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Effect of Heat Treatment on the Antioxidant Activity, Color, and Free Phenolic Acid Profile of Malt

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Green malt was kilned at 95 °C following two regimens: a standard regimen (SKR) and a rapid regimen (RKR). Both resulting malts were treated further in a tray dryer heated to 120 °C, as was green malt previously dried to 65 °C (TDR). Each regimen was monitored by determining the color, antioxidant activity (by both $ABTS^{\bullet+}$ and FRAP methods), and polyphenolic profile. SKR and RKR malts exhibited decreased L^* and increased b^* values above approximately 80 °C. TDR malts changed significantly less, and color did not develop until 110 °C, implying that different chemical reactions lead to color in those malts. Antioxidant activity increased progressively with each regimen, although with TDR malts this became significant only at 110−120 °C. The RKR malt $ABTS^{\bullet+}$ values were higher than those of the SKR malt. The main phenolics, that is, ferulic, *p*-coumaric, and vanillic acids, were monitored throughout heating. Ferulic acid levels increased upon heating to 80 °C for SKR and to 70 °C for RKR, with subsequent decreases. However, the levels for TDR malts did not increase significantly. The increase in free phenolics early in kilning could be due to enzymatic release of bound phenolics and/or easier extractability due to changes in the matrix. The differences between the kilning regimens used suggest that further modification of the regimens could lead to greater release of bound phenolics with consequent beneficial effects on flavor stability in beer and, more generally, on human health.

KEYWORDS: Malt; kilning conditions; color; antioxidant activity; phenolic acids

INTRODUCTION

Malting comprises three stages, that is, steeping (the moisture content of the grain increasing to 42−46%, the level at which germination occurs) (*1*), germination (resulting in high enzyme activity and endosperm modification with the minimum amount of growth) (*2*), and kilning, during which germination is terminated, the endosperm becomes brittle, and the rootlets are easily removed from the malt. By controlling the kilning conditions, some enzyme activity may be retained (*1*).

Kilning can be divided into five stages, that is, start-up, free drying, intermediate drying, bound-water removal, and curing:

1. Start-up warms both the kiln and the grain, and an air flow is established through the grain bed.

2. In free drying, an air-on temperature of 50−60 °C is applied to the base of the kiln. Free water is removed easily from the grain. The relative humidity (RH) of the air leaving the kiln (air-off) is high.

3. During intermediate drying, as the moisture of the grain decreases, the rate of drying of the grain decreases. The RH of the air-off falls, and air flows are reduced to allow the warm air to be in contact with the grain for a longer time, giving the moisture more time to reach the outside of the grain and to establish equilibrium with the air. The air-on temperature is increased for continual removal of moisture.

4. During the removal of bound water, as the grain moisture is reduced to approximately 12%, the air-on temperature is increased to 65−75 °C to encourage drying to continue, even though the air flow is reduced.

5. Curing begins as the grain moisture falls to 5−8%. Air-on temperature is increased further and the air flow reduced. This stage "cooks" the malt, reduces moisture content, and results in typical malt flavor and aroma. The temperatures used depend upon the type of malt being produced. Very little drying occurs in this stage, and high levels of air recirculation can be applied to the kiln.

Flavor instability, resulting in product deterioration, is a major problem for the brewing industry. Maintaining reducing power throughout brewing is important to limit off-flavor development in beer during storage (*3*). Compounds possessing antioxidant

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activity in barley, such as the phenolic species, are released during malting and mashing and transferred to the final product, that is, beer (*4*). Preservation of naturally occurring antioxidants of barley is therefore of great interest (*5*). Other compounds possessing reducing power are generated during kilning by the Maillard reaction (*6*), due to the production of, for example, reductones and melanoidins. Maintaining high levels of antioxidants during brewing, thereby limiting the formation of staling aldehydes, is crucial for beer stability.

Our previous studies (*7*) have demonstrated that the kilning regimen applied to malt affects both the antioxidant activity and the color of the final product. However, the changes in phenolic acid profile throughout kilning and the resultant effect of these changes upon antioxidant activity were not monitored. We (*8*) have previously reported that changes occur in the antioxidant activity of the free phenolic fraction extracted from green malt throughout heating. Small increases in antioxidant activity of the sample extracts were detected as the extract was heated between 80 and 100 °C. Whole malt grains may offer thermal protection to the phenolics during heating.

As a consequence, the aims of the current study were to monitor changes in antioxidant activity and the profile of phenolic acids of malts during kilning, up to 120 °C, and to assess the effect of three different kilning regimens on these malt parameters.

EXPERIMENTAL PROCEDURES

Materials. HPLC grade water and methanol were from Fisher Scientific (Leicester, U.K.). High-purity water from a Purite (High Wycombe, U.K.) Labwater RO50 unit was used for all chemical analyses. Glacial acetic acid, (+)-catechin, vanillic, chlorogenic, caffeic, *p*-coumaric, sinapic, and ferulic acids (all >99%), 2,4,6-tripyridyl-*s*-triazine (TPTZ), ferric chloride, and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were from Sigma (Poole, U.K.). 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) was from Aldrich (Gillingham, U.K.). Optic barley (2002 harvest), used to produce the malt, was supplied by BRI (Nutfield, Surrey, U.K.).

