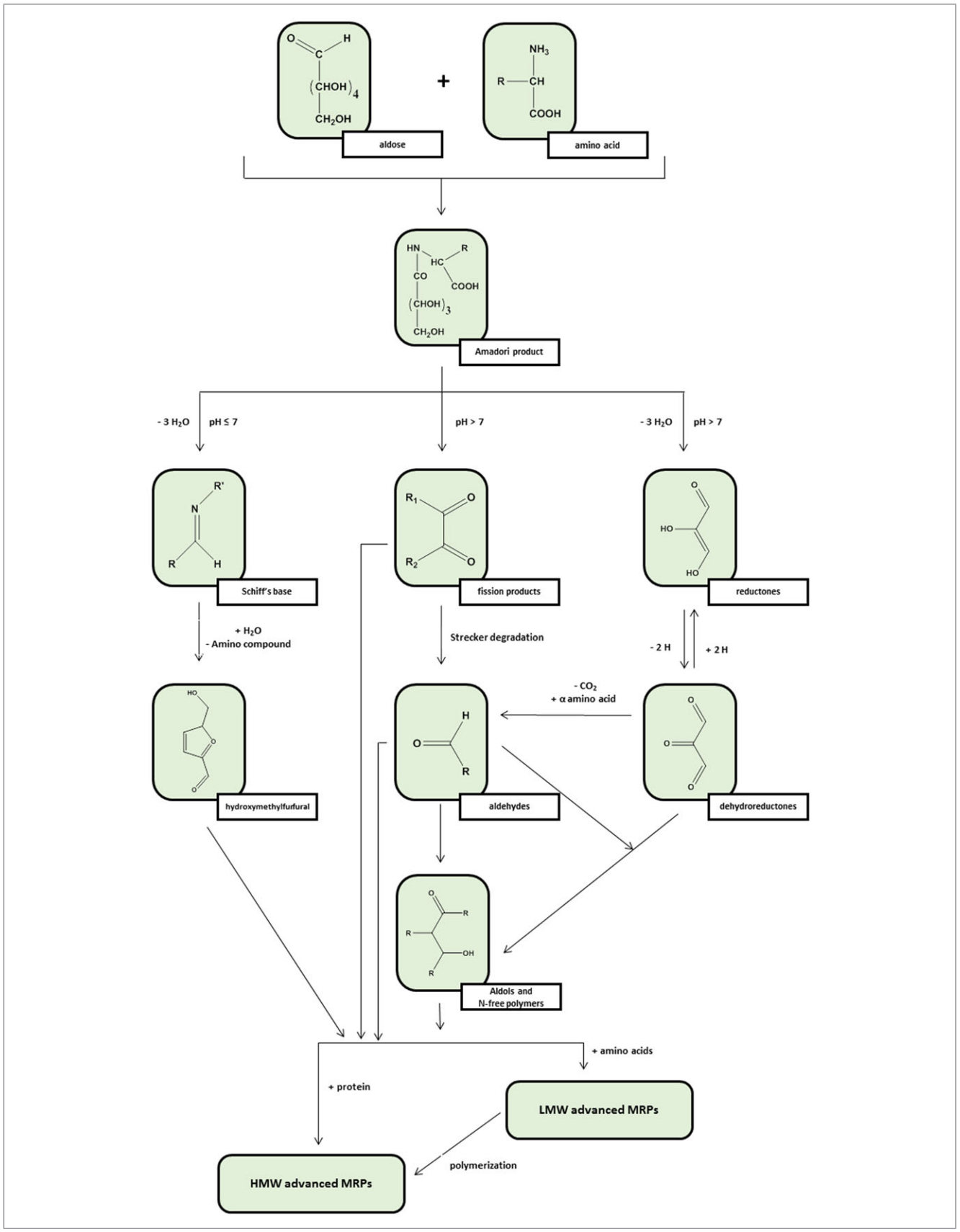

Figure 3–Formation of HMW advanced MRPs, during the Maillard reaction, according to (Wang and others 2011; Echavarría and others 2005).

