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Effect of Kilning on the Antioxidant and Pro-oxidant Activities of Pale Malts

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Pale malts were prepared using standard and rapid kilning regimes that differed in the temperature and moisture profiles in the kiln. Samples were taken over the last 9 h of kilning, that is, at 18, 20, 22, 25, and 27 h. Antioxidant activity, assessed by redox potential, scavenging of the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$), and ferric reducing/antioxidant power (FRAP), increased at moisture levels below 6.7% for both regimes. The 27 h malt exposed to the rapid regime (moisture content of 4.8%) had a higher activity than the 27 h standard regime sample (moisture content of 4.8%). None of the malts scavenged oxygen. Pro-oxidant activity profiles were different for the malts obtained using each regime and, at 27 h, the rapid procedure gave malt with higher activity. Levels of (+)-catechin and ferulic acid (the most abundant phenolic compounds identified) generally increased as the moisture content of malt fell below 6.7%. Differences in antioxidant and pro-oxidant activities of the 27 h malts are partly attributed to the Maillard reaction, as evidenced by lower *L** and higher *b** values and higher levels of Maillard-derived flavor compounds, in the sample obtained by the rapid procedure. Levels of lipid-derived flavor compounds were significantly higher after 27 h of kilning using the rapid procedure.

KEYWORDS: Malt; kilning; antioxidant activity; antioxidant methods; pro-oxidant activity; moisture

INTRODUCTION

Pale malt production is a three-stage process involving steeping (to initiate embryo growth), germination (to allow enzymatic modification of the endosperm), and kilning. Kilning involves drying the malt for up to 30 h and results in a stable product that can be readily handled, stored, and milled (*1*).

During the first 16−18 h of kilning, warm (40−65 °C) moist [40% relative humidity (RH)] air is passed through the grain, allowing continued growth of the malt and enzymatic modification of the endosperm. At the grain bed surface, the RH of the exhausted air falls and the temperature of the grain increases sharply (*1*), this stage being known as the "break point" (*2*). The final 5−8 h of kilning (curing) involves higher (typically 80−95 °C) temperatures, causing the development of color, flavor, and aroma (*3*). During kilning, the moisture content of the grain decreases from ∼45 to 5%, and its temperature simultaneously increases from 20 to ∼70−85 °C (*3*). Manipulation of processing time, temperature, and air flow gives malts with different physical, chemical, and biochemical properties (*3*).

Antioxidants reduce the rate of oxidation reactions. They may scavenge free radicals and active oxygen, act as reducing agents, and chelate metal ions. Antioxidants play a crucial role in maintaining beer flavor stability during processing and storage (*4*). Thus, the protection of the indigenous antioxidants of barley, including polyphenols such as (+)-catechin and ferulic acid, and the generation of new antioxidants during malting, including Maillard reaction products such as reductones (*5*) and melanoidins, are of great interest (*6*).

Recent reviews of methods available to assess the antioxidant properties of food systems reinforce the importance of using several procedures to predict antioxidant activity (*7*). There are very few literature reports of the effect of the kilning regime on the antioxidant and pro-oxidant properties of malts, and only one paper (*4*) deals with possible relationships among data sets obtained using different antioxidant methods applied to the same malts. However, Bamforth and co-workers have extensively studied and reviewed the effect of oxidation during brewing (*8*). Therefore, the main aim of the current study was to compare a range of methods for assessing the antioxidant (and pro-oxidant) properties of malts produced by two different kilning regimes. In addition, the levels of selected polyphenols, color properties, and selected flavor compounds formed were compared, and relationships among the various data sets were sought.

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Table 1. Kilning Conditions Used for the Production of SKM and RKM[a]

	SKM			RKM		
kilning time (h)	temp (°C) air-on	temp (°C) air-off	fan speed (%)	temp (°C) air-on	temp (°C) air-off	fan speed (%)
1–2	40	22–24	100	40	22–23	100
3–4	45	24–25	100	45	23–24	100
5–6	50	25–26	100	50	24–26	100
7–10	55	26	70	55	26–28	100
11–12	60	26	65	60	28–40	100
13–17	60	26–30	40	60	40–58	100
18–19	65	30–42	40	65	58–62	100
20	70	42–46	40	70	62–68	100
21	75	46–54	40	75	68–72	100
22	80	54–62	40	80	72–76	100
23–27	85	72–79	40	95	76–90	75[b]

[a] Samples were taken after the break point at 18, 20, 22, 25, and 27 (end) h. [b] Reduction of fan speed was necessary to raise the air-on temperature to 95 °C.

EXPERIMENTAL PROCEDURES

Materials and Reagents. High-purity water, produced in-house using a Purite (High Wycombe, U.K.) Labwater RO50 unit, was used for all chemical analyses. Acetonitrile, methanol, and trifluoroacetic acid (TFA) (all of HPLC grade) were from Fisher Scientific (Loughborough, U.K.). (+)-Catechin, ferulic acid, sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid), vanillic acid, *p*-coumaric acid, chlorogenic acid, caffeic acid, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,4,6-tripyridyl-*s*-triazine (TPTZ), ferric chloride, and saffron were from Sigma (Poole, U.K.). 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) was from Aldrich (Gillingham, U.K.).

Malt Preparation. Pale malts were prepared from optic barley in the 50 kg pilot plant at Brewing Research International (Lytel Hall, Nutfield, Surrey, U.K.). The steeping regime was 14 h wet/12 h air rest and 4 h wet/2 h air rest at 15 °C. The germination process used temperatures increasing from 15 to 18 °C over 4 days. Two different kilning regimes were used. The first was a standard procedure (SKM), using 90% air recirculation between 30 and 70% RH and 100% air recirculation below 30% RH, and a variable fan speed, resulting in malt with a color value of 4 °EBC. The second regime was a rapid procedure (RKM), which dried the malt at a higher rate, used no air recirculation, and employed the maximum fan speed throughout, resulting in more rapid removal of moisture and a malt with a color value of 5 °EBC (**Table 1**).