Malting. Green malt was prepared from Optic barley in the 50 kg pilot malting plant at BRI. Barley (50 kg) and water (150 L, at 16 °C) were mixed together to produce a well-modified green malt, by following a steeping regimen of 8 h wet/14 h air, rest, 5 h wet/18 h air rest, and 6 h wet/2 h air rest. The temperature within the steep/germination vessel rose from 16 to 17 °C over the steep/soak period. The grains were allowed to germinate for 5 days, during which time the grain temperature rose from 16 to 18 °C. Germination was arrested by kilning. A separate batch of green malt was prepared under identical conditions for each kilning trial, using barley from the same bulk container.

Kilning. Kilning was undertaken in the pilot plant kilns (Redler Ltd., Stroud, U.K.) at BRI using two regimens (**Table 1**). Using the standard kilning regimen (SKR), no air recirculation was used prior to the grain reaching break point at 17 h. As the percent RH in the kiln fell, the percent air recirculation was increased from 50 to 80% at the end of kilning. A final grain temperature of 95 °C was achieved. The rapid kilning regime (RKR) took the green malt through to break point very quickly. There was a rapid fall in the percent RH in the kiln after 13 h (**Table 1**), when the air-off temperature began to rise. This trial used 100% fan speed and 0% air recirculation in the early stages (1−4) of kilning. Samples of approximately 250 g were taken from the center of the grain bed at predetermined temperatures throughout kilning. The temperature was measured at the center of the grain bed immediately prior to sampling.

Postkilning Heat Treatment. SKR and RKR malts, both kilned to a final grain temperature of 95 °C, as well as malt kilned to 65 °C (using the SKR regimen stages 1−4), were subjected to postkilning heat treatment up to 120 °C on trays in an oven.

The oven (APV Mitchell dryer, model 7223, Carlisle, U.K.) was preheated to 95 °C. Grain (250 g) was spread thinly onto shallow metal trays (82 × 41 × 3 cm) and placed in the oven. The temperature was allowed to return to 95 °C. After 15 min at 95 °C, one tray was removed and the temperature increased to 100 °C. When the oven and grain had attained the new set temperature (~5 min), the samples were maintained at this temperature for 15 min and a further tray was removed. This procedure was repeated at temperatures of 5 °C intervals from 105 to 120 °C. The SKR malt kilned to 65 °C and oven treated was designated tray-dried regimen malt (TDR).

Table 1. Kilning Conditions Used for the Production of SKR and RKR Malts[a]

	SKR				RKR			
kilning stage	kilning time (h)	fan speed (%)	air-on temp (°C)	air-off temp (°C)	kilning time (h)	fan speed (%)	air-on temp (°C)	air-off temp (°C)
1	8	100	60	27–28	12	100	60	27–36
2	2	80	60	28–29	2	100	60	36–46
3	2	80	65	29–29	2	100	65	46–60
4	2	65	70	29–30	2	80	70	60–67
5	3.5	40	75	30–41	*b*	*b*	*b*	*b*
6	1	30	80	41–42	1	40	80	67–68
7	1	30	85	42–45	1	30	85	68–72
8	1	30	90	50–60	1	30	90	72–74
9	2	40	95	60–83	2	30	95	74–82
10	5	40	105	83–98	5	40	105	82–99

[a] SKR, standard kilning regimen; RKR, rapid kilning regimen. [b] This stage of kilning was omitted in RKR malt production.

All samples taken during malting were cooled, derooted, using a small-scale laboratory rotating derooter, and stored at room temperature in sealed polyethene bags prior to analysis (within 1 month).

Preparation of Malt Extracts. Hot water extracts were prepared for color measurement and acetate buffer extracts of malts were prepared for antioxidant analyses. Ethyl acetate extracts (of the acetate buffer extracts) were prepared for both antioxidant activity and HPLC analyses.

Hot water extracts were prepared for all malt samples. Malt (12 g) was ground in a coffee grinder (3 × 10 s) and transferred to a tared flask. Water (70 mL at 65 °C) was added, and the flask was stoppered and shaken vigorously manually, prior to being held in a shaking water bath at 65 °C for 1 h. The sample was cooled to room temperature, made up to 90 g with water, and filtered (Whatman no. 1). The filtrate was returned to the filter funnel and refiltered. The filtrate was analyzed for color (within 1 h).

Acetate buffer extracts were prepared as previously described (*9*). In brief, ground green malt (5 g) was homogenized in acetate buffer (50 mM, pH 5.4, 40 mL) for 2 min. The slurry was centrifuged (1500*g*, 10 min) and filtered (Whatman no. 1). Duplicate extracts were prepared for each malt sample. Samples were stored frozen prior to antioxidant analyses by FRAP and $ABTS^{\bullet+}$ assays (within 1 month).

The ethyl acetate extract was prepared as described by Pascoe et al. (*10*). In brief, the acetate buffer extract, prepared as described above, was acidified (to pH 1 using 600 μL of 1 M HCl and 0.5 g of potassium chloride), and the phenolic fraction was extracted with ethyl acetate (3 × 10 mL). The organic phase was centrifuged (2500*g*, 10 min), dried with anhydrous sodium sulfate (2 g for 2 h), and vacuum filtered (Whatman no. 1). The ethyl acetate fractions resulting from the two extractions were combined, and the solvent was removed using a rotary evaporator with the water bath set at 35 °C. The residue was then dissolved in methanol (2 mL). Duplicate extracts were prepared for each malt sample. Methanolic extracts were stored at 4 °C in sealed brown vials, prior to analysis (within 1 month).