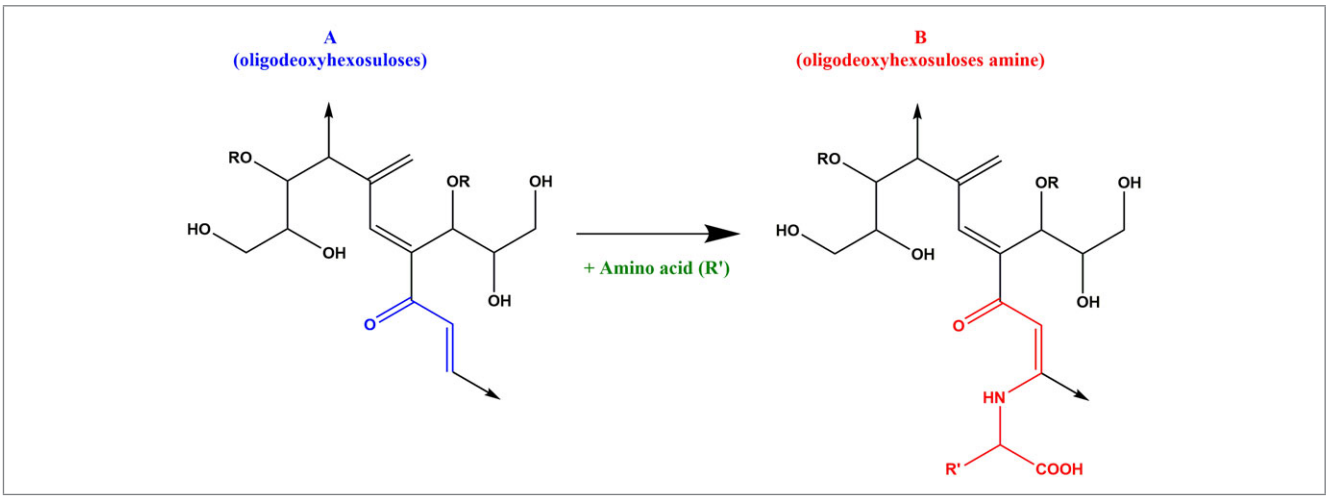

Figure 4–Proposed carbohydrate-based MLD structure adapted from Wang and others (2011).

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 (Fe^{2+}), 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 (O_2^{-}, HOO, H_2O_2, and $HO^{\bullet}$) 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 reductones 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 (Čechovská 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

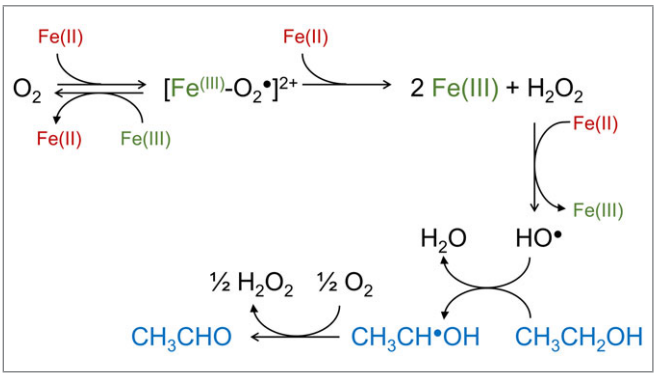

Figure 5–Proposed revised mechanism for the reduction of oxygen to water in an alcoholic beverage (adapted from Danilewicz (2013)).

oxidative damage (Qingming and others 2010). Among the polyphenols with antioxidant activity, catechin, caffeic acid, ferulic acid, and sinapic acid were reported as the principal contributors to the antioxidant properties of malt (Leitao and others 2011). So, the overall antioxidant activity of malt extracts largely depends on the phenolic content but, also on individual phenolic compounds (Qingming and others 2010; Leitao and others 2011). As demonstrated, while some phenolic compounds (such as catechin) exhibited high reducing power and metal-chelating activity, others can exhibit high radical-scavenging activity (such as ferulic acid) (Lu and others 2007).

