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Antioxidant Properties of Kilned and Roasted Malts

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Compounds possessing antioxidant activity play a crucial role in delaying or preventing lipid oxidation in foods and beverages during processing and storage. Such reactions lead to loss of product quality, especially as a consequence of off-flavor formation. The aim of this study was to determine the antioxidant activity of kilned (standard) and roasted (speciality) malts in relation to phenolic compounds, sugars, amino acids, and color [assessed as European Brewing Convention units (°EBC) and absorbance at 420 nm]. The concentrations of sugars and amino acids decreased with the intensity of the applied heat treatment, and this was attributed to the extent of the Maillard reaction, as well as sugar caramelization, in the highly roasted malts. Proline, followed by glutamine, was the most abundant free amino/imino acid in the malt samples, except those that were highly roasted, and maltose was the most abundant sugar in all malts. Levels of total phenolic compounds decreased with heat treatment. Catechin and ferulic acid were the most abundant phenolic compounds in the majority of the malts, and amounts were highest in the kilned samples. In highly roasted malts, degradation products of ferulic acid were identified. Antioxidant activity increased with the intensity of heating, in parallel with color formation, and was significantly higher for roasted malts compared to kilned malts. In kilned malts, phenolic compounds were the main identified contributors to antioxidant activity, with Maillard reaction products also playing a role. In roasted malts, Maillard reaction products were responsible for the majority of the antioxidant activity.

KEYWORDS: Kilned malt; roasted malt; antioxidant activity; 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging activity; oxygen radical absorbance capacity; Maillard reaction; capillary electrophoresis

INTRODUCTION

The malting process can be divided into steeping (initiation of embryo growth), germination (enzymatic modification of barley endosperm), and final heat treatment (kilning or roasting) of barley (*1*). The final applied heat treatment categorizes malts into kilned (standard) and roasted (speciality or colored) malts (*1*). Kilned malts possess low color and significant enzymatic activity. In contrast, roasted malts, such as black malt, possess high color and are characterized by a lack of enzymatic activity (*1*). Roasted malts are classified into those produced from green malt (e.g., cara and crystal malts) and those prepared from dry material (e.g., roasted barley and black and chocolate malts). Roasted barley is produced by direct roasting of barley without the steeping and germination stages. Black and chocolate malts are produced by roasting of kilned malt (*1*). Malt and malt extracts are used in the manufacture of a range of foods and beverages including breakfast cereals, cookies, bakery products, and milk drinks, but the most important use of malt is as a source of yeast-fermentable sugars for the preparation of alcoholic beverages, principally beer. Kilned malts are used for lager beer production. Brewers tend to use a mixture of mainly pale malts (>95%) with a small amount of speciality malts (<5%), which provide flavor and color to the final product (*2*). It has recently been reported that the use of speciality malts results in lower levels of fermentable sugars and amino acids in wort, attributed to both the consumption of sugars and amino acids in the Maillard reaction and the ability of Maillard reaction products (MRPs) to inhibit yeast metabolism (*3*).

Lipid oxidation is responsible for changes in the quality attributes of foods such as aroma, taste, color, texture, and nutritional value. Antioxidants are compounds having the ability to prevent or delay oxidation reactions (*4*) that lead to off-flavor development during processing and storage. Additionally, compounds with antioxidant activity may have potential health benefits, such as protective action against cardiovascular disease (*5*, *6*). The antioxidant activity of kilned and roasted malts comes

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Table 1. Production Conditions for Green, Pale, Lager, and Stewed Malts

sample	raw material	steeping conditions[a]	total steeping time (h)	germination conditions	max kilning temp (°C)	total kilning time (h)
barley	NA	NA	NA	NA	NA	NA
green malt	barley	12 h W; 12 h D; 12h W; 12 h D; 12 h W	60	4 days at 18 °C	NA	NA
pale malt	barley	7 h W; 10 h D; 8 h W; 10 h D; 6 h W; 4 h D	40	24 h at 18 °C; 24 h at 16 °C; 48 h at 14 °C	85	36
lager malt	barley	14 h W; 12 h D; 14 h W; 2 h D	42	4 days at 18 °C	87	28
stewed malt[b]	green malt	NA	NA	NA	NA	NA

[a] W, water; D, dry (air-rest); NA, not applicable. [b] Stewed malt was prepared by heating green malt at 65 °C for 45 min in a closed vessel.

from naturally occurring components of barley and heat-induced antioxidants (*7*). Naturally occurring components of barley with antioxidant activity are mainly polyphenols, for example, catechin, and phenolic acids, for example, ferulic and coumaric acids (*1*, *8*). Other naturally occurring antioxidants of barley include thiols, carotenoids, and vitamins (e.g., ascorbic acid). The amounts of these compounds are variety-dependent (*9*). Carotenoids and ascorbic acid are effective antioxidants but decrease in amount during kilning or roasting, owing to the high temperatures employed. The heat-induced antioxidants include melanoidins and reductones, which are formed by the Maillard reaction during kilning or roasting (*9*). Our recent work (*10*) on a model system comprising the reaction precursors glucose, proline, and ferulic acid indicates that colorless products formed by the reaction of all three of these precursors may also contribute to the antioxidant activity of kilned malts.