Color Analysis. Color measurements of the hot water extracts were determined using the Hunter Lab CIE *L*a*b** apparatus (*9*). Duplicate readings were obtained for single extracts only, due to limited sample size.

Antioxidant Activity. The antioxidant activity of the sample extracts was assessed by two methods. The $ABTS^{\bullet+}$ method was performed exactly as previously described (*9*). In summary, the chromophore solution (3.125 μL) and catechin standard or malt acetate buffer extract

(25 μL) were mixed and incubated at 25 °C, and the absorbance was measured at 734 nm. The ferric-reducing antioxidant potential (FRAP) assay was performed as described by Benzie and Strain (*11*), but modified according to Woffenden et al. (*9*). Malt extract (100 μL) and FRAP reagent (TPTZ; 2,4,6-tripyridyl-*s*-triazine, 3 mL) were mixed and incubated at 25 °C. After exactly 5 min from initial mixing, the absorbance of the reaction mix was measured at 593 nm. Catechin standard solutions and blanks (using acetate buffer in place of malt extract) were run for both assays. All methanolic extracts derived from ethyl acetate extraction were diluted 1:1 in acetate buffer (pH 5.4, 50 mM) prior to analysis. Duplicate extracts were prepared from each sample, and each extract was analyzed in triplicate. Calibration curves were prepared using (+)-catechin.

HPLC Analysis. Separations were performed using a Hewlett-Packard (HP, now Agilent Technology, Bracknell, U.K.) model 1050 series II LC with a quaternary solvent delivery system and equipped with an autoinjector and a UV−visible diode array detector. System control and data acquisition and analysis were performed using HP Chemstation software. An ACE 5Q 250 × 4.6 mm i.d. 5 μm column (HiChrom, Theale, U.K.), fitted with a 30 × 4.6 mm guard column (HiChrom), was used with gradient elution. Solvent A was water, and solvent B was methanol. Both solvents were adjusted to pH 2.3 using glacial acetic acid. Solvent B was increased linearly from 0 to 40% over 80 min. The flow rate was 1 mL/min, and the injection volume was 20 μL. Ferulic, sinapic, *p*-coumaric, and chlorogenic acids were monitored at 320 nm. Vanillic acid and catechin were monitored at 260 and 280 nm, respectively. Calibration curves for quantification were prepared from solutions of authentic compounds in the appropriate concentration range. All ethyl acetate extract samples and standards were passed through a 0.45 μm filter prior to analysis. Components were identified by matching their retention times and UV diode array spectra to those of the authentic standard. Duplicate extracts were prepared from each sample, and each extract was analyzed in triplicate.

RESULTS AND DISCUSSION

Processing Conditions. The air-on temperature profiles used in the two kilning regimens were identical, but the air-off temperatures, often used as a measure of the grain temperature, were different due to differences in fan speed and recirculated air levels (**Table 1**). This resulted in different air flows through the grain bed and therefore distinct grain-bed temperature profiles for the different kilning procedures. The actual grain temperature was not measured, but the air temperature at different levels within the grain bed was taken as the most accurate guide to actual grain temperature. The grain subjected to RKR reached break point at 13 h and that subjected to SKR at 19.5 h, but the drying time post break point was extended in RKR by using 100% recirculated air and lowered fan speeds for the final 10 h of kilning as the percent RH in the kiln had fallen to below 20%. Stage 5 was omitted for RKR malt as the grain had already reached break point.

It was important that the green malts used for the different heat treatments were as similar as possible, between batches, as amino acids and sugars are produced during modification and, as they are the precursors for conversion into Maillard reaction products in the later stages of kilning, the degree of modification in malting is important for color, flavor, and antioxidant activity development (*3*, *12*). Uneven modification of the grain may result in variations in the levels of phenolics present in malt (*13*) and in poor extraction during mashing (*14*). Shoot and rootlet growth in the green malt were not measured at the end of green malt production, but all malts were subjected to the same regimens, and an experienced maltster indicated that the green malts were as similar as possible to one another.

Moisture Content. Malts from SKR had higher moisture content (5.51%) at the end of heat treatment (**Table 2**), compared to the malts produced from RKR (3.54%) and TDR (4.65%). Although the total kilning time was 1 h less for RKR malt, at the end of kilning (95 °C) the RKR malt had a lower moisture content (4.61%) than the SKR malt (5.23%), due to the higher fan speeds used in stages 2−4. TDR malts were subjected to heat treatment for only 2.5 h, with temperature increasing from 65 to 120 °C, compared to >10 h for the same temperature increase in SKR and RKR malts.

Table 2. Effect of Heat Treatment on Moisture Content of SKR, RKR, and TDR Malts[a−c]

SKR			RKR			TDS		
time (h)	temp (°C)	moisture (%)	time (h)	temp (°C)	moisture (%)	time (h)	temp (°C)	moisture (%)
0.00	25	42.26	0.00	25	45.77	0.00		
19.00	40	6.26	16.50	67		0.00	65	6.03
20.25	70	7.13	18.00	70	6.25	0.25	70	5.72
22.20	80	5.12		80	6.00	0.50	80	6.35
23.50	85	4.77	18.50	85	4.65	0.75	85	5.88
24.30	90	6.27	25.00	90	4.20	1.00	90	5.80
27.50[d]	95[d]	5.23[d]	28.5[d]	95[d]	4.61[d]	1.25	95	5.92
27.75	100	4.18	28.75	100	4.03	1.50	100	6.12
30.00	105	4.38	29.00	105	5.38	1.75	105	5.96
30.25	110	5.41	29.25	110	3.60	2.00	110	6.32
30.50	115	5.32	29.50	115	5.19	2.25	115	5.94
30.75	120	5.51	29.75	120	3.54	2.50	120	4.65

[a] Moisture content data are the mean of triplicate determinations. [b] All temperatures measured at the center of the kiln. [c] Percent CV < 5%. [d] Denotes the end of kilning (95 °C).