The malting process is responsible for changes in the phenolic content of barley grains, with consequences in the overall antioxidant capacity. During germination the levels of phenolics in barley increase, as well as its antioxidant capacity (Sharma and Gujral 2010). Additionally, the enzymatic release of bound phenolics during malting also led to an increase of the total phenolic content as well as their antioxidant properties (Dvořáková and others 2008ba, 2008b). Thermal treatment steps have also a great impact on the antioxidant capacity of malt. Kilning was shown to be responsible for an increase of green malt antioxidant activity due to an increase of polyphenol levels (Chandra and others 2001). These differences were associated with higher levels of ferulic acid in heat treated malts (Inns and others 2007), which were considerably higher after kilning (from 12.5 to 21.9 and 7.8 to 56.1 μg/g dw in barley and malt, respectively). Accordingly, the total polyphenol and phenolic acids levels were positively correlated with reducing antioxidant power in beers produced from dark malts (Piazzon and others 2010). Since reducing antioxidant power strictly correlates with polyphenol and phenolic acid content, the higher levels of ferulic acid and other phenolics may be related with higher reducing capacity of dark beers produced from dark malts.

On the other hand, a decrease of the content of phenolic compounds during malting has also been reported. In fact, the production of beers using higher malt proportions resulted in lower oxidative stability and higher radical generation, which was related to lower levels of polyphenols in malt (Kunz and others 2012). This can be associated with lower levels of catechin and ferulic acid, which are responsible for a decrease of antioxidant capacity during steeping and in the early stages of germination (Lu and others 2007). Moreover, lower antioxidant activities of malt were also associated with a decrease of cathechin, prodelphinidin B3, and procyanidin B3 (Leitao and others 2012).

However, a decrease of the phenolic content during malting has been associated with an increase of the antioxidant properties from barley to malt. Lower polyphenols levels (up 49.6%) and total flavonoid content (up to 53.2%) after malt roasting were correlated with higher antioxidant properties (Sharma and Gujral 2011). For this reason, it was suggested that other compounds might contribute to the antioxidant capacity of malt. Also, a poor correlation between the antioxidant activity and the levels of catechin and ferulic acid was found in malt (Maillard and Berset 1995; Woffenden and others 2002). According to Goupy and others (1999), carotenoids (lutein and zeaxanthin) and tocopherols (α, β, and γ) are also natural antioxidants of barley and malt, whereas tocopherols were reported as good inhibitors of cooxidation of β-carotene in a linoleate model system with strong antioxidant capacity. Antioxidant capacity can increase during malting probably not only by the modification or the release of phenolic compounds, but also due to the formation of new antioxidants, such as MRPs.

MRPs have a huge impact on foods not only due their color and aroma, but also due to their health benefits and antioxidant properties. As recently presented, MRPs have exhibited important beneficial health effects, such as antiradical, antimutagenic, antimicrobial, antihypertensive, antiallergenic, antioxidant, and cytotoxic properties (Rufián-Henares and Morales 2007; Wang and others 2011; Echavarría and others 2012; Langner and Rzeski 2014; Pastoriza and Rufián-Henares 2014).

An evaluation of the antioxidant capacity of different beer samples has revealed that their antioxidant capacity, as assessed by ESR spectroscopy, significantly depended on extract contents and the color of beer (Polak and others 2013). These results suggest that colored compounds, mainly present in roasted malts, are responsible for an increase of the antioxidant capacity of malt and beer (Table 2 and 3). In fact, the higher antioxidant activity of malts was mainly attributed to the formation of MRPs upon heating, which is positively correlated with their color and MLD content (Chandra and others 2001; Coghe and others 2003).