Moisture Content of Malts. The moisture content of samples was measured using a Mettler (London, U.K.) LP16 infrared drier attached to a Mettler PM2000 laboratory balance, with all samples being analyzed in triplicate.

Preparation of Malt Extracts. For all antioxidant and pro-oxidant analyses (apart from oxygen consumption) acetate buffer extracts of the malts were prepared, and hot water malt extracts were prepared for color and flavor analyses as previously described (*9*). Extracts were frozen at −30 °C for <3 weeks prior to determination of redox potential and pro-oxidant activity. Preliminary experiments showed that measurements were not significantly affected by these storage conditions. Extracts were prepared fresh for the other analyses.

$ABTS^{\bullet+}$ Radical Scavenging Activity of Malt Extracts. The $ABTS^{\bullet+}$ was generated according to the method of Araki et al. (*10*), with modifications described by Woffenden et al. (*9*). Determinations were carried out as previously described (*9*). In summary, chromogen solution and malt extract or catechin standard were mixed and incubated at 25 °C, prior to measurement of the absorbance at 734 nm and determination of the radical scavenging activity relative to a standard [(+)-catechin]. Samples were analyzed in triplicate.

Redox Potential of Malt Extracts. Measurements were made using a platinum indicating electrode and a silver/silver chloride reference electrode, connected by a voltmeter (Hanna Instruments, model 8417, Milan, Italy). Calibration of the electrode was performed against a redox standard solution ($E° = 468$ mV at 25 °C). The electrode was inserted into a 50 mL three-neck flask containing a mixture of pale malt extract (10 mL) and distilled water (8 mL). Prior to analysis, oxygen was removed from the flask by continuous flushing with nitrogen for 10 min. The redox potential (E) was recorded for at least 15 min, until a stable reading was reached, that is, until E changed by <1 mV over 5 min. Samples were analyzed in triplicate.

Ferric Reducing/Antioxidant Power (FRAP) of Malt Extracts. The assay was carried out according to the method of Benzie and Strain (*11*). In summary, the TPTZ reagent (3 mL) and malt extract (100 μL) were mixed and incubated at 25 °C. The absorbance at 593 nm was measured exactly 5 min after initial mixing. (+)-Catechin standard solutions and blanks (using acetate buffer in place of malt extract) were run. Determinations were carried out in triplicate on duplicate extracts.

Oxygen Consumption of Malts. Ground malt (1 g) was introduced into a 10 mL capacity vial, hermetically sealed, and equilibrated at 18 °C for 48−52 h. Malts were analyzed for headspace oxygen consumption using a Fisons (Milan, Italy) model 8540 gas chromatograph, equipped with a thermal conductivity detector and two 2 m × 2 mm i.d. glass columns packed with Porapak QS 80−100 mesh. Significant operating conditions were as follows: column temperature, 100 °C; oven temperature, 120 °C; filament temperature, 170 °C; injector temperature, 180 °C; carrier gas flow rate, 27 mL min^{-1}. A headspace volume (0.4 mL) was injected using a precision sampling syringe (Dynatech, New York, NY) provided with a pressure lock system and a gas column (capacity = 0−1 mL). Chromatograms were recorded and analyzed using Chrom-Card for Windows software (version 1.14, Fisons, Milan, Italy). Duplicate extracts were analyzed, and the area of the oxygen peak of each malt was compared to that of an air blank.

Pro-oxidant Activity of Malt Extracts. Determination of pro-oxidant activity of malt extracts was performed as previously described (*12*). In summary, crocin was isolated from saffron and diluted in methanol to give a 0.144 mol/L crocin solution and then with 0.1 M phosphate buffer (pH 7) to obtain a 1.35×10^{-5} M solution ($A = 1.8$ at 443 nm). Crocin solution (2 mL) was mixed with malt extract (75 μL). The absorbance at 443 nm was monitored every 30 s for 10 min. Blank determinations were performed using 50 mM acetate buffer (pH 5.4) in place of malt extract. Results were expressed as V, where

$$V = \frac{A_0 - A_{10}}{AC_0 - AC_{10}}$$

and A_0 and A_{10} = absorbance of samples after 0 and 10 min, respectively, and AC_0 and AC_{10} = absorbance of blanks after 0 and 10 min, respectively.

Triplicate extracts were analyzed.

High-Performance Liquid Chromatography (HPLC). HPLC was performed on a Hewlett-Packard (HP, subsequently Agilent, Bracknell, U.K.) model 1050 series II LC with a quaternary solvent delivery system and an autoinjector and coupled to a UV−visible diode array detector. HP ChemStation software was used for system control, data acquisition, and analysis. A 250 × 4.6 mm i.d., 5 μm, Sphereclone C_{18} ODS column was used, connected to a 30 × 4.6 mm guard column packed with the same stationary phase (Phenomenex, Macclesfield, U.K.). Separations were performed using solvent A (0.05% TFA in acetonitrile) and solvent B (0.05% TFA in water). A linear gradient was run from 5% solvent A to 30% solvent A over 25 min. The column was washed with 80% solvent A between runs before a return to the initial solvent composition. The flow rate was 1 mL/min and the injection volume 30 μL. Separations were monitored at 280 nm, and data were collected from 200 to 600 nm. All solvents and samples were 0.45 μm filtered, prior to analysis. Calibration curves were prepared for standard compounds by injecting triplicate solutions once. Single extracts were injected in triplicate with the exception of the 27 h samples, for which triplicate extracts were injected in triplicate. Components were identified in malt samples by matching their retention times and UV diode array spectra with those of standard compounds.

