

# Relationships between Antioxidant Activity, Color, and Flavor Compounds of Crystal Malt Extracts

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Aqueous extracts were prepared from five barley crystal malts (color range 15–440 °EBC, European Brewing Convention units). Antioxidant activity was determined by using the 2,2'-azinobis(3-ethylbenothiazoline-6-sulfonic acid) (ABTS<sup>+</sup>) radical cation scavenging method. Antioxidant activity increased with increasing color value although the rate of increase decreased with increasing color value. Color was measured in CIELAB space. Extracts of the 15, 23, and 72 °EBC malts followed the same dilution pathway as did the 148 °EBC sample at higher dilution levels, indicating that they could each be used to give the same color by appropriate dilution. The 440 °EBC sample followed a different dilution pathway, indicating that different compounds were responsible for color in this extract. Fifteen selected volatile compounds were monitored using gas chromatography/mass spectrometry (GC/MS). Levels of methylpropanal, 2-methylbutanal, and 3-methylbutanal were highest for the 72 °EBC sample. When odor threshold values of the selected compounds were taken into account, 3-methylbutanal was the most important contributor to flavor. Relationships between levels of the lipid oxidation products, hexanal and (*E*)-2-nonenal, and antioxidant activity were complex, and increasing antioxidant activity for samples in the range of 15–148 °EBC did not result in reduced levels of these lipid-derived compounds. When different colored malt extracts were diluted to give the same *a*\* and *b*\* values, calculated antioxidant activity and amounts of 3-methylbutanal, hexanal, and (*E*)-2-nonenal decreased with increasing °EBC value.

**Keywords:** Malt; crystal malt; antioxidants; antioxidant activity; color; flavor

## INTRODUCTION

Malt is produced from germinating barley by a process involving steeping, germination, and application of a heat treatment, either kilning or roasting (1). The products available can be classified into two groups: pale malts and speciality malts. Pale malts are mildly heated (kilned at up to 95 °C) and are used as the main ingredient for the production of beer. Speciality or colored malts are subjected to roasting at temperatures of up to 250 °C. They are used to provide the flavor and color of beer and may also improve beer flavor stability due to the presence of compounds with antioxidant activity (2). They include crystal, brown, chocolate malts, etc. For crystal malts, a stewing step is included in the process prior to roasting to allow extensive carbohydrate and protein hydrolysis (1).

Malt contains various compounds with antioxidant activity. These include polyphenols from the barley and Maillard reaction products, the latter being generated during malting (3–5). In the production of colored malts, the temperatures experienced enhance the Maillard reaction leading to the formation of color, flavor, and antioxidant activity (2, 6). Maillard reaction products that may contribute to antioxidant activity in malt include compounds that behave as reductones and melanoidins (2, 4).

Components contributing to malt color include colored low molecular weight Maillard reaction products, mel-

anoidins, oxidized polyphenols, and products of sugar caramelization (7, 8); sugar caramelization only being important in very dark malts and roasted barley.

Investigations of crystal malt flavor have established that it possesses burnt, bready, malty, and chocolate-like odors (9). More than 200 components of crystal malt aroma have been identified, including various heterocycles (9, 10). In one study, 3-methylbutanal possessed the highest odor dilution factor (8000), and 2-methylbutanal and various pyrazines were also considered to be important aroma compounds (11).

Flavor stability is an important quality attribute of beer, and (*E*)-2-nonenal, formed by lipid oxidation in malt, is considered to be one of the main compounds responsible for beer staling (3). It is reported that the use of dark colored malt in beer results in better stability than when beer is made from pale malt alone (2, 12).

Development of color and antioxidant activity in malt are linked, with antioxidant activity increasing with color for crystal malts (4, 13). However, the relationship between these attributes is not linear. Antioxidant activity increases with color in various types of malt up to a certain color value, after which further increase in color either has no effect (14) or causes a decrease in antioxidant activity (4). Also, antioxidant activity per unit color diminishes with increasing color (13).

Flavor and color formation in heated foods are often considered to occur in parallel. However, Chandra et al. (15) demonstrated that, by careful adjustment of the roasting process, speciality malts of a specified color could be generated with different flavors.

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**Table 1. Roasting Conditions Used To Prepare Crystal Malts**

color (°EBC value <sup>a</sup> )	roasting time (min)	max product temp (°C)
15	125	112
23	175	113
72	160	130
148	150	140
440	155	165

<sup>a</sup> European Brewing Convention units.

No studies have been reported that consider the relationships between the formation of antioxidant activity, color, and flavor in malt. The aim of the current work was to study the relationships between antioxidant activity, color, and flavor in crystal malt of varying °EBC (European Brewing Convention units) value in order to aid the selection of malts with different antioxidant activity, color, and flavor combinations for brewing.