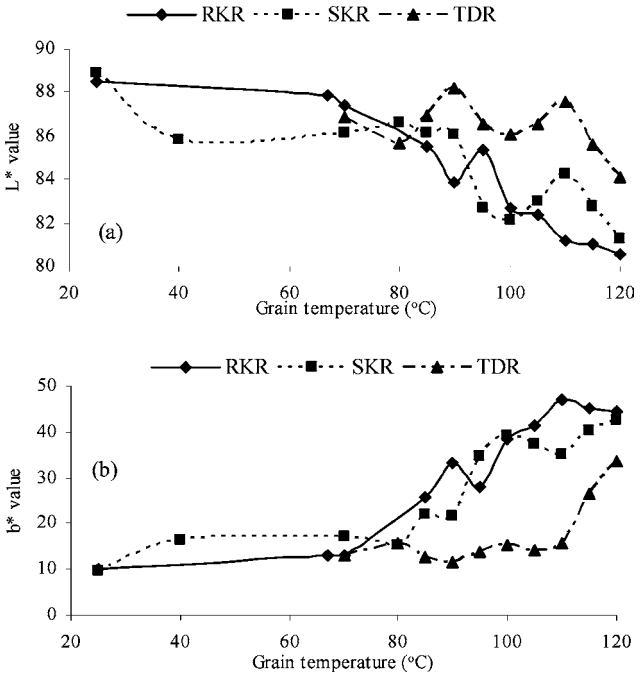

Figure 1. Changes in (**a**) *L** value and in (**b**) *b** value of hot water extracts from SKR, RKR, and TDR samples taken throughout heating. Data are the mean of duplicate determinations on a single sample due to limited sample size.

Color of Hot Water Extract. No significant differences were determined in the *a** values (redness) of hot water extracts of SKR, RKR, and TDR samples taken throughout heating, but there was a decrease in *L** value (lightness, **Figure 1a**) and an increase in *b** value (yellowness, **Figure 1b**). At grain temperatures of 70−80 °C, *b** values of the hot water extract from both SKR and RKR malts began to increase, and there were no significant differences in the *L** or *b** values of SKR and RKR samples at the end of the heat treatment (120 °C).

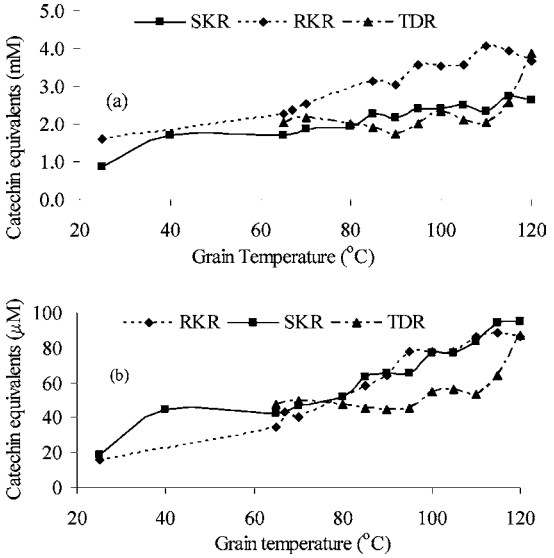

Figure 2. Effect of grain temperature on antioxidant activity of acetate buffer extracts of SKR, RKR, and TDR malts assessed by (**a**) $ABTS^{\bullet+}$ and (**b**) FRAP assays. All data are the mean of triplicate determinations on duplicate extracts. %RSD values are <10% for FRAP and <15% for ABTS data.

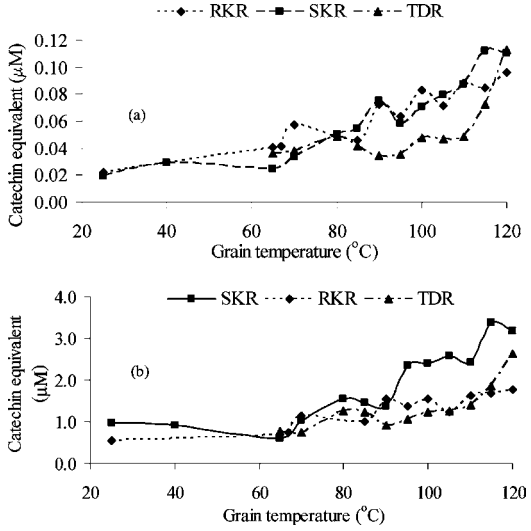

Figure 3. Effect of grain temperature on antioxidant activity of ethyl acetate extracts of SKR, RKR, and TDR malts assessed by (**a**) $ABTS^{\bullet+}$ and (**b**) FRAP assays. All data are the mean of triplicate determinations on duplicate extracts. %RSD values are generally <15% for FRAP and <20% for ABTS data.