Analysis of Color. Color measurements were obtained in CIEL*a*b* space by transmission spectrophotometry as previously described (*9*). Single analyses were carried out on triplicate extracts.

Table 2. Effect of Kilning on the Moisture Content (Percent) of Pale Malt[a–c]

kilning time (h)	SKM	RKM
18	7.5	6.7
20	6.8	6.5
22	6.7	6.0
25	5.5	5.1
27	5.3	4.8

[a] Average of single determinations on triplicate samples. [b] Percent coefficient of variation ≤2.2%. [c] Differences between SKM and RKM were significant ($p < 0.001$) at every sampling time, as determined by Student's *t* test.

Analysis of Flavor Compounds. Flavor compounds were isolated from 27 h SKM and RKM onto Tenax TA and subsequently analyzed by GC-MS as previously described (*9*). Relative amounts (RAs) of compounds were obtained from the mass spectral integration report, with reference to the internal standard:

$$\mathrm{RA} = \frac{\text{area of sample peak}}{\text{area of internal standard peak}} \times 100$$

Single analyses were performed on triplicate extracts.

Statistical Analysis. All data were corrected for differences in moisture content of the pale malt prior to analysis, statistical analysis was carried out using Student's *t* test (Excel 2000, Microsoft, Redmond, WA).

RESULTS

Processing Conditions. The air-on temperature profiles for SKM and RKM were the same up to 23 h. After 23 h, the temperature applied to RKM was 10 °C higher than that applied to SKM (**Table 1**). Despite little difference in the air-on temperature profile between the two regimes, the air-off temperature profiles (considered to be close to the temperature of the malt) were different, due to differences in the air recirculation conditions and fan speed (**Table 1**). These differences gave an earlier break point for RKM, resulting in a more intense heat treatment and significantly lower ($p < 0.001$) moisture contents at each sampling point (**Table 2**).

Redox Potential. The method was considered to be sufficiently sensitive to measure differences of >5 mV. **Figures 1a** and **2a** show that the main effect of kilning on redox potential was a significantly higher ($p < 0.001$) value (lower antioxidant activity) for 18 h SKM, with a moisture content of 7.5%.

$ABTS^{\bullet+}$ Radical Scavenging Activity. The ability of SKM to scavenge $ABTS^{\bullet+}$ decreased significantly (by 11%) between 18 and 22 h ($p < 0.01$) followed by significant increases between 22−25 h ($p < 0.01$) and 25−27 h ($p < 0.001$) (**Figure 1b**). Overall, the radical scavenging ability of the samples increased by 24% over the last 5 h of kilning. A significant increase in activity was observed for RKM between 18 and 27 h (18−22 h, $p < 0.01$; 22−25 h, $p < 0.001$; 25−27 h, $p < 0.001$), resulting in an overall 29% increase. Radical scavenging activity was significantly higher at each sampling time above 20 h ($p < 0.001$) for RKM, compared to SKM. When the moisture content was reduced below 6.7%, radical scavenging activity increased for both sets of samples (**Figure 2b**).

FRAP Assay. All of the extracts reduced Fe^{3+}-TPTZ to Fe^{2+}-TPTZ (**Figures 1c** and **2c**). There was no significant difference in FRAP value for SKM between 18 and 27 h or for RKM between 18 and 25 h, suggesting that moisture levels above 5.3% have no effect on the FRAP value. However, between 25 and 27 h (corresponding to a moisture reduction from 5.1 to 4.8%), a significant increase in activity (8 μM catechin equivalents, $p < 0.01$) was observed for RKM, resulting in a significantly higher ($p < 0.01$) FRAP value for the 27 h RKM, compared to 27 h SKM.

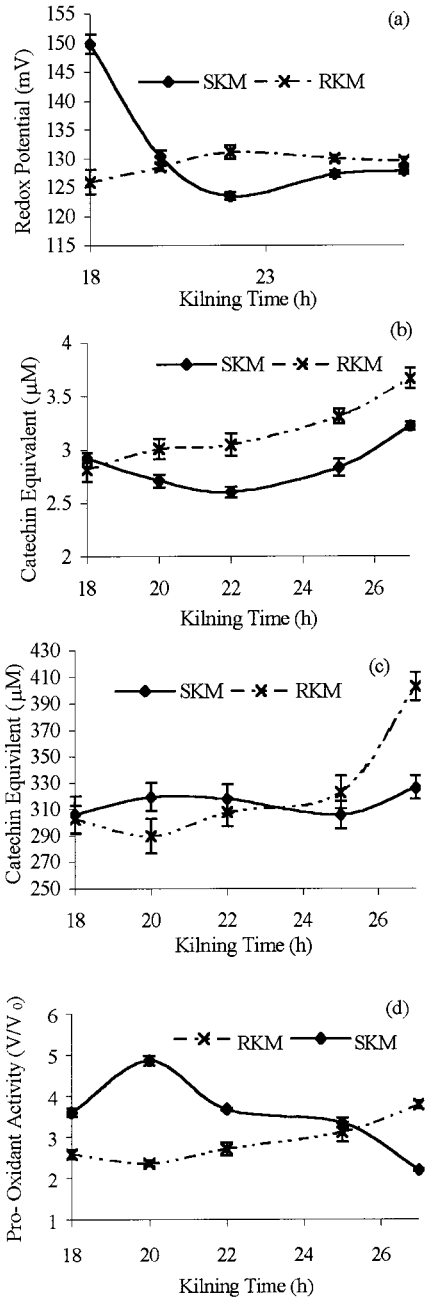

Figure 1. Effect of kilning time on antioxidant and pro-oxidant activities of SKM and RKM: (a) redox potential; (b) $ABTS^{\bullet+}$ radical scavenging activity; (c) FRAP value; (d) pro-oxidant activity. Error bars represent the standard deviation.