Development of antioxidant activity during malting, while maintaining activity of barley saccharolytic enzymes, is a great challenge for the malting industry. Levels of endogenous compounds possessing antioxidant activity need to be conserved (e.g., polyphenols) or maximized (reductones, melanoidins) during processing to avoid the addition of exogenous artificial antioxidants to the final product. The addition of such compounds is controlled by legislation. However, another important goal of malting is to retain sufficient activity of starch-degrading enzymes, for example, β-amylase, leading to high levels of fermentable sugars for brewing. The higher the antioxidant activity of malt, the higher the antioxidant activity of the final product, for example, beer. The majority of beer antioxidants come from malt; for example, 80% of beer polyphenols are derived from malt (*11*, *12*).

The majority of previous studies have focused on the antioxidant activity of kilned malts, including the effect of selected processing conditions. It has been established that the antioxidant activity of roasted malts increases with their color [European Brewing Convention units (°EBC)] (*13*, *14*) and has been attributed to the formation of MRPs, that is, reductones and melanoidins (*15*). For example, Woffenden et al. (*14*) demonstrated that the antioxidant activity of crystal malts (color range of 15−440 °EBC) increased in parallel with color value. However, there is very limited information in the literature concerning the relationship between antioxidant activity and color development in malt as measured by absorption values at a single wavelength (420 nm) (*16*). Furthermore, limited attention has been paid to relationships between antioxidant activity, phenolic compounds, sugars, amino acids, and thermally generated compound data sets for both kilned and roasted malts (*17*).

Therefore, the objectives of this study were to determine the antioxidant activity and the color of kilned and roasted malts, to monitor changes in composition (i.e., phenolic compounds, sugars, amino acids, thermally generated compounds) of kilned and roasted malts, and to compare the antioxidant activity of malts with changes in composition and color to allow an improved understanding of the main changes that lead to gain or loss of antioxidant activity.

Table 2. Production Conditions for Roasted Malts

sample	raw material	process time (min)	max product temp (°C)
black malt	lager malt	125	228
chocolate malt	lager malt	115	220
cara malt	green malt	173	107
crystal malt	green malt	165	140
roasted barley	barley	123	229

MATERIALS AND METHODS

Chemicals and Reagents. All chemicals and reagents were of the purest grade available. Ferulic acid (4-hydroxy-3-methoxycinnamic acid), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, glacial acetic acid, sodium acetate trihydrate, sodium dihydrogen orthophosphate dehydrate, dipotassium hydrogen orthophosphate, ethyl acetate, hydrochloric acid, potassium chloride, and sodium sulfate were obtained from Sigma (Poole, U.K.). Methanol was obtained from Merck (Darmstadt, Germany). Disodium fluorescein and sodium tetraborate decahydrate were from Fluka (Gillingham, U.K.). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was from Aldrich (Gillingham, U.K.). High-purity water was produced in-house using a Purite (High Wycombe, U.K.) Labwater R050 unit and was used throughout.

Production and Characterization of Malt Samples. Barley and malt samples were provided by Brewing Research International (Redhill, U.K.) except for the pale malt sample, which was provided by Muntons Malted Ingredients (Stowmarket, U.K.). The processing conditions are shown in **Tables 1** and **2**. The moisture content of the malts was determined by the loss in mass on drying under defined conditions (*18*). The measurement of the hot water extract (HWE) of pale and lager malts used a constant-temperature infusion mashing procedure of a coarsely ground sample (0.7 mm) (*18*). The determination of the HWE of roasted malts used a mashing procedure and a mixed grist (*18*). The color was determined in °EBC units using a color comparator (*18*).

Preparation of Acetate Buffer Extracts and Ethyl Acetate Extracts. Barley/malt samples (15 g) were frozen in liquid nitrogen for 15 min and ground in a coffee grinder (Moulinex, Paris, France) for 30 s (3 × 10 s). Samples (10 g) were homogenized with acetate buffer (50 mM, pH 5.4, 40 mL) for 2 min using a pestle and mortar (kept on ice). The slurry was centrifuged (Centaur 2, MSE Scientific Instruments, Crawley, U.K.) at 0.6*g* for 10 min. The supernatant was filtered through a Whatman (Maidstone, U.K.) no. 1 filter paper, to remove any particulate material, to give acetate buffer extracts (30 mL). Ethyl acetate extracts were prepared from acetate buffer extracts by acidification (600 μL of 1 M HCl, pH 1, 0.5 g of potassium chloride) and extraction with ethyl acetate (3 × 10 mL) to give the phenolic fraction. The organic phase was centrifuged at 0.3*g* for 10 min, and the excess water was removed by drying with anhydrous sodium sulfate (2 g) for 2 h. The dried organic layer was filtered through a Whatman no. 1 filter paper, under vacuum. The ethyl acetate was removed using a rotary evaporator (water bath at <30 °C). The residue was dissolved

in methanol (2 mL) to give the ethyl acetate extracts, which were enriched phenolics fractions. Triplicate acetate buffer and ethyl acetate extracts were prepared.