The patterns of the L^* and b^* data are similar, but not identical, for the SKR and RKR malts, but different from that of the TDR malts (**Figure 1**). The decrease in L^* values and the increase in b^* values were significant only in the later stages of heat treatment (110−120 °C) as the grain moisture level fell below 6%. The data are in line with our previous findings (*7*) illustrating that hot water extracts from rapidly kilned malt were darker and yellower than standard kilned malt extracts.

Colored compounds are generated in the SKR and RKR malt samples at temperatures above 80 °C, but in the TDR sample not until 110 °C, probably as a result of the different heating conditions. It seems that different chemical reactions lead to the formation of colored compounds in the TDR samples compared to in the SKR and RKR ones. Because levels of amino acids and reducing sugars increase during the steeping and germination stages of malting (*12*), the same components were present in all green malts prior to kilning. Colored compounds are produced throughout kilning, the final color of the malt depending upon the final kilning temperature, but the higher temperatures utilized to induce color development in roasted barley or chocolate and black malts were not used in this study (*15*).

Antioxidant Activity. There was an increase in antioxidant activity of acetate buffer extracts from SKR, RKR, and TDR malts taken throughout heating as monitored by both the $ABTS^{\bullet+}$ and FRAP assays (**Figure 2**). The antioxidant activity of the malt extracts increased with increasing temperature and decreasing moisture content in all heating regimens.

The antioxidant activity measured by the $ABTS^{\bullet+}$ assay on acetate buffer extracts of RKR was significantly higher than for SKR and TDR, both at the end of kilning (95 °C) and at the end of postkilning heat treatment (120 °C), but no significant differences were found in antioxidant activity as measured in the same samples using the FRAP assay. Both RKR and SKR malts increased significantly in antioxidant activity throughout the heat treatment, whereas TDR acetate buffer extracts exhibited significant increases in antioxidant activity, measured by both the $ABTS^{\bullet+}$ and FRAP assays, only in the final stages of heat treatment (110−120 °C). Acetate buffer extracts from RKR malt exhibited a greater increase in the antioxidant activity by the $ABTS^{\bullet+}$ method during postkilning heat treatment compared to the SKR malt. The results determined using the FRAP assay indicate that there was no difference in the final reducing power of the samples regardless of processing conditions.

When the grain was subjected to RKR, the grain moisture content fell sooner and at a lower temperature, the increase in antioxidant activity taking place at between 70 and 80 °C, compared with 80−85 °C for the SKR samples. The decrease in L^* and increase in b^* values also occurred at a lower temperature in RKR than SKR, indicating that the processing was affecting the development of compounds responsible for both color and antioxidant activity. This supports the data of Woffenden et al. (*7*).

The TDR samples had higher moisture contents and lower color than the other trial samples, which indicates that for the increase in color the lower moisture content may be more important than heat treatment. A linear correlation was found between color and antioxidant activity for the TDR samples, by both the $ABTS^{\bullet+}$ and FRAP assays for the acetate buffer extracts. Relationships between color and antioxidant activity have been found in both coffee (*16*) and malts (*12*). Liegeois et al. (*17*) reported the relationship to be unpredictable in malts, due to the variety of reactions that can occur depending upon processing conditions. A linear relationship between color (determined as °EBC units) and antioxidant activity (determined using the $ABTS^{\bullet+}$ assay, up to 400 °EBC units) was demonstrated by Chandra et al. (*12*) and Woffenden et al. (*7*). The increase in antioxidant activity was considered to be due mainly to the generation of colored Maillard reaction products (MRPs).

Although the antioxidant activity of the ethyl acetate extracts from SKR, RKR, and TDR malts tended to increase throughout heating, as monitored by both the $ABTS^{\bullet+}$ and FRAP assays (**Figure 3**), no significant differences were obtained in the radical scavenging antioxidant activity of ethyl acetate extracts of finished malts heated to 120 °C by the different regimes. At 95 °C, the TDR extract, which had been heated in the oven and not kilned from 65 to 95 °C, had a lower antioxidant activity than the SKR and RKR extracts (**Figure 3a**).

Data from the $ABTS^{\bullet+}$ assay indicate that the different heat treatments had only a limited effect upon the generation of

radical scavengers. Both SKR and RKR samples exhibited a peak in antioxidant activity as the samples were heated to 90 °C, although overall the antioxidant activity continued to increase with postkilning heat treatment. At the end of kilning (95 °C), the TDR ethyl acetate extract, which had not been subjected to traditional kilning from 65 to 95 °C, had a significantly lower antioxidant activity than the SKR and RKR sample extracts.

When ethyl acetate extracts were submitted to the FRAP assay, SKR malts exhibited greater reducing power at temperatures above 90 °C than those of the RKR and TDR malts (**Figure 3b**), the rate of increase of antioxidant activity of the SKR extracts being significantly greatest. As with the acetate buffer extracts, significant increases in antioxidant activity in TDR ethyl acetate extracts were detected only in the final stages of heat treatment (ABTS$^{\bullet+}$ and FRAP), as the grain temperature increased from 110 to 120 °C.

It is not possible to superimpose the graphs of antioxidant activity for the different kilning regimes, regardless of the type of extract, nor the antioxidant activity (**Figures 2** and **3**) from the same samples as determined by different assays. Increases in antioxidant activity, determined by both the ABTS$^{\bullet+}$ and FRAP methods and for all heating regimens, are neither continuous nor smooth. This indicates that the formation of compounds with antioxidant activity occurs at different rates throughout the kilning regimes. Assays reflect different aspects of antioxidants; FRAP measures the total reducing power (*11*, *18*) and ABTS$^{\bullet+}$ the radical scavenging ability of the sample (*19*).