The *in vitro* antiradical properties of water-soluble components of natural and roasted barley were determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and the linoleic acid-β-carotene system. Also, *ex vivo* antiradical properties were evaluated in rat liver hepatocyte microsomes against lipid peroxidation induced by CCl4. In this particular experiment, it was demonstrated that components in natural barley were are weak antioxidants, being capable to react against reactive peroxyl radicals but they offered little protection against stable DPPH radicals derived from peroxidation in microsomal lipids. Contrariwise, roasted barley are able to efficiently scavenge free radicals. The barley grain roasting process induces the formation of soluble MRPs with powerful antiradical activity (Papetti and others 2006). Recently, Sharma and Gujral (2011) have shown that the antioxidant activity of barley was significantly higher after roasting (16.8% to 108.2%), as well as reducing power and metal-chelating activity (by up to 77.5% and 78.9%, respectively). The differences were attributed to the development of MRPs, since heat-treated samples exhibited a higher nonenzymatic browning index (315% to 774%). In agreement, aqueous pale malt extracts exhibited a slightly higher electrochemical reducing capacity compared to raw barley, while roasted malts showed almost a 13-fold higher reducing capacity, whereas MRPs contributed 55% of the total reducing power. In comparison, malt-derived phenolics were responsible for around 50% and 40% of the reducing power of beers produced form pale and dark malt, respectively (Čechovská and others 2012).

Table 2–Antioxidant compared with pro-oxidant potential of barley and malt.

Sample	Process	Extract	Antioxidant assay	Overall antioxidant properties	Main contributors	References
Barley	Germination	80% methanol	DPPH scavenging activity	Increased antioxidant activity	Phenolic compounds	(Sharma and Gujral 2010)
	—	80% acetone	ABTS and DPPH scavenging activities Reducing power	Antioxidant activity	Phenolic compounds	(Zhao and others 2006; Zhao and others 2008)
	—	80% methanol	DPPH, superoxide and hydroxyl scavenging activity Oxygen radical absorbance capacity Inhibition of lipid peroxidation	Antioxidant activity	Phenolic compounds	(Madhujith and others 2006; Madhujith and Shahidi 2007)
Barley and malt	Malting	Ethyl acetate	ABTS scavenging activity	Decreased antioxidant activity	Phenolic compounds	(Leitao and others 2012)
		Wort	Ferric reducing power ABTS scavenging activity	Increased antioxidant activity	Phenolic compounds	(Dvořáková and others 2008b)
		70% methanol	Ferric reducing power ABTS scavenging activity	Increased antioxidant activity	Phenolic compounds	(Dvořáková and others 2008a)
		80% acetone	ABTS and DPPH scavenging activities Reducing power Metal chelating activity	Increased antioxidant activity	Phenolic compounds	(Lu and others 2007)
		Ethyl acetate	DPPH scavenging activity Linoleic acid β-carotene assay	Increased antioxidant activity	Phenolic compounds Carotenoids and tocopherols	(Goupy and others 1999)
		Ethyl acetate	ABTS scavenging activity Ferric reducing power	Increased antioxidant activity Increased antioxidant activity	Phenolic compounds MRPs	(Inns and others 2007) (Inns and others 2011)
		Acetate buffer	ABTS scavenging activity Ferric reducing power Pro-oxidant activity	Increased antioxidant activity Pro-oxidant behavior	MRPs	(Woffenden and others 2002)
		Acetate buffer	ABTS scavenging activity	Increased antioxidant activity	Phenolic compounds MRPs	(Chandra and others 2001)
		Ethyl acetate Acetate buffer	ABTS scavenging activity ORAC assay	Increased antioxidant activity	Phenolic compounds MRPs	(Samaras and others 2005)
	Roasting	Ethyl acetate Acetate buffer	ABTS scavenging activity ORAC assay	Increased antioxidant activity	MRPs	(Samaras and others 2005)
		80% methanol	DPPH scavenging activity Reducing power Metal chelating activity	Increased antioxidant activity	Possible MRPs	(Sharma and Gujral 2011)