Oxygen Consumption. There was no significant difference ($p > 0.1$) among oxygen peak areas for any of the samples and the blank, indicating that the malts do not have the ability to scavenge free oxygen.

Pro-oxidant Activity. Pro-oxidant activity of SKM increased significantly ($p < 0.001$) by 33% between 18 and 20 h (**Figure 1d**). Longer heating times resulted in a significant decrease at each subsequent sampling interval (20−22 h, $p < 0.001$; 22−25 h, $p < 0.01$; 25−27 h, $p < 0.001$). Between 20 and 27 h, pro-oxidant activity was reduced by 54%, to levels below those at 18 h. In contrast, no significant difference in pro-oxidant activity was observed for RKM between 18 and 20 h, whereas

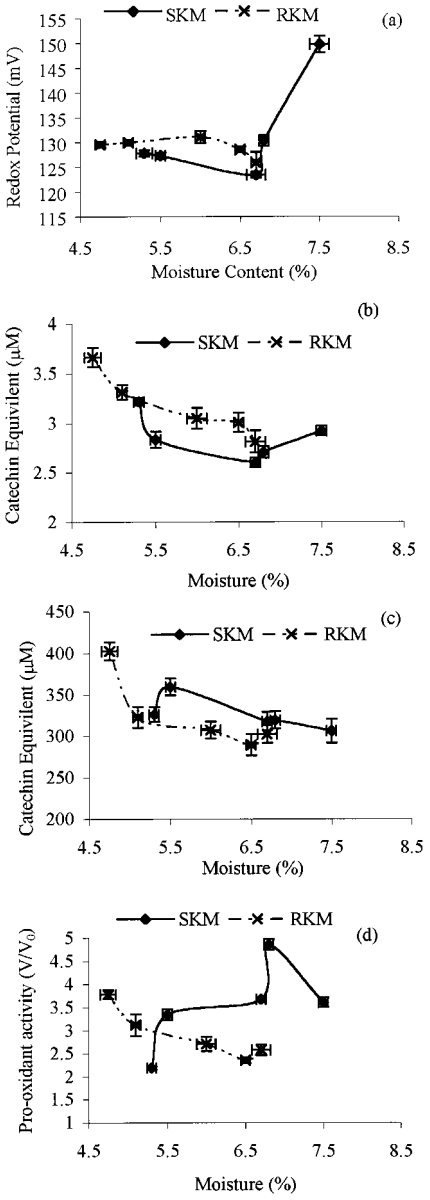

Figure 2. Effect of moisture content on antioxidant and pro-oxidant activities of SKM and RKM: (a) redox potential; (b) $ABTS^{\bullet+}$ radical scavenging activity; (c) FRAP value; (d) pro-oxidant activity. Error bars represent the standard deviation.

significant increases were obtained between 20 and 22 h ($p < 0.05$) and between 25 and 27 h ($p < 0.01$) (**Figure 1d**). Between 20 and 27 h, pro-oxidant activity increased by 61% for RKM. SKM possessed significantly higher activities ($p < 0.001$) than RKM at 18, 20, and 22 h but significantly lower activity at 27 h ($p < 0.001$). Although the relationship between antioxidant activity and moisture was the same for both kilning regimes, the relationship between pro-oxidant activity and moisture was different for each regime. There was no correlation between moisture and pro-oxidant activity for SKM, whereas pro-oxidant activity increased with decreasing moisture for RKM (**Figure 2d**).

HPLC Analysis of Polyphenols. For standard compounds, the coefficient of variation (CV) values were <1.5% and plots of concentration against peak area gave r^2 values of >0.99. All of the compounds were resolved, except ferulic acid and sinapic acid, which coeluted. Ferulic acid occurs in ~11 g/L amounts in beer, and it is considered to originate exclusively from the malt (*13*), whereas sinapic acid occurs in only trace amounts in

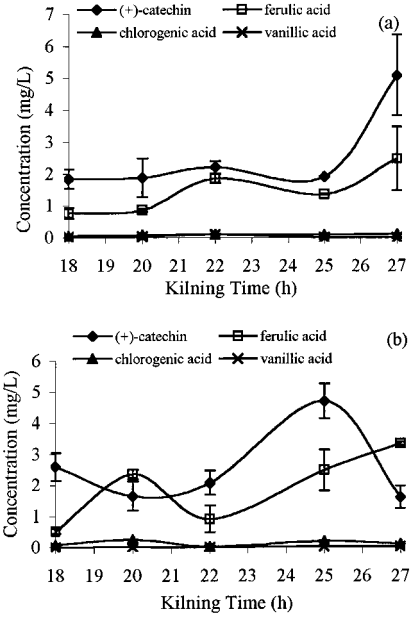

Figure 3. Effect of kilning time on levels of (+)-catechin, ferulic acid, chlorogenic acid, and vanillic acid: (a) SKM; (b) RKM. Error bars represent the standard deviation.

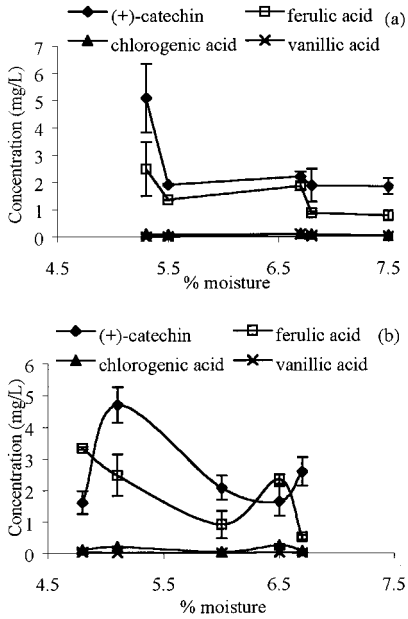

Figure 4. Effect of moisture content on levels of (+)-catechin, ferulic acid, chlorogenic acid, and vanillic acid: (a) SKM; (b) RKM. Error bars represent the standard deviation.

malt (*3*). Therefore, in the current study, this peak was considered to be due mainly, if not exclusively, to ferulic acid.