The antioxidant activity of the acetate buffer extract is derived only from the components that are soluble in the aqueous phase, and, as such, is a measure of the antioxidant activity of malt which can be transferred to the wort. The antioxidant activity in methanolic extracts of malt has been previously discussed (*5*), but some of these compounds cannot contribute to the antioxidant activity of the wort because of a lack of water solubility. The medium into which the antioxidant components are extracted has an effect upon the measured antioxidant activity of the sample extract. Differences in antioxidant activity of aqueous and methanolic extracts from several cereals, including barley, have been demonstrated (*20*). More phenolics are extracted into methanol than into water (as determined using the Folin−Ciocalteu method), and consequently methanolic extracts exhibit higher antioxidant activity than aqueous ones (measured by the ABTS $^{\bullet+}$ assay).

In this study, all ethyl acetate extracts were derived from an initial acetate buffer extract, and the ethyl acetate extracts constituted a concentrated form of the phenolics present in the acetate buffer extract. In all experiments, the methanolic solution resulting from the ethyl acetate extract was diluted with acetate buffer, as it has been demonstrated (*21*) that standards and samples must be prepared in the same medium to avoid discrepancies.

The antioxidant activity of the acetate buffer extract of malt is representative of the antioxidant activity of malt in the mashing stage of brewing. The ethyl acetate extract is a fraction of it, enriched in phenolic compounds. However, in addition to phenolic compounds, it will contain other components extractable by ethyl acetate, including some low molecular mass Maillard reaction products, some of which possess antioxidant activity (*5*).

Quantitative data concerning the contribution of the phenolic fraction of malt to the overall antioxidant activity are not available, but it has been described as "small" (*5*). From our

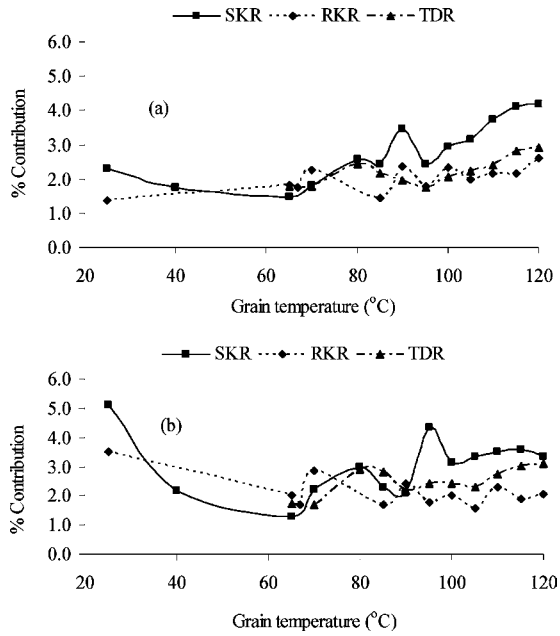

Figure 4. Effect of grain heating on the percent contribution to the ethyl acetate extract antioxidant activity to that of the acetate buffer extract determined by (**a**) ABTS$^{\bullet+}$ and (**b**) FRAP assay. The data are calculated from the mean antioxidant activity data described in **Figures 2** and **3**.

study, the percent contribution of the ethyl acetate extract to total antioxidant activity of the malt is indeed small (<5%; **Figure 4**), but there is variation both throughout each heat treatment and among the three different heat treatments, indicating different patterns of release of bound phenolics in response to the different temperature and moisture contents of the malts resulting from the variation in kilning conditions. Generally, there was an increase in the percent contribution throughout heat treatment. The percent contribution (ABTS$^{\bullet+}$ data) was higher for the SKR than for the RKR and TDR malts (**Figure 4a**).

An increase in the percent contribution was given by both the SKR and RKR malts as the grain temperature increased from 85 to 90 °C, but not by the TDR malts, indicating that the increase might be associated with kilning procedures. The greater increase in the percent contribution in the postkilning heat treatment of the malt (95−120 °C) for the SKR samples indicates the generation of more ethyl acetate extracted radical scavengers in malts prepared using this regimen rather than the RKR or TDR.

When antioxidant activity was determined using the FRAP assay (**Figure 4b**), a higher percent contribution was again noted for malts from SKR at grain temperatures of >90 °C, in line with the ABTS$^{\bullet+}$ assay results. The percent contribution was high in both the SKR and RKR green malts. It then decreased as the green malt was heated, indicating that components of the green malt possessing antioxidant activity were destroyed in the early stages of kilning (25−90 °C). This decrease, with increasing grain temperature, was barely apparent in the ABTS$^{\bullet+}$ data, suggesting that radical scavenging was not important during this phase.