#### EXPERIMENTAL PROCEDURES

**Materials and Reagents.** Barley for the malts (variety Optic) was grown in the U.K. and harvested in the spring of 1999. It was stored under ambient conditions in a warehouse for ~4 months before use. High-purity water, produced in-house using a Purite (High Wycombe, U.K.) Labwater RO50 unit, was used for all chemical analyses. (+)-Catechin and ABTS were from Sigma (Poole, U.K.). AAPH and 1,2-dichlorobenzene were from Aldrich (Gillingham, U.K.). Tenax traps were supplied by SGE (Milton Keynes, U.K.).

**Preparation of Malts and Malt Extracts.** Crystal malts of specified color value were prepared from germinated barley by stewing at 165 °C for 40 min followed by roasting using the conditions summarized in Table 1. For antioxidant analysis, acetate buffer (pH 5.4) malt extracts were prepared to assess antioxidant activity at the pH of beer. Malt was placed in liquid nitrogen for 15 min and immediately ground in an electric coffee grinder for 30 s (3 × 10 s). The temperature of the malt was < -10 °C. Extracts were prepared by homogenizing ground malt (5 g) with 50 mM pH 5.4 acetate buffer (40 mL) in a pestle and mortar for 2 min on ice (temperature < 4 °C throughout extraction). The use of subambient temperatures minimized degradation of antioxidant compounds. The homogenate was centrifuged (Centaur 2, MSE Scientific Instruments, Crawley, U.K.) at 3240g for 5 min, and the resulting supernatant was filtered through a Whatman (Maidstone, U.K.) No. 1 filter paper. The filtrate (~30 mL) was stored on ice until analysis. Malt extracts were prepared in duplicate.

Hot water malt extracts were prepared for all other analyses to mimic the conditions used for extraction of crystal malts at the beginning of the brewing process. Malt was ground in a coffee grinder for 30 s (3 × 10 s). Ground malt (10 g) was mixed with 65 °C water (68 mL), stirred for 15 min in a 65 °C water bath and centrifuged at 3000 rpm for 5 min, and the resulting supernatant was filtered through a Whatman No. 1 filter paper. Fresh malt extracts were prepared in triplicate for each analysis.

**Antioxidant Assay.** The 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS<sup>•+</sup>) radical cation method was used, and the ABTS<sup>•+</sup> radical cation was generated according to Araki et al. (16) with some modifications. Chromophore solution, consisting of ABTS (150 μM) and AAPH (2 mM) in 50 mM pH 5.4 acetate buffer, was incubated at 55 °C for 1 h in the dark and cooled to 25 °C. Chromophore solution (3.125 mL) and antioxidant solution (malt extract or standard, 25 μL) were mixed and incubated at 25 °C. The absorbance at 734 nm was taken exactly 14 min after initial mixing with reference to an acetate buffer blank. Blanks were run using acetate buffer in place of the antioxidant solution. All determinations were carried out in triplicate for each of the

duplicate extracts. Sample and catechin standard solutions were diluted with acetate buffer such that, after introduction of a 25-μL aliquot of the dilute solution, they produced 15–80% inhibition of the absorbance observed for the blank.

The percentage inhibition of absorbance at 734 nm was calculated as follows:

$$\% \text{ inhibition} = 100 \times \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}}$$

where  $A_{\text{Blank}}$  and  $A_{\text{Sample}}$  are the absorbance values for the blank and antioxidant solution, respectively, at 734 nm.

Antioxidant activity, expressed in catechin equivalents using a catechin calibration line in the range of 0–50 μM, was defined as the concentration of catechin with activity equivalent to the malt extract at a concentration of 1 g/L:

$$\text{antioxidant activity } (\mu\text{M}) = \frac{\% \text{ inhibition for the sample (1 g/L)}}{\% \text{ inhibition for the standard}}$$

Catechin was used as the standard because it is the polyphenol contributing most to antioxidant activity in malt (1).