[a] *(Continued)*

Table 2–Continued

Sample	Process	Extract	Antioxidant assay	Overall antioxidant properties	Main contributors	References
Malt	Roasting	Wort	DPPH radical scavenging activity Ferric reducing power	Increased antioxidant activity and reducing power	MRPs	(Magalhães and others 2011)
		Wort	DPPH scavenging activity Fe-dipyridyl assay	Increased reducing capacity	HMW MLD (>70 kDa)	(Coghe and others 2006)
		Wort	Reducing power assays	Increased reducing capacity	MRPs	(Coghe and others 2003)
		Wort	Radical intensity measured by ESR spectroscopy	Increased radical formation Increased lipid oxidation	Possible MRPs	(Hoff and others 2014)
		Wort	Radical intensity measured by ESR spectroscopy	Increased radical intensity	MRPs	(Hoff and others 2012)
		Wort	Ferric reducing power Metmyoglobin assay Deoxyribose assay	Increased reducing power and scavenging properties Decreased antioxidant capacity in a	HMW MLD (>300 kDa)	(Carvalho and others 2014)
		Wort	DPPH scavenging activity Linoleic acid β-carotene assay	Increased antioxidant activity Fenton system	HMW MRPs (>300 kDa)	(Papetti and others 2006)
	-	Wort	ABTS scavenging activity	Antioxidant activity	Phenolic compounds	(Leitao and others 2011)
		80% acetone	Hydroxyl and superoxide radicals scavenging activity Ferric reducing power Protection against ROS induced lipid, protein and DNA damage	High *in vitro* and in *in vivo* antioxidant activities	Phenolic compounds	(Qingming and others 2010)
		Wort	DPPH scavenging capacity Radical intensity measured by ESR spectroscopy	High reductive power and radical formation induction	MRPs	(Wunderlich and others 2013)
		Methanol	Electrochemical capacity DPPH scavenging assay	High reducing capacity	MRP	(Čechovská and others 2012)

Table 3–Antioxidant compared with pro-oxidant potential of barley and beer.

Sample	Antioxidant assays	Overall antioxidant properties	Main contributors	References
Beer	Copper induced oxidation of human LDL *in vitro*	Inhibition of LDL oxidation and reduce oxidative modification of LDL *in vivo*	MRPs	(Dittrich and others 2009)
	DPPH radical intensity measured by ESR spectroscopy	High antioxidant capacity	Phenolic compounds Colored compounds	(Polak and others 2013)
	ABTS scavenging activity Ferric reducing power N,N-dimethyl-p-phenylenediamine dihydrochloride assay	High reducing power	Phenolic compounds	(Piazzon and others 2010)
		High antioxidant activity	Phenolic compounds MLD	(Rivero and others 2005)
	Deoxyribose assay	Antioxidant activity	MLD (>10 kDa)	(Morales 2005)
	Anti-oxidative potential determined by ESR	Increase reducing power and decrease endogenous anti-oxidative potential	MRPs	(Furukawa Suárez and others 2011)
	Radical intensity measured by ESR spectroscopy	Pro-oxidant effect	MRPs	(Nøddekær and Andersen 2007)
	Radical intensity measured by ESR spectroscopy	Low oxidative stability		(Kunz and others 2012)
	Radical intensity measured by ESR spectroscopy	Strong reducing properties Hydroxyl radical generation	MRPs	(Kunz and others 2013)

Malt obtained by using the hottest kilning regimen possessed higher antioxidant activity due to higher levels of MRPs (Inns and others 2011). Also, antioxidant contribution of MRPs was higher for malts kilned using a rapid regimen (Woffenden and others 2002). Even though the development of radical-scavenging and reducing activities coincided with color formation in the early caramelization phase, higher roasting temperatures did not continuously produce MRPs with antioxidant activity (Coghe and others 2006). Herein, the existence of at least 2 types of Maillard reaction-related antioxidants in malt has been proposed: redox indicator-reducing antioxidants and radical-scavenging antioxidants (Coghe and others 2003). In fact, MRPs can contribute to the antioxidant capacity of malt due to their metal-chelating properties, reducing power, and radical-scavenging properties (Sovrano and others 2006; Wang and others 2011; Echavarría and others 2012). Accordingly, the antioxidant properties of kilned and roasted malts have been demonstrated, in several cases, as having the capacity to quench radicals or to reduce redox indicators (Woffenden and others 2002; Coghe and others 2003; Samaras and others 2005).