UV−Visible Spectrophotometry. The absorbance of triplicate acetate buffer extracts and ethyl acetate extracts was measured at 420 nm in a 1-cm glass cuvette using a Perkin-Elmer (Beaconsfield, U.K.) Lambda 5 UV−vis scanning spectrophotometer connected to a thermostatically controlled (25 °C) chamber and an automatic sample positioner and equipped with UV Winlab software (Perkin-Elmer).

Mashing. Malt or barley (25 g) and pale malt (25 g) were milled together to produce coarse grounds. Water (360 mL, <65 °C) was added to the malt grounds, and the resultant mash was transferred to a preheated (65 °C) water bath for 2 h with continuous stirring. At the end of mashing, the sample was cooled to 20 °C and filtered to give the wort.

Free Amino Acid Analysis. Wort (500 μL) was diluted with trichloroacetic acid (2.5%, 2.5 mL) and centrifuged at 2012*g* for 5 min. Residual particulates were removed by passing the supernatant through a 0.2 μm filter. Filtrate (150 μL) was analyzed. The amino acids were separated by ion exchange chromatography, using a strong cation-exchange column (Aminex A8, 250 mm × 4.5 mm, Bio-Rad, Hemel Hempsted, U.K.) and a stepwise gradient with five lithium citrate buffers of increasing pH (pH 2.8, 68 min; pH 3.8, 65 min; pH 4.2, 80 min; pH 9.0, 30 min; pH 2.8, 40 min). The flow rate was 0.37 mL/min. Amino acids were derivatized postcolumn with ninhydrin, and separations were monitored at 440 and 570 nm. Amino acids were quantified by reference to a solution of standard amino acids, and data were expressed as milligrams per 100 g of malt (dry basis).

Sugar Analysis. Wort (50 mL, diluted 1:500 and 1:2000) was passed through a 0.2-μm filter. Each filtrate was placed into a 5-mL autosampler vial, and 40 μL was injected. Sugars were analyzed by using a Dionex ion chromatography system (DX500, Dionex, Sunnyvale, CA) composed of an autosampler (model AS-50), a gradient pump (model GP-50) with on-line degas, and a pulsed amperometric detector. The separation was accomplished on a 250 mm × 4 mm i.d. CarboPac PA10 anion-exchange column (Dionex) coupled to a 50 mm × 4 mm i.d. CarboPac PA10 guard column (Dionex). The mobile phase gradient ran from 5 mM sodium acetate in 0.1 M NaOH to 170 mM sodium acetate in 0.1 M NaOH with a flow rate of 0.8 mL/min. Sugars were quantified by reference to a solution of standard sugars, and data were expressed as milligrams per 100 g of malt (dry basis).

Analysis of Malt Polyphenols. Separation of polyphenols in ethyl acetate extracts of malts was conducted by capillary electrophoresis (CE). The CE system (Agilent, Bracknell, U.K.) was equipped with a diode array detector, an autosampler, and ChemStation software for data collection, data analysis, and system control. The fused silica capillary (Agilent) was of 48.5 cm total length (40 cm to the detector) with an internal diameter of 50 μm. Separations were performed at 25 °C and 25 kV. Samples were injected at 50 mbar for 5 s. Separations were monitored at 200, 280, 325, and 420 nm, and spectra were collected between 190 and 600 nm. The capillary was conditioned before use as follows: 30-min flush with 1 M NaOH followed by a 20-min flush with 0.1 M NaOH and 15 min with water. Finally, the capillary was flushed for 15 min with running buffer (borate, 50 mM, pH 9.5, containing 20% methanol). Two injections were carried out for each replicate sample. Between runs, the capillary was flushed with 0.1 M NaOH for 3 min and with running buffer for 3 min. The identification of phenolic compounds was based on matching of the diode array UV−visible spectra and migration times of kilned phenolic compounds with those of sample components. Spiking of malt extracts with kilned compounds was performed to confirm identifications. The identified compounds were quantified using the kilned calibration curves constructed for each compound.

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) Radical Cation ($ABTS^{\bullet+}$) Scavenging Activity. The antioxidant activity of acetate buffer extracts and ethyl acetate extracts was determined according to the $ABTS^{\bullet+}$ decolorization assay (*19*) as previously reported (*20*). A standard calibration curve was constructed by plotting percentage inhibition against concentration of ferulic acid. The antioxidant activity of the extracts was calculated in ferulic acid equivalents using the calibration curve. Triplicate determinations were performed for the standards, and each replicate sample was analyzed once.