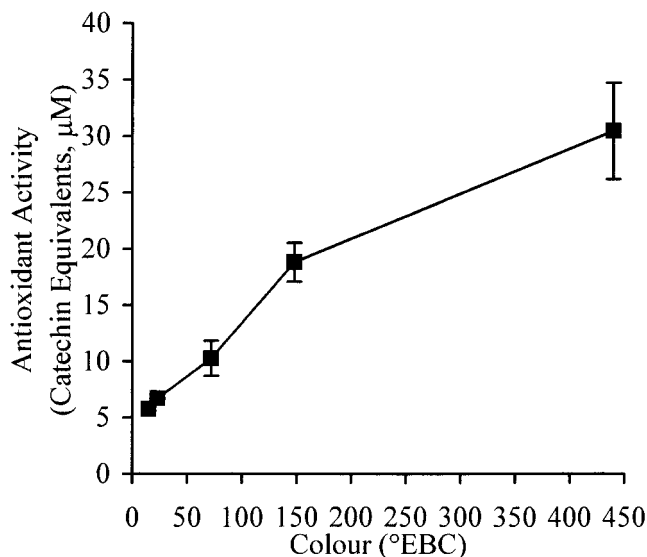
**Color Assessment.** Color measurements were obtained in CIELAB space by transmission spectrophotometry using a Hunter Colorquest spectrophotometer (Reston, VA) and a 10-mm glass cell (Hellma Ltd, Southend-on-Sea, U.K.) with the spectrophotometer set to illuminant D<sub>65</sub> and 10° standard observer. Color dilution profiles were obtained by serially diluting malt extracts with water until a complete lack of chromatic color was obtained. Visual assessment of the samples in the 10-mm glass cell were observed informally by one assessor in a viewing cabinet under artificial daylight fluorescent illumination. Single analyses were carried out on triplicate extracts.

**Analysis of Flavor Compounds.** Malt extract (10 mL) was placed in a 250-mL collection flask in a 40 °C water bath. One microliter of a methanolic solution of 1,2-dichlorobenzene (130 μg/mL, internal standard) was injected onto the Tenax trap, and the collection apparatus was assembled. Volatile compounds were collected for 45 min using a nitrogen flow rate of 40 mL/min. Blank collections used water in place of malt extract in the sample flask. After the collections, the Tenax trap was connected directly to the nitrogen supply for 5 min to remove any residual moisture.

Analyses were performed in triplicate using a Hewlett-Packard (Bracknell, U.K.) 5972A mass spectrometer interfaced with a HP 5890 GC, connected to a PC loaded with HP G1034C (version C.01.05) GC/MS workstation software. Tenax traps were inserted into the CHIS (SGE) thermal desorption port of the GC, which was equipped with a fused-silica column coated with CP-SIL8, film thickness 0.25 μm, length 60 cm, i.d. 0.25 (Chrompak, London, U.K.). Volatile components were desorbed at 280 °C onto the front end of the column and held at 0 °C for 5 min by means of a subambient accessory. The helium flow rate was 1.0 mL/min. The column temperature was increased to 40 °C at a rate of 40 °C/min and held for 2 min. The temperature was further increased to 200 °C at a rate of 4 °C/min rise and to 250 °C at a rate of 10 °C/min and held at 250 °C for 15 min. The injection port and detector temperatures were maintained at 280 °C. Significant MS operating conditions were as follows: ionization mode, electron impact; electron energy, 70 eV; ionization current, 50 μA; source temperature, 165–175 °C; scan range, 32–450 m/z with 1.82 scans/s. Compounds were identified by comparing their mass spectra to those held in the mass spectrometer data system library. Relative amounts of compounds were obtained from the mass spectral integration report with reference to the internal standard (1,2-dichlorobenzene):

$$\text{relative amount} = \frac{\text{area of sample peak}}{\text{area of internal standard peak}} \times 100$$

Experimental linear retention indices (LRI) were calculated



**Figure 1.** Relationship between antioxidant activity and color (European Brewery Convention, °EBC, values) for crystal malt extracts.

with reference to the retention times for a series of standard alkanes ( $C_6$ – $C_{22}$ ) run under the same GC/MS conditions and compared to those of authentic compounds or published in the literature (17, 18). Positive identifications were obtained when both MS and LRI data matched those for the authentic compounds. When only MS data were available, identities were considered to be tentative.