At grain temperatures up to 90 °C, there were increases in the percent contribution of 0.3, 0.4, and 0.2% for the SKR, RKR, and TDR malts respectively, indicating that compounds possessing reducing power were being generated at a similar rate in all three heating trials (**Figure 4**). However, as the grain temperature was increased from 90 to 95 °C, there was a marked

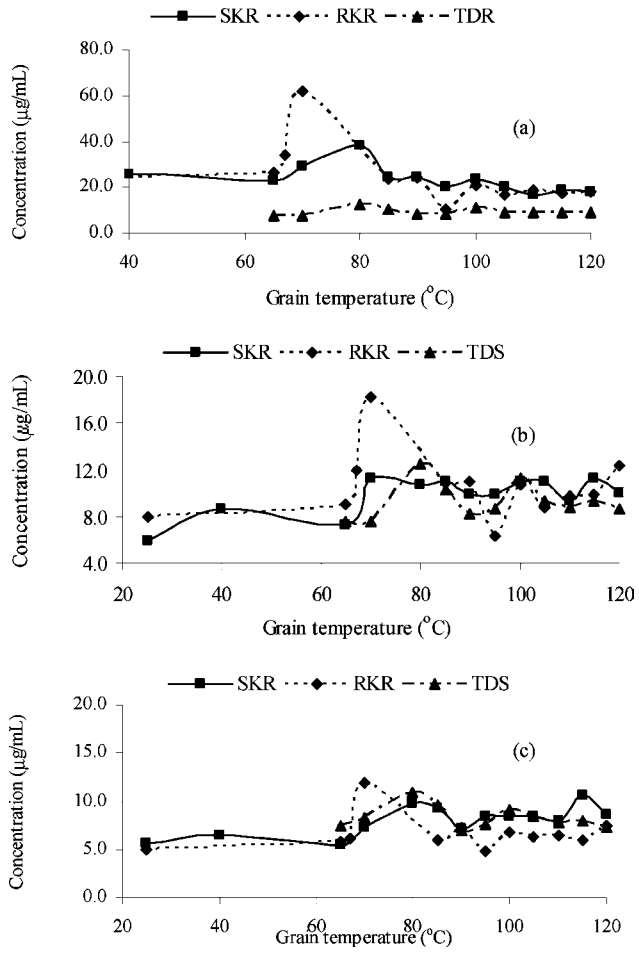

Figure 5. Effect of grain temperature on the level of (**a**) ferulic acid, (**b**) *p*-coumaric acid, and (**c**) vanillic acid in malt. Data are the mean of duplicate analyses on each of two extracts. %RSD values are generally <15% for ferulic acid.

increase in the percent contribution of the ethyl acetate extract to the acetate buffer extract for the SKR malt, which was not detected for either the RKR or TDR malt. This indicates that conditions were more favorable for the generation of radical scavengers in SKR malts at temperatures above 90 °C.

Phenolics. The major phenolics detected in this study were ferulic, *p*-coumaric, and vanillic acids, in line with the literature (*5*, *22*, *23*). Catechin and vanillic, *p*-coumaric, and ferulic acids were identified by retention time and spectral matching in ethyl acetate extracts prepared from the acetate buffer extracts of malt taken throughout the different heating regimens. A further peak (λ_{max} = 320 nm), present in all malt extracts and eluting close to ferulic acid, was also monitored. The chromatograms became increasingly more complex as kilning progressed.

Levels of ferulic acid increased significantly as the grain temperature increased to 80 °C in SKR and to 70 °C in RKR (**Figure 5a**). These increases correspond to decreases in moisture from 7.13 to 5.12% for SKR malt and from 6.25 to 6.00% for RKR malt. As the grain temperature increased further, with moisture decreasing in consequence, there were decreases in the detected level of ferulic acid. No significant differences were detected in the levels of ferulic acid in TDR malt extracts throughout heating.

The concentration of *p*-coumaric acid, although much lower than that of ferulic acid, changed throughout kilning, depending upon the heat treatment (**Figure 5b**). For SKR and RKR malts,

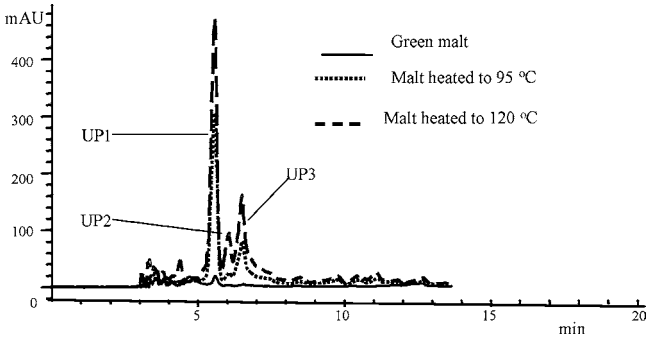

Figure 6. HPLC chromatogram (280 nm) of ethyl acetate extracts prepared from SKR green malt and SKR malt heat treated to 95 and 120 °C. Three new peaks (UP1, UP2, and UP3) were detected in the chromatograms of the 95 and 120 °C sample extracts.

the maximum concentration of *p*-coumaric acid was detected at 70 °C. The levels decreased as the grain temperature increased further from 70 to 85 °C. No significant differences in the level of *p*-coumaric acid were detected in SKR and RKR malt during postkilning heating (95−120 °C), nor were any significant differences detected in levels of *p*-coumaric acid in TDR extracts throughout the heating.

Levels of vanillic acid were similar to those for *p*-coumaric acid (**Figure 5c**). The maximum level of vanillic acid was detected at 70 °C for RKR and at 80 °C for both SKR and TDR malts. No significant differences in vanillic acid concentration were detected between the different finished malts.

Catechin was a small peak, the peak area of which increased with grain temperature. However, as heating progressed this peak was not fully resolved, catechin appearing as a shoulder on an adjacent peak. This prevented catechin from being readily quantified.