Table 3. Moisture, Hot Water Extract (HWE), and Color (°EBC) of Malt Samples[a]

sample	moisture (%)	HWE (L °/kg)	color (°EBC)
barley	13.7	nd[b]	nd
green malt	46	nd	nd
stewed malt	38	nd	nd
pale malt[c]	4.7	nd	5
lager malt	4.8	nd	5
cara malt	7.9	292	16
crystal malt	4.6	293	180
black malt	1.2	267	1400
chocolate malt	1.1	282	830
roasted barley	0.8	264	1200

[a] Values are single determinations of single extracts. [b] Not determined. [c] Pale malt was provided by Muntons Malted Ingredients (Stowmarket, U.K.).

Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC assay developed by Ou et al. (*21*) was also used to determine the antioxidant activity of acetate buffer extracts and ethyl acetate extracts. The antioxidant capacity of the extracts was calculated in ferulic acid equivalents using the standard calibration curve. Triplicate determinations were performed for the standards, and each replicate sample was analyzed once.

Statistical Analysis. Statistical analysis was performed using Minitab 13.1 for Windows (Minitab Ltd., Coventry, U.K.). One-way analysis of variance (ANOVA) was used to determine if there were any statistically significant differences between the mean values. Tukey's test was then used to determine which mean values were different. A p value of <0.05 was considered to denote a statistically significant difference.

RESULTS AND DISCUSSION

Malt Analysis (Moisture, HWE, and °EBC). Data for malt analysis are given in **Table 3**. The moisture, HWE, and color values of the malt samples are in line with those reported in the literature (e.g., refs *1*, *22*, and *23*).

Visible Absorption Data. Malt color is normally described in terms of °EBC values that are determined by comparing the kilned malt extract to a series of standard glass disks. This is based on the visual discernment of the assessor and his/her ability to express the value as an integer. Therefore, in the current study, absorbance measurements at 420 nm for all malt acetate buffer extracts and ethyl acetate extracts were obtained, in addition to °EBC values. A wavelength of 420 nm was selected to investigate the formation of colored compounds. It is representative of the wavelengths used to monitor colored compounds formed by the Maillard reaction. The absorbance readings of acetate buffer and ethyl acetate extracts of malts are illustrated in **Figure 1**. The absorbance values at 420 nm for barley, green, stewed, pale, and lager malts are quite low. Significantly higher values were obtained for cara malt, whereas the highest values were found for crystal, black, and chocolate malts and roasted barley. Apparently, higher quantities of colored MRPs were extracted from crystal, black, and chocolate malts and roasted barley, resulting in higher absorbance values. Between 5 and 6% of color was extracted from the acetate buffer extracts into the ethyl acetate.

Free Amino Acid Analysis. The levels of amino acids in worts prepared from the malt samples are given in **Table 4**. The total level of amino acids is in line with literature reports (*24*), and there is an inverse relationship with the degree of heat treatment, that is, roasted barley, chocolate malt, black malt ≪ crystal malt < cara malt < pale malt < lager malt. This is likely

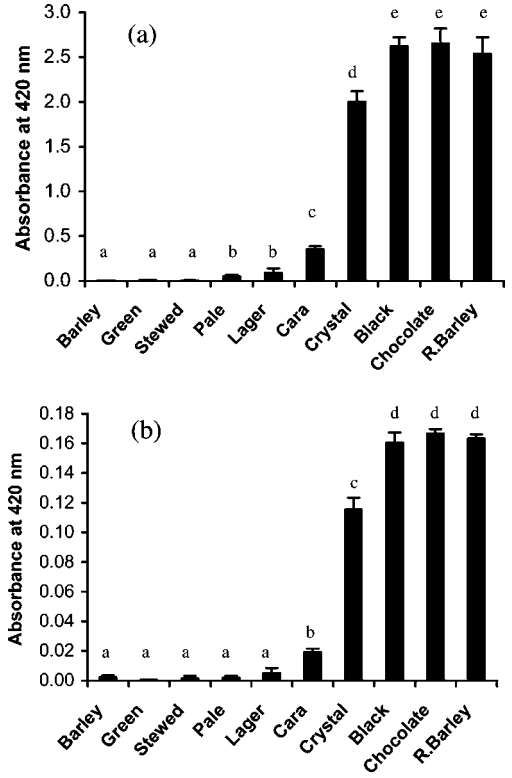

Figure 1. Absorbance at 420 nm of malt extracts. (**a**) Acetate buffer extracts. Values for crystal, black, and chocolate malts and roasted barley were obtained using samples diluted with acetate buffer (1:3) and corrected for the dilution factor. (**b**) Ethyl acetate extracts. Error bars represent the standard deviation of mean values for triplicate samples. Bars with the same letters indicate no significant difference ($p > 0.05$).