## RESULTS AND DISCUSSION

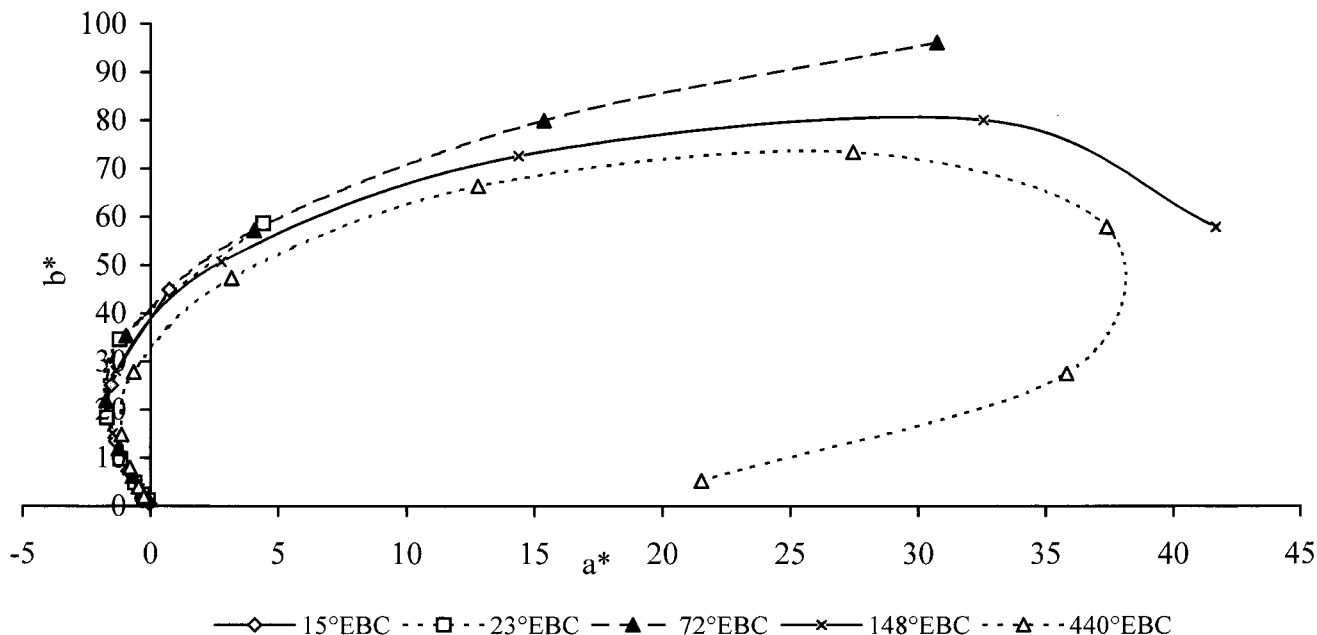
**Antioxidant Activity.** Antioxidant activity increased with European Brewing Convention values (°EBC) (Figure 1). Increases in antioxidant activity of extracts prepared from malts with increasing color have been reported over the range of 0–250 American Society of Brewing Chemists values (°ASBC) for crystal malts (4) and up to ~500 °EBC for different malt types (14). The higher antioxidant activity reported for more highly colored malts in the current study may be related to their higher contents of reductones and melanoidins produced during roasting (19).

The rate of increase in antioxidant activity was lower at the higher color values (Figure 1). (The calculated increase between 15 and 23 °EBC was 0.12 µM catechin equiv/°EBC as compared to 0.04 µM catechin equiv/°EBC between 148 and 440 °EBC.) It would be interesting to confirm this finding by analyzing additional malt extracts in the range of 148–440 °EBC. The decrease in the rate of development of antioxidant activity could be due to Maillard reaction products formed during the intermediate stages of the reaction, i.e., low molecular weight reductones, having the strongest antioxidant properties. Further reaction of these compounds may lead to their progressive transformation, through the Maillard reaction, into compounds with lower antioxidant activity (20). Polyphenols, including flavan-3-ols, flavonols, and phenolic acids, as well as carotenoids and tocopherols also contribute to the antioxidant activity of kilned, pale malts (5, 21). It seems likely that these compounds will also contribute to the antioxidant activity of the malts in the current study, at least those of lower color value. With progressive heating, they may become degraded or react further to give compounds with less antioxidant activity.