Coghe and others (2003) have suggested that the initial steps of the Maillard reaction led to the production of antioxidants with quenching properties, while redox-reducing antioxidants are formed during malt color development and the late stages of the Maillard reaction. Heat-induced advanced HMW MLDs have shown a 4-fold higher reducing power and a 3-fold higher antioxidant capacity, as tested by the metmyoglobin assay, compared to LMW compounds. As demonstrated, they can act as antioxidants by scavenging radical species or by having reducing properties (Carvalho and others 2014). However, during mild and intermediate roasting only reductive capacity increased while intensive roasting led to an increase of DPPH radial-scavenging capacity. During continuous roasting at high temperatures (above 157 °C) the redox indicator-reducing capacity stagnated while scavenging properties decreased (Coghe and others 2006).

The soluble HMW fraction (>10 kDa) isolated from Maillard reaction model systems and beer by ultrafiltration are able to scavenge hydroxyl radicals, but no correlation between browning and scavenging efficiency was found, meaning that chromophore residues linked to MLD are not responsible for the observed effect (Morales 2005). Moreover, Morales (2005) has shown that beer-isolated MLD displayed the same properties of model MLD obtained from the combination of sugar (glucose or lactose) with amino acids. However, another study has shown that chromophores linked to the beer MLD skeleton largely contribute to their peroxyl radical-scavenging properties (Morales and Jiménez-Pérez 2004). Malts roasted using temperatures above 150 °C exibith lower antiradical activity comparing to malts roasted at lower temperatures for longer periods (Coghe and others 2006). This indicates that structural groups responsible for the antiradical properties are involved in the advanced stages of nonenzymatic browning reactions that occur at high temperatures. So, according to Coghe and others (2006), the maximum antiradical activity appears to be more related to a specific end-temperature than to a specific malt color.

The higher reducing power observed for roasted malts could also come from the reaction between phenolic compounds with MRPs (Samaras and others 2005; Bekedam and others 2008). In coffee, the levels of caffeic accids correlated with melanoidin levels, indicating that they are incorporated in melanoidins not linked via its carboxyl group (Bekedam and others 2008) . Samaras and others (2005) have also found that ferulic acid can react with Maillard reaction intermediates, which are formed from glucose and

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 (Rufián-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 H_2O 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 (Boivin 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 MLD 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 (Nøddekær and Andersen 2007; Cortes and others 2010; Hoff and others 2012; Kunz and others 2013; Wunderlich and others 2013) (Table 2 and 3).

The temperature applied during kilning has a direct influence on the generation of stable organic radicals in malt. Dark malts kilned at high temperatures are responsible for higher concentrations of radicals in wort and beer, causing to lower oxidative stability in dark beers compared to pale beers (Cortes and others 2010). These results indicate that there is a direct link between organic radicals generated by the kilning temperature during the malting process, the content of MRPs and the oxidative stability of the resulting beer (Cortes and others 2010). The influence of MRPs on the oxidative stability of beer was also evaluated by mixing lager beer with dark beer. Dark beer induced a pro-oxidative effect, determined by ESR spectroscopy using spin trapping, reducing the oxidative stability of lager beer and resulting in shorter lag phases for radical formation and increased rates of oxidative reactions (Nøddekær and Andersen 2007). Accordingly, roasted malt was much more unstable than Pilsener malt and exhibited higher radical intensity by ESR, with a negative effect on the oxidative stability of wort and beer caused by increased radical formation (Hoff and others 2014). Dark worts were found to be less stable with high radical intensities and high iron content, contrary to light worts that were less reactive toward oxidation with low radical intensity and low iron content. Other authors also suggested that MRPs formed during malt roasting are pro-oxidant in sweet wort due to the formation of radicals by a Fenton mechanism in the presence of iron or copper cations (Hoff and others 2012). 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, Nøddekær 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 Fe^{3+} to Fe^{2+}, 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 as 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 Fe^{3+}, 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).

Melanoidins 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 melanoidins in conditions of high temperature and low pH (Hashimoto 1972). The melanoidin-mediated oxidation of higher alcohols, associated with the oxidation of isohumolones and unsatured 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 developing 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 ($C_{18:2}$), 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 nonbiological 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).