Table 4. Free Amino Acid Analysis of Worts Prepared from Malt Samples (Milligrams per 100 g of Malt)[a]

amino acid	green malt	lager malt	pale malt[b]	cara malt	crystal malt	black malt	chocolate malt	roasted barley
aspartic acid	5.35	5.25	2.10	2.13	nd	nd	nd	nd
hydroxyproline	nd[c]	nd	nd	nd	nd	nd	nd	nd
threonine	2.68	4.20	2.10	1.60	0.52	0.51	0.51	0.51
serine	4.01	4.20	2.10	1.60	0.52	nd	nd	nd
glutamic acid	4.01	6.30	3.14	1.07	1.05	nd	nd	nd
asparagine	8.03	10.49	3.14	4.26	2.10	nd	nd	nd
glutamine	14.72	15.74	6.29	2.67	0.52	0.51	0.51	0.51
proline	16.05	30.43	9.43	9.06	3.67	0.51	nd	nd
glycine	1.34	2.10	1.05	0.53	0.52	0.51	0.51	0.51
alanine	4.01	5.25	3.14	1.60	1.57	0.51	nd	nd
valine	6.69	7.35	3.14	2.67	1.57	0.51	0.51	0.51
cystine	nd	nd	nd	nd	nd	nd	nd	nd
methionine	1.34	2.10	1.05	0.53	0.52	0.51	0.51	0.51
isoleucine	4.01	4.20	2.10	0.53	0.52	0.51	0.51	0.51
leucine	8.03	8.39	4.19	1.60	0.52	0.51	0.51	0.51
tyrosine	4.01	6.30	3.14	1.60	0.52	0.51	0.51	0.51
phenylalanine	6.69	8.39	4.19	2.13	1.05	nd	nd	1.03
γ-aminobutyric acid	5.35	4.20	2.10	1.07	nd	nd	nd	nd
ornithine	nd	nd	nd	nd	nd	nd	nd	nd
lysine	5.35	5.25	3.14	0.53	0.52	nd	nd	0.51
tryptophan	2.68	3.15	1.05	1.07	0.00	nd	nd	nd
histidine	2.68	4.20	1.05	0.53	0.52	nd	nd	nd
arginine	4.01	9.44	4.19	2.13	1.05	1.03	nd	1.03
total	111.04	146.90	61.84	38.91	17.29	6.18	4.12	6.68

[a] Values are single determinations of single extracts (dry basis). [b] Pale malt was provided by Muntons Malted Ingredients (Stowmarket, U.K.). [c] Not detected.

to be related to the degree of the Maillard reaction and is in agreement with the literature (*3*). Moreover, the degree of modification of barley endosperm during germination plays an important role in the concentrations of free amino acids. During germination, modification of the barley grain takes place. Enzymes (proteases) responsible for protein degradation (proteolysis) are active during germination, leading to an increase in amino acid levels. Proteases, such as carboxypeptidase and exo- and endopeptidases, are also active during the first stages of kilning (*1*), during which a low temperature (60 °C) is applied. Therefore, levels of amino acids are higher in worts prepared from green, pale, and lager malts, compared to worts from the other malt samples. Proline, an imino acid, was the most abundant free amino/imino acid in worts from all malt samples, except those of highly roasted malts, followed by glutamine, whilereas lower levels were detected for the other amino acids. Proline is known to be the most abundant amino/imino acid in worts prepared from pale/lager malts (*1*, *24*). The exact concentration of amino/imino acids in worts made from malt depends on many factors such as barley variety, growing conditions, season of crop, malting conditions (steeping, germination, kilning), and method of extraction of free amino acids.

Table 5. Sugar Analysis of Worts Prepared from Malt Samples (Grams per 100 g of Malt)[a]

sugar	barley	green malt	pale malt[b]	lager malt	cara malt	crystal malt	black malt	chocolate malt	roasted barley
glucose	0.24	1.74	3.15	3.71	5.4	3.99	nd	nd	nd
fructose	nd[c]	0.39	0.34	0.4	0.23	0.52	nd	nd	nd
sucrose	0.96	nd	2.69	3.68	2.73	2.64	nd	nd	nd
maltose	10.7	11.94	42.02	42.82	19.46	13.91	0.18	0.49	0.1
maltotriose	1.05	0.91	7.65	6.69	6.33	4.81	0.38	0.77	0.26
total	12.95	14.97	55.85	57.3	34.15	25.87	0.56	1.26	0.36

[a] Values are single determinations of single extracts (dry basis). [b] Pale malt was provided by Muntons Malted Ingredients (Stowmarket, U.K.). [c] Not detected.

Sugar Analysis. Worts were prepared by mashing each malt sample with an adjunct (pale malt). The levels of sugars in the malts are given in **Table 5**. Maltose was the most abundant sugar. It accounted for >50% of the total sugars in worts prepared using cara and crystal malts and for >70% in worts prepared from barley and green, pale, and lager malts. When highly roasted malts were used, maltose and maltotriose were the only sugars detected in the worts. Glucose, fructose, and sucrose were detected in all worts except those prepared from highly roasted malts. The concentration of total sugars in wort increased in the order roasted barley, chocolate malt, black malt $\ll$ barley malt < green malt < crystal malt < cara malt $\ll$ pale malt < lager malt. The same order applied to the majority of the individual sugars. The concentration of total sugars was much higher in worts prepared using lager malt or only pale malt, compared to the other samples. For worts made from highly roasted malts (roasted barley, black malt, and chocolate malt), the concentration of total sugars was <1.3 g/100 g. The intensity of the heat treatment applied during kilning/roasting of malts is the major factor affecting the concentration of sugars in worts prepared from them, and there is an inverse relationship between heat treatment and concentration of sugars, in line with recent results (*3*). Moreover, the applied heat treatment affects the enzymatic activity of malts. During germination and kilning, starch degradation occurs due to the action of enzymes including α- and β-amylase, limit dextrinase, phosphorylase, and α-glucosidase (maltase), leading to an increase in the concentration of sugars (*1*, *2*, *24*). On the contrary, highly roasted malts are characterized by a lack of any enzymatic activity (*1*), due to the high temperatures involved in their production. For these malts the disappearance or lower amounts of sugars in their worts is due to their consumption in the Maillard and caramelization reactions (*3*).