**Color Assessment.** Color dilution profiles of crystal malts are important since these malts are used in

brewing at low levels to modify beer color (and flavor). Thus, an understanding of the effect of dilution on color is crucial to the selection of malt of appropriate color (°EBC) value as well as level of use. The effects of dilution on the color properties of the malt extracts are shown in Figure 2. Successive points on each locus represent the data obtained for serial dilutions of the samples. Serial dilutions of each extract modified the  $a^*$  and  $b^*$  values until readings of zero were obtained for both coordinates (no chromatic color remaining). The loci for extracts prepared from the 15, 23, and 72 °EBC malt samples are very similar. This suggests that they contained colorants of the same nature that were present at increasing concentration in extracts of increasing °EBC value. The hue was modified with progressive dilution from orange/brown to less orange (increasing  $+b^*$ ) and more green (increasing  $-a^*$ ) to reach a maximum hue angle of 96–97°. This was confirmed by visual assessment (Table 2). The 440 °EBC sample followed a different dilution pathway (Figure 2), suggesting that the compounds responsible for color in this extract were different from those in the lower colored malts and that the increase in °EBC value cannot be attributed solely to an increase in concentration of colored compounds but also to their nature. The 1:4 dilution of the 440 °EBC extract had  $a^*$  and  $b^*$  values of 37.39 and 57.9, respectively, and was visually orange/brown (Table 2). The colors of the 1:2 dilution and the undiluted extract have low chroma and became progressively dark brown causing a decrease in  $a^*$  and  $b^*$  values, where these values measure the nature of the color but not its darkness. The 148 °EBC sample exhibited a dilution pathway that was different from those of the malt extracts of lower °EBC value and the 440 °EBC sample; i.e., at high levels of dilution, the locus was similar to those of the lower °EBC malts, while at higher concentrations, the locus lay closer to that of the 440 °EBC sample (Figure 2). The different dilution pattern observed for the 440 °EBC extract as compared to the 15, 23, and 72 °EBC samples might be expected to be different due to different pH values. However, this was not the case since the pH of all of the extracts was 7.2. Therefore, the different dilution profiles could be due to the stronger heat treatment used for the production of the more highly colored malts favoring different Maillard reaction pathways. MacDougall and Granov (22) reported that, for an aqueous xylose–glycine model system, the color locus was dependent on the extent of heating and concentration of sugars, with high levels of heating resulting in decreased  $a^*$  and  $b^*$  values. Alternatively, the higher temperatures attained by the malt during production of the more highly colored samples may have resulted in sugar caramelization. The highly complex materials that result from caramelization have intense red-brown colors (8) and may be responsible for the change in hue toward the red-brown region observed for the 440 °EBC sample in Figure 2.

**Analysis of Flavor Compounds.** Total amounts of all the compounds whose areas were integrated by the computer varied little with increasing °EBC value. Levels of 15 compounds were monitored. They included compounds responsible for both desirable (e.g., 3-methylbutanal) and undesirable (e.g., (*E*)-2-nonenal) aromas in malt. Relative amounts of the selected compounds in the different malt extracts are given in Table 3. Three Strecker aldehydes, i.e., methylpropanal



**Figure 2.** *a\** and *b\** color loci for serial dilutions of extracts of crystal malts of varying European Brewery Convention (°EBC) value.

**Table 2. Visual Assessment of Malt Extracts**

dilution <sup>b</sup>	color value <sup>a</sup> (°EBC)				
	15	23	72	148	440
0	yellow	yellow/orange	orange/brown	red/brown	dark brown
1:1	green	yellow	orange	orange/brown	red/brown
1:2	light green	green	yellow/orange	orange	red/brown
1:4	green tint	light green	yellow	yellow/orange	orange/brown
1:8	colorless	green tint	green	yellow	orange
1:16		colorless	light green	green	yellow/orange
1:32			green tint	light green	yellow
1:64			colorless	green tint	green
1:128				colorless	light green
1:256					green tint
1:512					colorless

<sup>a</sup> European Brewing Convention units. <sup>b</sup> Serial dilution of malt extract with water.