Three new peaks (UP1−3) were detected in the SKR and RKR trial sets (λ_{max} = 280 nm) as the temperatures were increased, indicating that they contain new compounds formed during kilning (**Figure 6**). These peaks appeared in TDR HPLC chromatograms as the grain temperature increased to >110 °C. Retention times of the peaks do not match the retention times of authentic commercial samples of vanillin or 4-vinylguaicol, both of which are degradation products of ferulic acid.

A further unidentified peak (UP4) eluted after ferulic acid (29 min, not shown in **Figure 6**), and its spectrum is very similar to that of ferulic acid. Changes in the level of UP4 occurred throughout heat treatment of the malt (not shown). The maximum concentration of UP4 was detected in grain heated to 70 °C for RKR and to 80 °C for SKR. Some differences in the levels of this peak were detected throughout heat treatment for the TDR extracts. There was an increase in the peak area as the grain was heated from 65 to 85 °C. However, with small peak areas and high standard deviations, the increases were not significant.

The observed increase in free phenolics in the early stages of kilning (up to 80 °C) could be due to either enzymatic release of bound phenolics and/or easier extractability of the free phenolics due to changes in the matrix as discussed by Maillard and Berset (*5*). However, beyond 80 °C the decreases in the moisture content of the malt are not significant, and therefore alterations to the matrix are less likely and enzymatic activity would also be limited. Therefore, an alternative explanation is required for the increases observed at high temperatures.

Ferulic acid esterases, which release bound ferulic acid from the matrix, have been isolated from various sources. They have

been determined to have optimum activity at 40−65 °C (*24*−*26*). This implies that such enzymes would not exhibit substantial activity in the later stages of kilning. However, many enzymes used in brewing (α- and β-amylases) are denatured in solution at temperatures at which they survive during kilning (*24*). Ferulic acid esterases, isolated from kilned malt, have been found to be active and effective in the release of ferulic acid from methyl ferulate as substrate (*24*, *27*), showing that they can withstand kilning conditions.

If, as suggested by Maillard and Berset (*5*), the moisture content of the malt affects the matrix and therefore the ease of extractability of the phenolics, the matrix in the RKR sample would be changed more rapidly than that in the SKR sample, as the moisture content decreased to 6% in 16.5 (RKR) and 19.5 h (SKR). This would then affect the level of free phenolic acids. The peak concentration of phenolic acids was attained at a grain temperature of 70 °C in RKR malts and at a grain moisture content of 6%, but peak phenolic concentration was attained at 80 °C in the SKR malts. Therefore, changes in the matrix may have enhanced extractability at moisture contents down to 6%, but, below that, the matrix is not likely to have been affected.

The matrix is also subject to enzyme activity, which will be effective over a range of temperatures and moistures. It may be active over a longer time during the SKR, but may be more active during the shorter RKR. Enzymes work together, and it may be that some enzymes breaking down the matix are active only during short periods, before other enzymes break bonds to release the phenolic acids.

The concentrations of both the various free phenolic acids and antioxidant activity of the malts varied throughout kilning. The correlation coefficient determined for the antioxidant activity (measured by the $ABTS^{\bullet+}$ assay) and total phenolics for the ethyl acetate extract for SKR samples was low (0.0108), but a higher correlation (0.962) was obtained between antioxidant activity and total phenolics in the heating time up to 80 °C. Such a strong positive correlation between the free phenolics and antioxidant activity of the ethyl acetate extract in the initial stages of kilning implies that, during this period, phenolics are responsible for the majority of malt antioxidant activity.

During kilning, phenolic compounds are destroyed or, as in coffee, they may react with free radicals from the Maillard reaction and be incorporated into browning products (*28*, *29*), which exhibit antioxidant activity. Oxygenation and heat treatment in wine can promote the progressive polymerization of phenols to form brown macromolecules, which retain antioxidant activity (*30*). Therefore, similar reactions during kilning could result in some antioxidant activity in the finished malt relating to the original phenolic compounds of the malt.

The heat treatment applied to green malt affects the color, antioxidant activity, and phenolic acid profile of the final malt. Comparison of SKR and TDR malts, which differ in heat treatment at temperatures between 65 and 95 °C, shows that kilning induces the formation of compounds responsible for color and antioxidant activity. Processing conditions can affect the rate of release of the bound phenolics from green malt. As the grain was rapidly pushed through to break point, the maximum level of ferulic acid was detected at a lower temperature, 70 °C, for RKR, as compared to 80 °C for SKR malt.

No difference in radical scavenging ability at the end of kilning (95 °C) was detected between the SKR and RKR malts, but differences between the two malt antioxidant activities when determined by the FRAP assay were apparent. This indicates that the formation of components with reducing power is affected by the processing conditions in the early, prebreak, stages of kilning. The high correlation between phenolic compound concentration and antioxidant activity, at temperatures up to 80 °C, implies that the phenolic constituents of malt are the major contributor to antioxidant activity when kilning up to this temperature, but, as the grain temperature is increased further, their contribution becomes less important, Maillard products probably participating progressively more prominently.

The contribution of the free phenolic acids constitutes only a small percentage (5%) of the total antioxidant activity of malt. However, as they are naturally occurring, effective antioxidants, their fate throughout kilning is important. Maximizing their presence in finished malt is likely to help to maintain flavor stability in beer as well as having implications for human health and disease. Future work is planned to attempt to increase the level of these active compounds in malted barley, which is likely to be beneficial to the brewing industry.

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