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 peroxyl 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

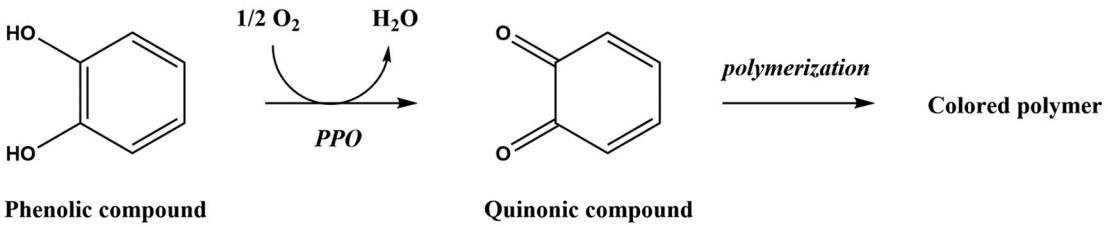

Figure 6–The action of polyphenol oxidase (PPO).

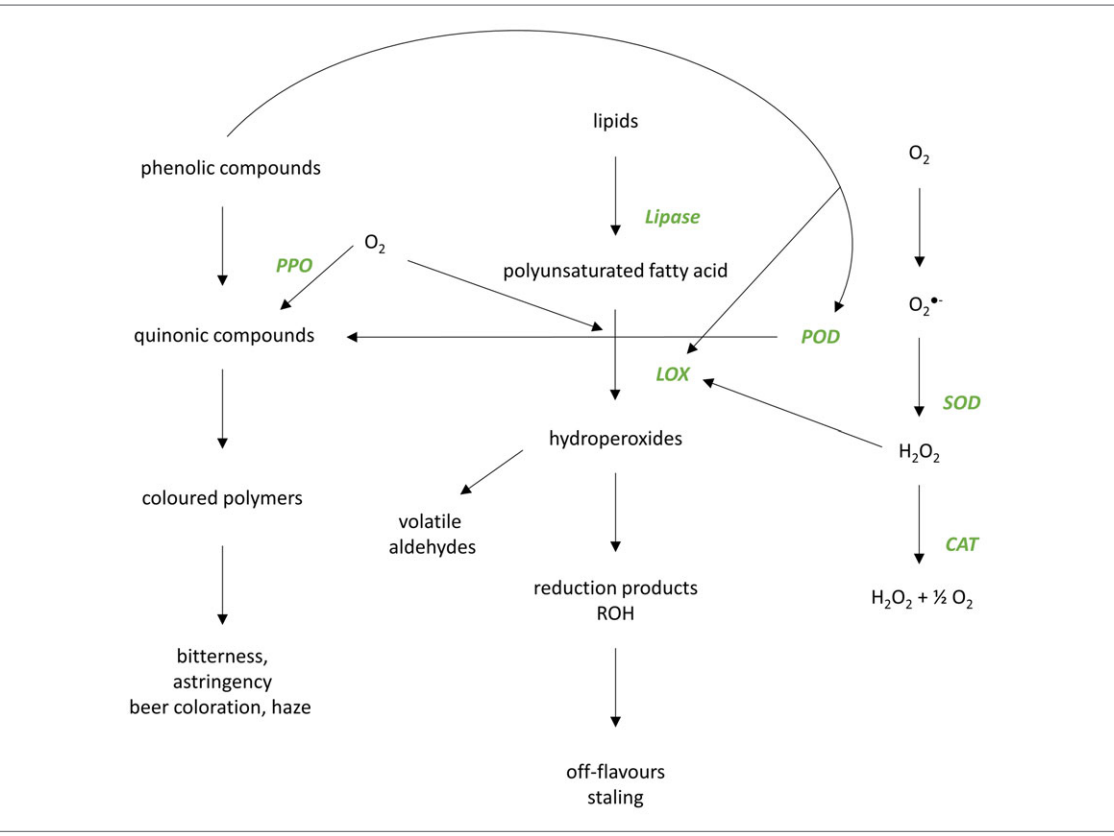

Figure 7–Implication of pro- and antioxidant enzymes in the malting and brewing process (Boivin 2001).

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 the 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 transference 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 increases during malting in consequence of an increase of phenolic compounds. On the other hand, MRPs 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.

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