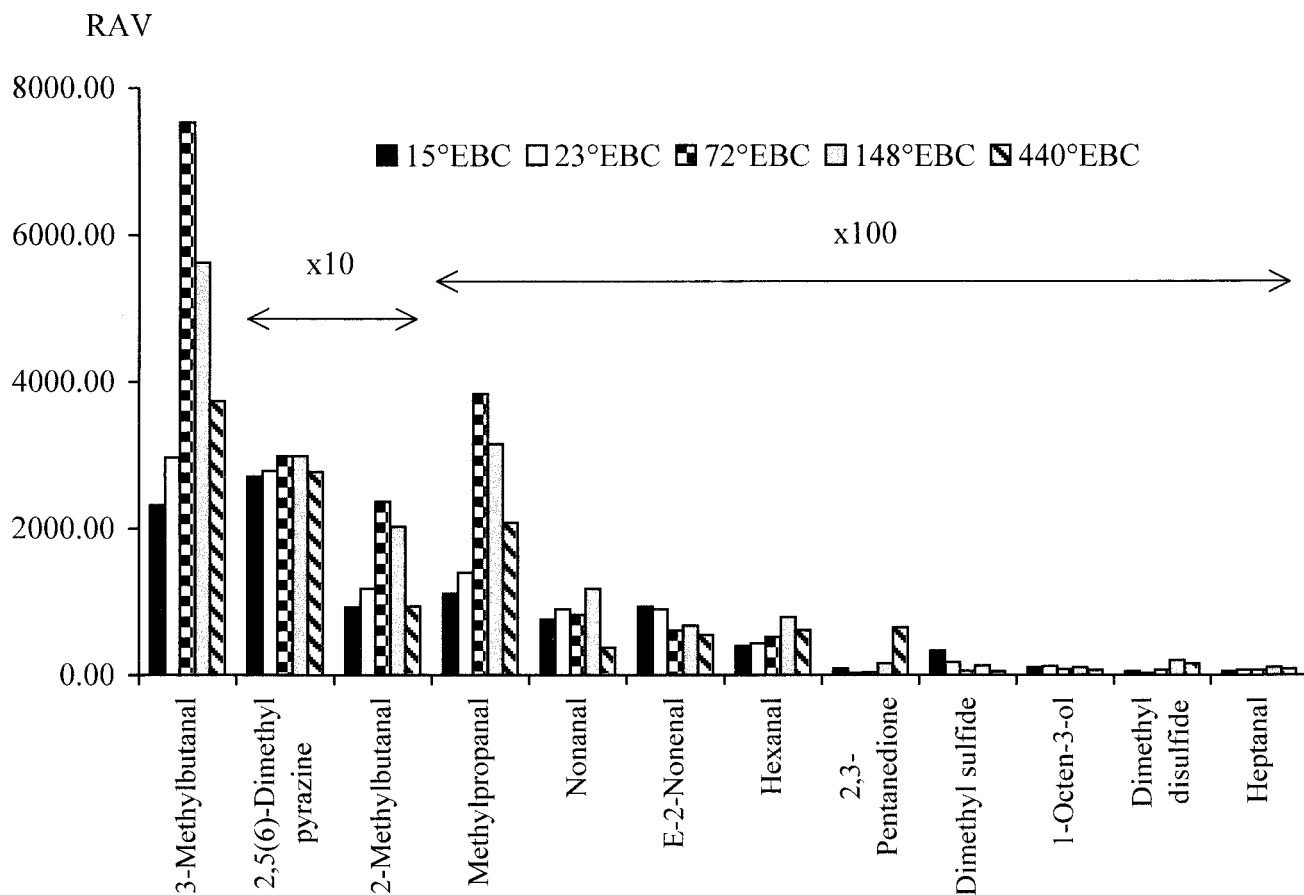
**Table 3. Flavor Compounds of Malt Extracts**

compound	LRI <sub>Exp</sub> <sup>a</sup>	LRI <sub>Lit</sub> <sup>b</sup>	odor threshold <sup>c</sup> (μg/L)	relative amount <sup>d</sup> of color (°EBC value <sup>e</sup> )				
				15	23	72	148	440
dimethyl sulfide		308	0.3	10	5	1	3	1
methylpropanal		552	10	11	140	383	315	208
3-methylbutanal	658	655	0.35	812	1039	2635	1970	1309
2-methylbutanal	669	665	3.7	342	437	875	750	347
2,3-pentanedione	702	702	30	28	8	12	48	196
pentanal	704	695	1500	2	3	4	14	14
dimethyl disulfide	736	727	12	6	3	9	24	18
hexanal	800	795	10.5	42	46	55	83	64
methylpyrazine	840	837	100	2	2	4	12	11
2-furfural	851	848	3000	44	112	283	704	1375
heptanal	902	898	5.8	3	4	4	6	5
2,5(6)-dimethylpyrazine	930	926	0.02	5	2	6	2	6
1-octen-3-ol	987	986	1	10	12	8	10	7
nonanal	1103	1104	5	38	45	41	59	19
(E)-2-nonenal	1174	1172	0.08	8	7	5	5	4

<sup>a</sup> Calculated linear retention indices. <sup>b</sup> Linear retention indices obtained for authentic compounds analyzed on the same GC column or from the literature (17, 18). <sup>c</sup> Values quoted from Rychlik et al. (23). <sup>d</sup> Relative amounts calculated as described in the Experimental Procedures. Figures quoted are the means of triplicate analyses. Coefficients of variation usually <20%. <sup>e</sup> European Brewing Convention units.

and 2- and 3-methylbutanal, as well as 2,3-pentanedione and furfural were present in the greatest amounts. Levels of the former three compounds were greatest in the 72 °EBC sample, while amounts of furfural increased throughout the color range. Beal and Mottram

(11) reported similar behavior for 3-methylbutanal in crystal malt. Hexanal and nonanal (deriving from lipid) were also prominent compounds, while other lipid degradation products (pentanal, heptanal, 1-octen-3-ol, and (E)-2-nonenal) and dimethyl sulfide were present



**Figure 3.** Relative aroma values (RAVs) of monitored flavor compounds of extracts of crystal malts of varying European Brewery Convention ( $^{\circ}$ EBC) value.