Table 6. Concentration (Micrograms per 100 g ± SD) of Free Phenolic Compounds Identified in Ethyl Acetate Extracts of Malts (Dry Mass Basis)[a]

phenolic compound	barley	green malt	stewed malt	pale malt[b]	lager malt	cara malt	crystal malt	black malt	chocolate malt	roasted barley
catechin	231.3 ± 10.9	59.8 ± 0.9	55.5 ± 0.4	688.4 ± 20.4	677.9 ± 20.5	350.3 ± 10.4	nd[c]	nd	nd	nd
ferulic acid	175.7 ± 12.1	67.4 ± 0.9	52.3 ± 0.8	357.8 ± 12.1	375.5 ± 11.1	123.6 ± 10.5	124.9 ± 10.8	nd	nd	nd
p-coumaric acid	19.9 ± 1.2	nd	nd	84.6 ± 1.5	103.2 ± 15.2	nd	nd	nd	nd	nd
vanillic acid	49.0	nd	nd	nd	nd	106.4 ± 10.3	92.6 ± 8.8	112.9 ± 10.1	45.8 ± 0.5	58.0 ± 8.1
homovanillic acid	nd	nd	nd	nd	nd	nd	nd	275.8 ± 15.2	201.6 ± 17.9	275.6 ± 22.1
p-hydroxyphenyl-acetic acid	28.4 ± 0.9	nd	nd	19.2 ± 8.2	2.6 ± 0.1	nd	nd	nd	nd	nd
4-vinylguaiacol	nd	nd	nd	nd	nd	nd	nd	33.6 ± 0.8	26.7 ± 0.7	43.9 ± 0.7
total	504.4	127.2	107.8	1150.0	1159.2	580.3	217.5	422.2	274.1	377.6

[a] Values are the means for triplicate extracts ± SD. [b] Pale malt was provided by Muntons Malted Ingredients (Stowmarket, U.K.). [c] Not detected.

Free Phenolic Compounds Analysis. The concentrations (micrograms per 100 g) of free identified phenolics in the ethyl acetate extracts of the malts are given in **Table 6**. Phenolic compounds identified in barley were ferulic, *p*-coumaric, vanillic, and *p*-hydroxyphenylacetic acids and catechin. Ferulic acid and catechin were present in the highest concentrations, in line with the literature (*25*). Hernanz et al. (*26*) used HPLC to determine the levels of hydroxycinnamic acids and the dimers of ferulic acid in 11 barley varieties. The extraction method included alkaline hydrolysis to release bound phenolics, followed by ethyl acetate extraction. According to their findings, the levels of ferulic and *p*-coumaric acids ranged between 36 and 62 mg/100 g and between 8 and 26 mg/100 g, respectively. These levels are higher than those determined in the current investigation; total (free plus bound) levels were measured in ref *26*, whereas we measured only the free phenolics.

The total concentrations of free identified phenolic compounds are quite similar for pale and lager malts and higher than that in barley, largely due to increased levels of catechin and ferulic acid in the malt samples. It has been reported that ferulic acid is the main bound phenolic acid in barley and green malt (*27*). Certain enzymes are active during the first stage of kilning (*1*) and, as kilning proceeds, some of them are partly or completely denatured, depending on their individual heat stabilities and the intensity of the heat treatment. However, some enzymes survive kilning (*24*), including ferulic acid esterase (*28*). This enzyme is able to release bound ferulic acid from barley cell walls, resulting in an increase of concentration of ferulic acid in kilned malts. Neither catechin nor ferulic acid was identified in black or chocolate malt or roasted barley (highly roasted malts). Vanillic and homovanillic acids and 4-vinylguaiacol were detected in these highly roasted malts. Vanillic and homovanillic acids, as well as 4-vinylguaiacol, come from the degradation and/or metabolism of other phenolic compounds (e.g., ferulic acid) (*8*, *29*, *30*). Furthermore, the thermally induced melanoidins were visualized as a broad hump (*31*) migrating between 7 and 11 min in the electropherograms (e-grams) of the ethyl acetate extracts of the highly roasted malts.