The effects of kilning time and moisture content on the concentrations of (+)-catechin, ferulic acid, chlorogenic acid, and vanillic acid are shown in **Figures 3** and **4**. Two unidentified peaks (**U1** and **U2**) were also detected. They had spectra (**Figure 5**) that resembled those of (+)-catechin and ferulic acid, respectively. They were larger than any of the peaks due to the identified phenolic compounds, and the effects of kilning time and moisture content on their HPLC peak areas are shown in **Figures 6** and **7**.

For SKM, the concentration of (+)-catechin (between 18 and 25 h) changed very little (**Figure 3a**). In contrast, there was a significant increase ($p < 0.05$) of 3.09 mg/L between 25 and

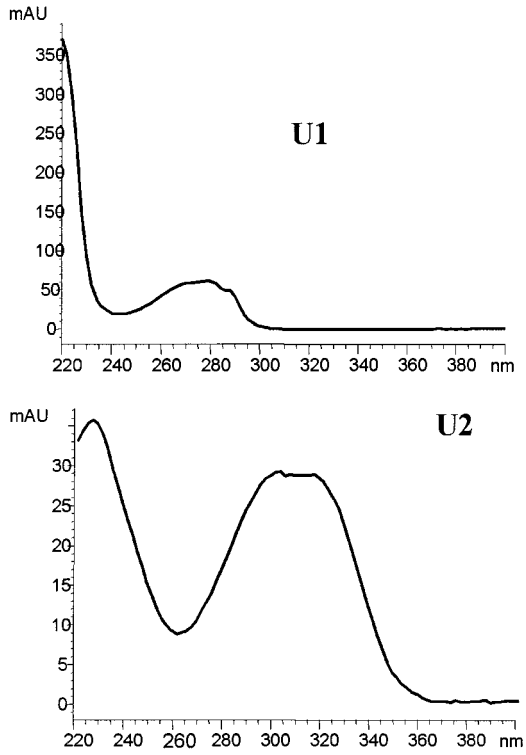

Figure 5. UV spectra of peaks **U1** and **U2**.

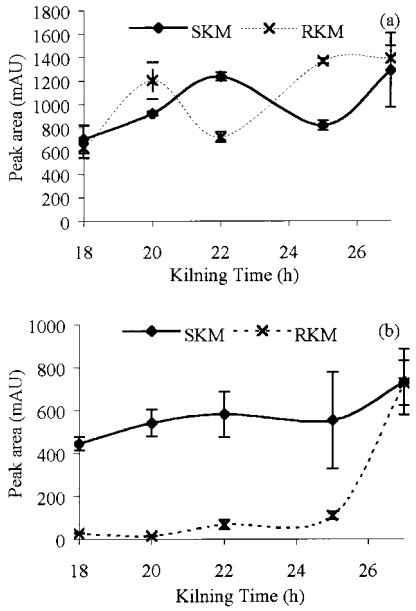

Figure 6. Effect of kilning time on areas of peaks (a) **U1** and (b) **U2**. Error bars represent the standard deviation.

27 h, corresponding to a moisture content of <5.5% (**Figure 4a**). For RKM, the (+)-catechin concentration was not significantly different between 18 and 22 h but, between 22 and 25 h, there was a significant increase of 2.7 mg/L (corresponding to a decrease in moisture content from 6.0 to 5.1%), followed by a significant decrease of 3.1 mg/L between 25 and 27 h (corresponding to a further reduction in moisture content to 4.8%) (**Figures 3b** and **4b**). When the two kilning regimes were compared, levels of (+)-catechin were not significantly different ($p > 0.1$) between 18 and 22 h but became significant at 25 h ($p < 0.001$) and 27 h ($p < 0.05$).

Moisture appears to have an important effect on levels of ferulic acid, regardless of the kilning regime. **Figure 4** shows

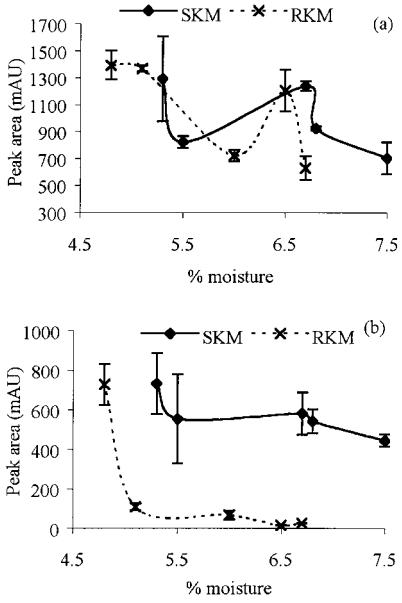

Figure 7. Effect of moisture content on areas of peaks (a) **U1** and (b) **U2**. Error bars represent the standard deviation.

that levels increased significantly ($p < 0.001$) as the moisture decreased to 6.7% in SKM (corresponding to 22 h) or to 6.5% in RKM (corresponding to 20 h). Then levels decreased significantly ($p < 0.05$) when the moisture fell further to 5.5% in SKM (corresponding to 25 h) or to 6.0% in RKM (corresponding to 22 h) and finally increased nonsignificantly for the remainder of each kilning process. When data for the 20−25 h SKM and RKM are compared, ferulic acid was significantly higher for RKM at 20 h ($p < 0.001$) and 25 h ($p < 0.05$) but significantly lower ($p < 0.05$) at 22 h (**Figure 3**).

The concentrations of chlorogenic acid and vanillic acid were too low (<0.25 mg/L) to be amenable to interpretation.