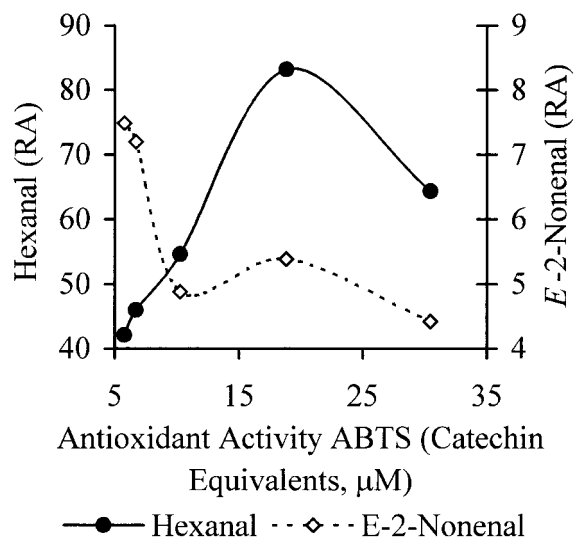
in the flavor isolates at much lower levels. Amounts of most of them were highest in the 148  $^{\circ}$ EBC sample. Thermally induced reactions, after the formation of these compounds and the Strecker aldehydes, resulting in the formation of other flavor compounds and/or colorants may account for their lower relative amounts in samples of higher color value. The precursor amino acids of methylpropanal, 2-methylbutanal, and 3-methylbutanal, i.e., valine, leucine and isoleucine, may also become exhausted. Amounts of the two monitored pyrazines were very low and tended to increase with  $^{\circ}$ EBC value. (*E*)-2-Nonenal and dimethyl sulfide tended to decrease with  $^{\circ}$ EBC value, suggesting that they may be lost during roasting. This suggests that the formation of (*E*)-2-nonenal may also be inhibited by melanoidins, which possess antioxidant activity (7), the levels of which will increase with color value.

The importance of a particular component to flavor is a combination of the amount present and its odor threshold value. Relative aroma values (RAVs) for each compound were obtained by dividing relative amounts by the odor threshold value. These data (Figure 3) provide information about the aroma impact of each compound in each sample. (RAVs for pentanal, furfural and methylpyrazine were below 0.2 and have been omitted from Figure 3.) 3-Methylbutanal was the most important contributor to flavor because its RAV is 10–20-fold higher than the second most important contributor (2,5(6)-dimethylpyrazine). 3-Methylbutanal is reported to be an important contributor to the overall malty character of malt, along with methylpropanal and 2-methylbutanal (11, 24). RAVs for methylpropanal and 2-methylbutanal were also high (Figure 3). 2,5(6)-

Dimethylpyrazine was the second most important contributor to flavor (Figure 3), and it contributes roasted notes to malt (25). Relative amounts of (*E*)-2-nonenal were low but, due to its low odor threshold value of 0.08  $\mu$ g/L (23), it was one of the more important contributors to malt flavor.

**Effect of Antioxidant Activity on Lipid Oxidation Products.** Lipid oxidation products, such as hexanal and (*E*)-2-nonenal, may form during germination, subsequent thermal processing, and storage. Figure 4 demonstrates the relationship between antioxidant activity and levels of two of the lipid oxidation products, hexanal and (*E*)-2-nonenal, in the malt extracts. (Pentanal, heptanal, and nonanal all demonstrated similar behaviors to that of hexanal.) Hexanal levels increased with antioxidant activity up to  $\sim 20$   $\mu$ M catechin equiv (corresponding to 148  $^{\circ}$ EBC) and then decreased up to  $\sim 30$   $\mu$ M catechin equiv (corresponding to 440  $^{\circ}$ EBC). Levels of (*E*)-2-nonenal decreased with increasing antioxidant activity to approximately 10  $\mu$ M catechin equiv (corresponding to 72  $^{\circ}$ EBC), after which the very low levels remained fairly constant. Increasing levels of the monitored alkanals, up to an antioxidant activity of  $\sim 20$   $\mu$ M catechin equiv (corresponding to 148  $^{\circ}$ EBC), indicates that lipid oxidation was increasing with the degree of roasting up to the conditions used to prepare the 148  $^{\circ}$ EBC sample.

The different relationship between levels of hexanal and those of (*E*)-2-nonenal levels and antioxidant activity can be explained as follows. Hexanal and 2,4-decadienal are the major products, respectively, of the 13- and 9-hydroperoxides of linoleic acid (21). These hydroperoxides are formed in approximately equal



**Figure 4.** Relationship between relative amounts (RA) of hexanal and (*E*)-2-nonenal and antioxidant activity for extracts of crystal malts of varying European Brewery Convention ( $^{\circ}$ EBC) value.