Antioxidant Activity. Two radical scavenging methods, the $ABTS^{\bullet+}$ and the ORAC assays, were employed to assess the antioxidant activity of malt extracts. The antioxidant activity of the acetate buffer extracts and ethyl acetate extracts as measured by the $ABTS^{\bullet+}$ and ORAC assays and expressed as ferulic acid equivalents are given in **Figure 2**. Antioxidant activity increased in the order barley ≈ green malt ≈ stewed malt ≤ pale malt ≈ lager malt < cara malt < crystal malt < black malt ≈ chocolate malt ≈ roasted barley. The same order of antioxidant activity for both acetate buffer extracts and ethyl acetate extracts was found by the ORAC assay.

This is the first study to examine the antioxidant activity of a range of malts using the ORAC assay. This assay involves the use of the peroxyl radical ($ROO^{\bullet}$), a "natural" radical that is formed in biological systems undergoing oxidation. Many studies in the literature present contradictory results concerning the antioxidant activity of food, due to the use of different assays that are based on different oxidative mechanisms. In contrast, studies on the antioxidant activity of kilned and roasted malts usually give similar results for different antioxidant methods. Our data are in line with those of previous studies (e.g., refs *13*, *14*, and *32*) reporting that the antioxidant activity of malts increases in the order barley ≤ green malt ≤ pale malt < cara malt < amber malt < brown malt < light crystal malt < medium crystal malt < dark crystal malt < chocolate malt ≤ black malt ≤ roasted barley. The same order of antioxidant activity has been obtained for the malts by various methods, that is, FRAP assay (*33*), leucomethylene blue assay (*13*), and linoleic acid oxidation method (*32*), as well as the $ABTS^{\bullet+}$ and ORAC assays applied in the current study, suggesting that malt antioxidant compounds possess activity against different oxidative mechanisms. The incorporation into foods and beverages of kilned and roasted malts at levels that do not adversely affect the flavor or color of the final product may delay or prevent lipid oxidation and extend product shelf life.

Antioxidant Activity and °EBC Data. The antioxidant capacity of malts ($ABTS^{\bullet+}$ value) increased with °EBC value, in line with previous studies (*13*, *32*). However, the $ABTS^{\bullet+}$/°EBC ratio fell with increasing malt color (**Figure 3**), indicating the increasing contribution to antioxidant activity made by colored malt components with degree of heat treatment. The antioxidant activity of malts of low color (lager and pale malts) is largely due to compounds that do not absorb in the visible region, for example, polyphenols and reductones. Our recent work (*10*) on a glucose−proline−ferulic acid model system heated at 60 °C for up to 24 h suggests that colorless compounds formed by the reaction of all three precursors might also contribute to the antioxidant activity in these kilned malts. Compounds that contribute to color development of malt samples include MRPs, polyphenol oxidation products, and caramelization products. The finding that the antioxidant activity of malts per color (°EBC) unit is largest for lager malt and decreases at higher color values is in line with data reported in the literature (*14*). The lightly colored (and mildly flavored) pale malts may be useful as a source of antioxidants in foods and beverages that possess mild flavor and pale color.

Antioxidant Activity and Visible Absorption Data. The low absorbance values obtained for pale and lager malts demonstrated that color production during kilning is not high. The temperatures involved during kilning are <100 °C, resulting in