Levels of **U1** demonstrated a significant increase ($p < 0.05$) between 18 and 22 h, followed by a significant decrease ($p < 0.05$) between 22 and 25 h and an increase ($p < 0.05$) between 25 and 27 h in SKM (**Figure 6a**). In RKM, a significant increase ($p < 0.05$) was observed between 18 and 20 h, followed by a significant decrease ($p < 0.05$) between 20 and 22 h and a significant increase between 22 and 25 h. There was no significant change between 25 and 27 h. Plots of moisture content against peak area values for **U1** (**Figure 7a**) were remarkably similar to those obtained for ferulic acid (**Figure 4**).

Amounts of U2 were not significantly different between 18 and 27 h in SKM or between 18 and 25 h in RKM, but a significant increase ($p < 0.001$) was observed between 25 and 27 h for RKM (**Figure 6b**). Amounts of U2 were significantly higher for SKM than for RKM at all time points except 27 h, when the value for RKM increased to that of SKM. **Figure 7b** shows that levels of U2 were significantly higher for SKM than for RKM over the entire moisture range examined.

Color. No significant difference ($p > 0.05$) in a^* value (positive values denoting redness) was observed during kilning for either SKM or RKM. L^* (lightness/darkness values) and b^* values (positive values denoting yellowness) showed no significant difference between 18 and 25 h, but significant ($p < 0.001$) decreases in L^* and increases in b^* values occurred between 25 and 27 h of kilning for both regimes (**Figure 8**). Differences between the 27 h SKM and RKM samples were not significant, but the b^* value was significantly higher ($p < 0.01$) for RKM.

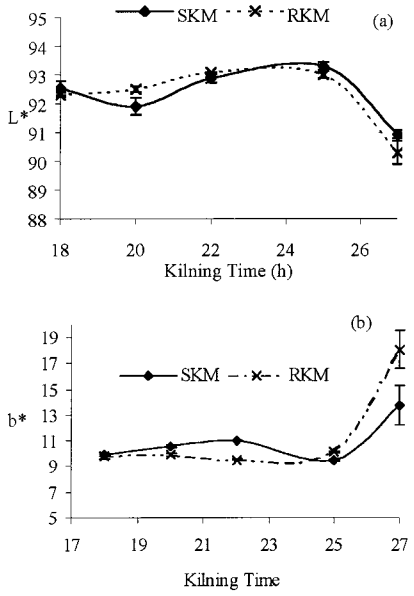

Figure 8. Effect of kilning time on (a) *L** and (b) *b** values of SKM and RKM samples. Error bars represent the standard deviation.

Table 3. Effect of Kilning Regime on the Relative Amount of Flavor Compounds in the Finished Malt (27 h)

	relative amount[a]	
compound[b]	SKM	RKM
ethanol	25	37
dimethyl sulfide	<1	27
methylpropanal[c]	29	171
butanedione	8	13
2-butanone	23	13
2-methyl-1-propanol[c]	15	28
3-methylbutanal[c]	225	1603
2-methylbutanal[c]	110	813
pentanal[d]	18	39
3-methyl-1-butanol[c]	25	46
2-methyl-1-butanol[c]	11	43
1-pentanol[d]	22	58
hexanal[d]	79	197
2-furfural[c]	6	64
(*E*)-2-hexanal[d]	5	69
1-hexanol[d]	7	24
heptanal[d]	16	67
(*E*)-2-heptenal[d]	5	73
benzaldehyde[d]	9	104
1-octen-3-ol[d]	7	54
octanal[d]	11	94
nonanal[d]	20	203
(*E*)-2-nonenal[d]	4	167
decanal[d]	7	130

[a] Data quoted are the means of triplicate analyses, and the coefficient of variation was <25% for all analyses. [b] Compounds were positively identified by matching sample MS and LRI data with those of standard compounds injected on the same stationary phase. [c] Maillard reaction product. [d] Lipid oxidation product.

Flavor Compounds. Significantly higher ($p < 0.05$) RAs of all of the monitored compounds were observed for 27 h RKM compared to 27 h SKM (**Table 3**).

DISCUSSION

Effect of Kilning on Antioxidant Activity. Plots of the data (**Figure 1a−c** and **2a−c**) obtained for the same malt samples using the three methods of determining antioxidant activity cannot be superimposed and even indicate different behaviors in some cases. The data reflect differences in the nature of the compounds responding to the different antioxidant assays, as well as the changing composition of the malts during kilning. Compositional differences may be due to the degradation or other reaction of compounds, for example, phenolics, the generation of compounds, for example, via the Maillard reaction, or the interaction of phenolics or their degradation products with Maillard compounds. Maillard et al. (*6*) observed an increase in antioxidant activity (inhibition of methyl linoleate oxidation) for malts, compared to barley.

Measurement of the antioxidant activity of a food is not easy, due to the complexity of the system and the range of possible oxidative mechanisms (*7*). Most antioxidant assays measure a specific type of activity, and thus particular groups of compound, with different methods yielding different information. The use of a range of antioxidant methods provides insights into the possible types of antioxidant activity operating in a system. The total reducing power of a system may be measured using a redox electrode, which measures the ability of a system to donate an electron to a species of known redox potential (*14*). It is a thermodynamic measurement, and therefore the activity of any slow-acting antioxidants is taken into account. Data from the $ABTS^{\bullet+}$ procedure give a kinetic measure of the ability of the antioxidants of a system to scavenge free radicals (*10*), but slow-acting antioxidants are not measured. FRAP values indicate the total reducing capacity of the electron-donating antioxidants of a system but, because no oxidizable substrate is used, no information is provided concerning the protective effects of the antioxidants (*7*). Again, being a kinetic measurement, FRAP values do not provide information about the slow-acting reducing agents (*15*).