amounts on linoleic acid autoxidation. (*E*)-2-Nonenal is formed by isomerization of (*Z*)-3-nonenal, also formed from the 9-hydroperoxide. Aldehydes formed by lipid oxidation are themselves able to autoxidize with the unsaturated compounds reacting at a faster rate than their saturated counterparts (27). Autoxidation of 2,4-decadienal yields hexanal as the major product (28). Therefore, it would appear that the general increase in hexanal level up to an antioxidant activity of  $\sim 20 \mu\text{M}$  catechin equiv is due to the accumulation of hexanal as a result of its low ability to autoxidize and to its formation, both from the 9-hydroperoxide of linoleic acid, via 2,4-decadienal, and directly from the 13-hydroperoxide. The general decrease in amount of (*E*)-2-nonenal up to an antioxidant activity of  $\sim 20 \mu\text{M}$  catechin equiv is due to its relatively high rate of autoxidation (27). At catechin equivalent values in excess of  $\sim 20 \mu\text{M}$ , the decrease in amounts of both compounds may be due to the production of high levels of Maillard reaction products with antioxidant properties. The small amounts of lipid remaining after roasting to higher color values and volatilization of the monitored compounds may also account for their presence at lower levels in these malts.

**Relationship between Antioxidant Activity, Color, and Flavor.** The CIELAB results show that some of the malt extracts possess similar color loci. Therefore, the antioxidant activity and amounts of 3-methylbutanal, hexanal, and (*E*)-2-nonenal were calculated by dividing the data for extracts prepared from malts of the relevant color values (Table 3 and Figure 1) by the appropriate dilution factor (Table 4) required to obtain two predetermined sets of  $a^*$  and  $b^*$  values (Table 4). The data suggest that the use of lower colored malts to produce the same color as a more highly colored sample will result in higher flavor intensity and higher antioxidant activity. It has been reported that the antioxidant activity of malts per unit  $^{\circ}$ EBC is higher for malts of low color and decreases at higher color values (2, 4, 6) in line with the data reported here (Figure 1).

In conclusion, antioxidant activity of malt extracts increased in parallel with color value, although the rate of increase decreased as the color became darker. This

**Table 4.** Effect of Color on Calculated Levels of Antioxidant Activity and Selected Flavor Compounds

color ( $^{\circ}$ EBC value <sup>a</sup> )	antioxidant acty <sup>b</sup> ( $\mu\text{M}$ catechin equiv)	relative amount		
		3-methylbutanal <sup>c</sup>	hexanal <sup>c</sup>	( <i>E</i> )-2-nonenal <sup>c</sup>
$a^* = 4; b^* = 50$ (Visual Assessment, Orange)				
23	0	6.71	1039	46
72	4	2.56	659	14
148	8	2.35	246	10
$a^* = -1; b^* = 25$ (Visual Assessment, Green/Yellow)				
15	2	2.89	406	21
23	4	1.68	259	12
72	16	0.64	165	3
148	32	0.59	62	3

<sup>a</sup> European Brewing Convention units. <sup>b</sup> Calculated by dividing the data for the malt of the relevant color value in Figure 1 by the dilution factor given in column 2. <sup>c</sup> Calculated by dividing the data for the malt of the relevant color value in Table 3 by the dilution factor given in column 2. <sup>d</sup> Trace; relative amount less than 1.

study has demonstrated for the first time that malt samples with color values of 15, 23, and 72  $^{\circ}$ EBC and low dilutions of the 148  $^{\circ}$ EBC sample can all be used to induce the same color at appropriate dilution in water because they have similar dilution pathways when assessed in CIELAB space. The 440  $^{\circ}$ EBC sample did not follow the same dilution pathway, indicating the presence of different colorants. The yield of total volatiles was independent of malt color value. Levels of Strecker aldehydes, i.e., methylpropanal, 3-methylbutanal, and 2-methylbutanal, were greatest for the 72  $^{\circ}$ EBC sample, suggesting that intermediate colored crystal malts may be better at inducing malty notes. 3-Methylbutanal, 2,5(6)-dimethylpyrazine, 2-methylbutanal, and methylpropanal had the greatest flavor impact in the samples, and (*E*)-2-nonenal was also prominent. Malt extract antioxidant activity had little effect on the levels of volatile lipid oxidation products, including (*E*)-2-nonenal. When malt extracts were diluted to the same  $a^*$  and  $b^*$  values, the lower colored malts had more antioxidant activity and higher levels of 3-methylbutanal, hexanal, and (*E*)-2-nonenal.

#### ACKNOWLEDGMENT

The authors thank Douglas MacDougall for advice and the Chief Executive of Brewing Research International for permission to publish this paper.

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Received for review May 7, 2001. Revised manuscript received August 9, 2001. Accepted August 10, 2001. The Biotechnology and Biological Sciences Research Council (U.K.) and the Brewing Research International (U.K.) provided funding (via a studentship to H.M.W.).

JF010583B