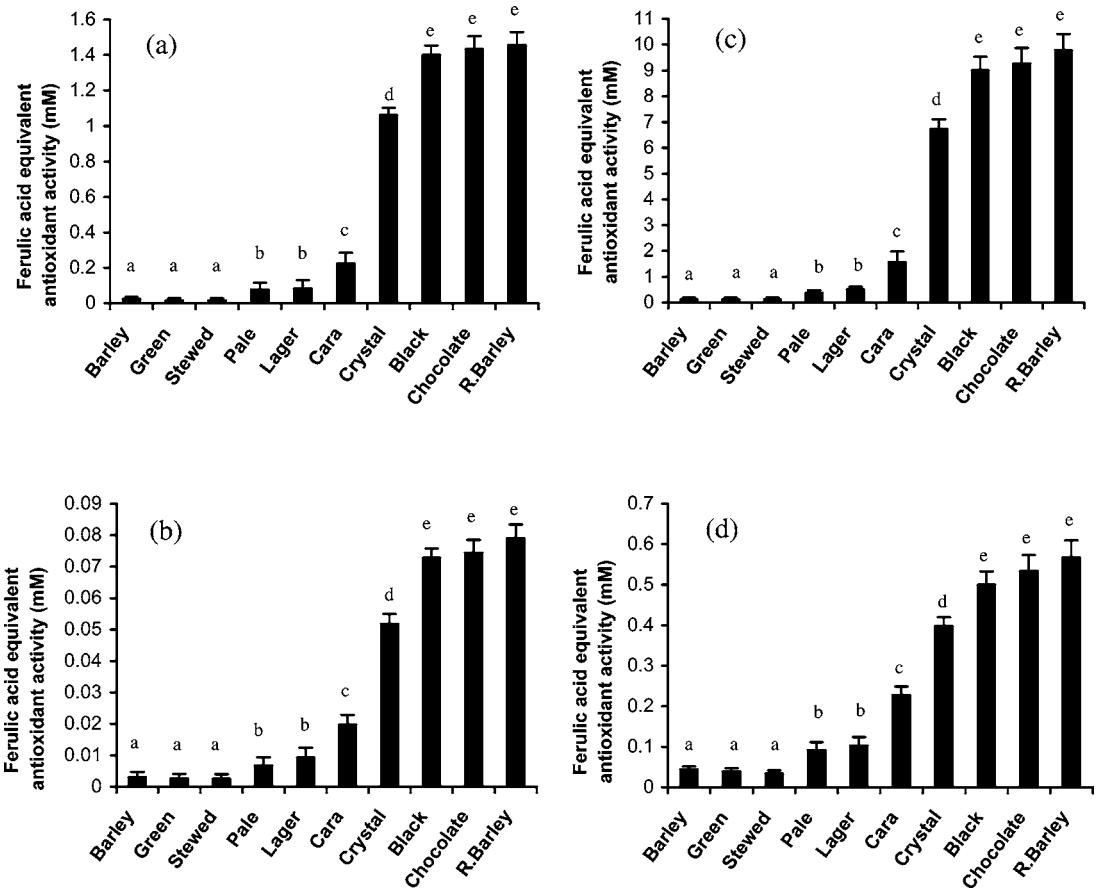

Figure 2. Antioxidant activity of malt extracts: (**a**) $ABTS^{\bullet+}$ scavenging data for acetate buffer extracts; (**b**) $ABTS^{\bullet+}$ scavenging data for ethyl acetate extracts; (**c**) ORAC assay data for acetate buffer extracts; (**d**) ORAC assay data for ethyl acetate extracts. Error bars represent the standard deviation of mean values for triplicate samples. Bars with the same letters indicate no significant difference ($p > 0.05$).

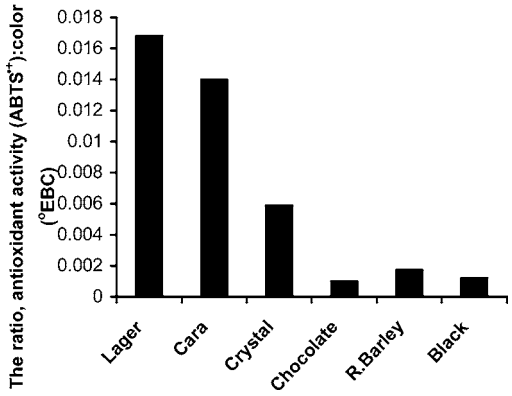

Figure 3. Ratio of the antioxidant activity ($ABTS^{\bullet+}$) to color (°EBC) of the acetate buffer extract of malts.

very low levels of browning. Therefore, early or intermediate stage low molecular mass Maillard reaction products, such as reductones, plus phenolic compounds are likely to make more important contributions to antioxidant activity for kilned malts. A wavelength of 420 nm is frequently used to monitor the formation of colored MRPs, including melanoidins, that are known to possess antioxidant activity, in line with the data presented in **Figure 3**.

Antioxidant Activity and Sugar and Amino Acid Analyses. Negative correlations were found between antioxidant activity ($ABTS^{\bullet+}$) and both sugar ($r = -0.9585$) and amino acid concentration ($r = -0.9690$). This was expected because amino acids and sugars participate in the Maillard reaction, resulting in the formation of MRPs with antioxidant activity (*17*). The antioxidant activity of barley and green malt comes exclusively from natural antioxidants, because no heat is applied. Pale and lager malts were produced under similar malting (steeping, germination, and kilning) conditions and, as expected, possessed similar levels of antioxidant activity. Both natural antioxidants (e.g., phenolic compounds) and LMM MRPS (e.g., reductones), formed from the Maillard reaction during kilning, contribute to antioxidant activity. Cara and crystal malts, which were produced by roasting of green malt, showed significantly higher antioxidant activity than barley and green, pale, and lager malts. The high roasting temperatures involved are responsible for the generation of MRPs such as melanoidins and reductones, which, together with residual phenolic compounds and their thermal degradation products, are responsible for the greater antioxidant activity in these malts, compared to kilned malts. The highest antioxidant activities were obtained for roasted barley and black and chocolate malts. The antioxidant activity of these samples may come from colored MRPs, especially melanoidins.

In conclusion, this study investigated the antioxidant activity of kilned and roasted malts in relation to their color and their profile of phenolic compounds, sugars, amino acids. It is the first comprehensive study reporting the profiles of various components for a wide range of malts. It shows for the first time that antioxidant activity and browning (absorbance at 420 nm) of both the acetate buffer extracts of malts and the ethyl acetate extracts prepared from them increase in parallel. In addition, the relative contribution to antioxidant activity from the phenolic compounds was greater for the kilned malts, whereas the relative contribution of the MRPs increased with

degree of roasting and was greatest for the black and chocolate malts and roasted barley.

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