Changes in antioxidant activity with kilning time, observed from the redox potential, $ABTS^{\bullet+}$, and FRAP data, may be attributed in part to modifications of phenolic compounds in the malt. The most abundant phenolics identified were (+)-catechin and ferulic acid, in line with previous studies on malt (*4*, *6*). Plots of catechin concentration against both time of kilning and moisture content (**Figures 3** and **4**) show a behavior fairly similar to that of the $ABTS^{\bullet+}$ and FRAP data (**Figures 1b**,**c** and **2b**,**c**). However, differences between the catchin and antioxidant data in the current study include a decrease in catechin concentration but an increase in antioxidant activity for the 27 h (4.8% moisture) RKM sample. Also, variations between $ABTS^{\bullet+}$ values for SKM and RKM were greater than for the FRAP values and concentrations of (+)-catechin. Differences may be partly attributed to degradation or further reaction of (+)-catechin under the relatively high-temperature/low-moisture conditions experienced by 27 h RKM. Although there are no previous reports of the relationship between (+)-catechin concentration and antioxidant activity in malts, levels of total flavan-3-ols extracted from malt by organic solvent have previously been shown to correlate positively with $DPPH^{\bullet}$ scavenging activity (*4*).

Decreases in redox potential and increases in radical scavenging ability of samples toward the end of kilning may be due in part to the increased antioxidative activity of oligomerized polyphenols, including (+)-catechin, compared to their monomeric counterparts (*16*). There is no information in the literature concerning the relationship between polyphenol oligomerization and iron reduction. Increased antioxidant activity of polyphenol oligomers may be attributed to their increased ability to donate a hydrogen atom and/or to support an unpaired electron through delocalization (*17*). However, the rate at which polyphenol oligomers act as antioxidants is lower than that of their

monomeric counterparts (*18*). The lower levels of (+)-catechin coupled with the higher antioxidant activity (ABTS$^{\bullet+}$ and FRAP data) of 27 h RKM, compared to 27 h SKM, suggest that the lower rate of action of any polyphenol oligomers did not reduce the ABTS$^{\bullet+}$ or FRAP values. This is in line with Hagerman et al. (*16*), who showed that formation of the catechin trimer in a model system caused an increase in ABTS$^{\bullet+}$ scavenging ability. Polyphenol oxidase-mediated oligomerization of polyphenols is likely to be affected by moisture level, which will influence both enzyme activity and substrate mobility (*19*). At the end of kilning, levels of (+)-catechin were about twice as high in SKM (5.3% moisture) as in RKM (4.8% moisture), suggesting that differences in the kilning regimes, including moisture conditions, may have affected (+)-catechin levels in these samples.

Plots of ferulic acid concentration against kilning time were similar to those obtained for the ABTS$^{\bullet+}$ and FRAP data, in line with previous reports. Evolution of total bound phenolic acids during kilning approximately paralleled antioxidant activity using a method based on the autoxidation of methyl linoleate (*6*), and positive, nonlinear relationships have been demonstrated between antioxidant activity and both free and bound total phenolic compounds for extracts of malt rootlets (*20*). In the current study, differences between SKM and RKM were apparent. Over the moisture range 5.5−6.7%, RKM showed more activity than SKM in the ABTS$^{\bullet+}$ assay, whereas the opposite was the case for the FRAP assay. Release of ferulic acid, a free radical scavenger (*21*), from lignin and arabinoxylans during kilning at up to 80 °C also contributes to antioxidant activity in malt (*6*). Release is aided by hydrolytic enzymes (*6*) and is also affected by moisture (*22*); levels of ferulic acid increased in oats after steaming and drying at 100 °C (*23*). Yields of ferulic acid (and other phenolic acids) from malt are known to be affected by the barley, kilning conditions, and extraction solvent (*4*).

The unidentified compound **U1** showed behavior very similar to that of ferulic acid, and it is suggested that U1 could be a phenolic acid, a ferulic acid degradation product, or a component formed on reaction of ferulic acid with another compound.

Levels of the unidentified compound **U2** increased between 18 and 27 h of kilning for both regimes. Higher amounts were extracted from SKM, and levels increased mainly in the final stages of kilning, notably for RKM. Plots of **U2** peak area against moisture were similar to plots of the FRAP data, but the FRAP value for 27 h RKM was higher than the level of **U2**, compared to 27 h SKM, probably due to other components of RKM contributing to antioxidant activity.

Although, in general, levels of the monitored compounds and antioxidant activity increased over the last 9 h of kilning [the exception being (+)-catechin in 27 h RKM], correlations between the monitored compounds and antioxidant activity were poor, in line with previous studies (*6*). In addition to the polyphenols and phenolic acids commonly measured in malt, carotenoids and tocopherols, present in barley (*4*), as well as Maillard reaction products generated on kilning, may contribute to the antioxidant activity in malt.

The air-off temperature (representative of the temperature of the malt) was higher for RKM than for SKM over the last 16 h of kilning, and the moisture content of the malt was lower for RKM at each sampling time between 18 and 27 h. The conditions experienced by RKM favor the Maillard reaction, and products of the Maillard reaction that possess antioxidant activity include reductones and melanoidins, both of which are free radical scavengers and reducing agents (*24*−*27*). Melanoidin production during kilning is unlikely to be high, due to the temperature being <100 °C, and it is noteworthy that the pale malts exhibited very low levels of browning (4−5 °EBC). Thus, early or intermediate stage low molecular mass Maillard reaction products, such as reductones, are likely to make a more important contribution to antioxidant activity here, and any contribution made by melanoidins would be higher for RKM, especially the 27 h sample, than for SKM.

Oxygen consumption occurs in severely heated Maillard systems and in systems heated for prolonged times (140−260 h) at low temperatures (40 °C) (*28*, *29*). Melanoidins are the most likely Maillard products to consume oxygen (*29*). The oxygen consumption data obtained in the current study suggest that storing ground pale malt in air should not affect its antioxidant activity.

Comparison of Color, Flavor Compound, and Antioxidant Activity Data Sets. Darker and more yellow colors, evidenced by lower *L** and higher *b** values, respectively, and higher levels of Maillard-derived flavor compounds were observed for RKM, suggesting that the Maillard reaction had progressed further in malts subjected to the RKM regime compared to the SKM process and that melanoidins are likely to be more important contributors to antioxidant activity in RKM, especially 27 h RKM, than in SKM, as discussed above. Thus, melanoidins could account for the discrepancy between the (+)-catechin concentration and ABTS$^{\bullet+}$ and FRAP values in 27 h RKM. Reductones are colorless compounds and are potent free radical scavengers in aqueous solution (*30*). They are also likely to account for some of the discrepancy between the profiles of phenolic compounds and those of antioxidant activity.

Levels of all monitored flavor compounds formed by lipid oxidation were higher at 27 h in RKM than in SKM, presumably due to the higher temperature and air circulation conditions experienced by RKM during kilning. The air-on temperatures between 23 and 27 h were 85 and 95 °C, respectively, for SKM and RKM (**Table 1**), leading to air-off temperatures of 72−79 and 76−90 °C, respectively. The higher observed levels of antioxidant activity in 27 h RKM, assessed by the ABTS$^{\bullet+}$ and FRAP procedures, together with the higher levels of Maillard reaction products, for example, melanoidins, assessed by the *L** and *b** values, do not appear to be sufficient to suppress lipid autoxidation in this malt.

Effect of Kilning on Pro-oxidant Activity. The different profiles of pro-oxidant activity with kilning time for SKM and RKM suggest that the different processing regimes may lead to different mechanisms and/or rates of formation of radicals from the Maillard reaction. This might result in different reactions occurring between radicals and antioxidants in SKM and RKM. The pro-oxidant assay assesses all of the radicals in a system, including those stabilized by resonance that are not considered to possess pro-oxidant activity (*31*). The profile of pro-oxidant activity for SKM is consistent with the changes in antioxidant activity observed by all three antioxidant assays.

The increase in pro-oxidant activity of SKM between 18 and 22 h, corresponding to a drop in moisture content from 7.5 to 6.7%, could be due to lipoxygenase activity yielding peroxy, alkoxy, and/or alkyl radicals (*19*). The equivalent time-point RKM samples were subjected to more severe heating and were of lower moisture content (6.0−6.7%), both of which could account for any reduction in lipoxygenase activity (*32*). A greater rate of lipid autoxidation in 27 h RKM could account for higher pro-oxidant activity, as well as higher levels of lipid oxidation flavor compounds, as discussed above.

Pro-oxidant activity of the malt extracts may also be attributed to, for example, flavonoids (*33*), procyanidins (*16*), and certain Maillard reaction products (*24*). Several flavonoids can autoxidize, generating reactive oxygen species (*33*). Many phenolic compounds function as antioxidants only at high concentrations, whereas, at lower levels, they may exert pro-oxidant effects (*33*). Procyanidins also have some pro-oxidant effect at low concentrations, although less than the monomeric form (*16*).

The development of low molecular mass compounds with pro-oxidant activity occurs in the intermediate stage of the Maillard reaction (*24*). These compounds may include aminoreductones (*30*) and carbonyls (*34*). Their levels depend on the intensity and duration of heat treatment because, when low temperatures are applied, the reaction steps contributing to the formation of pro-oxidant compounds last longer than with high-temperature treatments (*35*). Melanoidins may also exert pro-oxidant activity (*24*). The pro-oxidant activity of all these compounds depends on their concentration and the prevailing conditions.

Both the $ABTS^{\bullet+}$ and the pro-oxidant activity data are kinetic measurements, and thus a comparison of the data for the two kilning regimes is of interest. For SKM, between 18 and 20 h, pro-oxidant activity increased while antioxidant activity decreased (**Figure 1b**,**d**). The subsequent decrease in pro-oxidant activity, accompanied by an increase in antioxidant activity, between 22 and 27 h, may be explained by either the further reaction of pro-oxidant compounds or the production of compounds with antioxidant activity as kilning progresses. The relationship between antioxidant and pro-oxidant activities in RKM was different, an increase in antioxidant activity being paralleled by an increase in pro-oxidant activity (**Figure 1b**,**d**).

In conclusion, the kilning regime applied to malt affects the antioxidant properties, color, and profile of flavor compounds in the final product. Plots of antioxidant activity against kilning time or moisture are different for redox potential, $ABTS^{\bullet+}$, and FRAP data, reflecting the differential responses of the various malt components in the assays. Antioxidant activity increased below moisture levels of 6.7% for both SKM and RKM. Correlations between levels of (+)-catechin or ferulic acid and antioxidant activity were poor, suggesting that other components, for example, polyphenol oligomers and Maillard reaction products, also contribute to antioxidant activity. RKM used higher temperatures and lower humidity processing conditions and resulted in darker and more yellow malts with higher levels of flavor compounds (formed via both the Maillard reaction and lipid oxidation) than SKM. It is suggested that Maillard reaction products (reductones and melanoidins) contribute to antioxidant activity, especially in the most strongly heated sample (27 h RKM). Although the highest levels of antioxidant activity were observed in 27 h RKM, they were insufficient to suppress lipid oxidation products, the levels of which increased in this malt. All malts exhibited pro-oxidant behavior, values being higher for RKM than for SKM in the finished product.

Future work should focus on two areas. First, the response of malt components in the antioxidant and pro-oxidant assays is required. This should include the study of melanoidins (including different relative molecular mass fractions), reductones, and other Maillard reaction products, as well as polyphenols and their thermal reaction products, tocopherols and carotenoids. Such a study should include the effect of component concentration on response. Second, the role played by individual malt components/fractions in inhibiting the generation of malt off-flavor compounds derived from lipid oxidation requires clarification.

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