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Influence of Malt Roasting on the Oxidative Stability of Sweet Wort

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ABSTRACT: Influence of malt roasting on the oxidative stability of sweet wort was evaluated based on radical intensity, volatile profile, content of transition metals (Fe and Cu) and thiols. Malt roasting had a large influence on the oxidative stability of sweet wort. Light sweet worts were more stable with low radical intensity, low Fe content, and ability to retain volatile compounds when heated. At mild roasting, the Fe content in the wort increased but remained close to constant with further roasting. Dark sweet worts were less stable with high radical intensities, high Fe content, and a decreased ability to retain volatiles. Results suggested that the Maillard reaction compounds formed during the roasting of malt are prooxidants in sweet wort. A thiol-removing capacity was observed in sweet wort, and it was gradually inhibited by malt roasting. It is possibly caused by thiol oxidizing enzymes present in the fresh malt.

KEYWORDS: *malt, sweet wort, roasting, oxidative stability, thiols, electron spin resonance spectroscopy, volatiles*

INTRODUCTION

Raw materials as well as the brewing process have a large influence on the quality and storage stability of the resulting beer. It has become evident that the extent of oxidation during the initial stages of beer brewing has a major influence on the flavor stability of the final beer.[1] Cortes et al.[2] found correlation between oxidative stabilities of boiled wort and the oxidative stabilities of the corresponding beers using electron spin resonance (ESR) spectroscopy. In both wort[3] and beer,[4] transitional metals were found to have significant effects on the oxidative stability as trace levels of Fe and Cu act as catalysts in radical generation and oxidation reactions during beer aging.

Malt is the major constituent in beer, and malt roasting results in different types of beer by influencing aroma and color. Previous malt studies have been carried out on commercial malts introducing uncertain variations due to different barley varieties and production conditions.[2,5] A standardized study of the influence of roasting alone has not been carried out. The influence of malt roasting on beer stability is preferably studied in wort as sulfite produced during fermentation has an impact on beer stability that may mask the influence of the roasting.

Thiols have recently been suggested to have an important role in oxidative processes in beer and in beer stability[6] and were recently quantified in beer with a correlation to the oxidative stability measured by ESR spectroscopy.[7] The significance of thiols in wort has not been investigated, and it remains uncertain where in the brewing process the thiols are introduced and whether they have an influence on the oxidative stability of sweet wort.

A correlation between radical intensity measured by ESR spectroscopy and stale flavor in beer has been found;[8,9] however, the possible correlation between radical intensity and the volatile profile of wort or the oxidative changes in wort over time have not been investigated. Some studies suggest that Maillard reaction products produced during malt roasting have antioxidative capacity and a positive influence on the oxidative stability of wort and beer.[5,10] However, in other studies these compounds were found to have a negative influence on wort and beer stability.[2,4] The purpose of this study was to test the effect of malt roasting on the oxidative stability of sweet wort using a standardized roasting process. The sweet worts were evaluated based on Fe and Cu content and thiol levels, whereas radical intensity and volatile profile were determined in both fresh and heat treated sweet worts.

MATERIALS AND METHODS

Chemicals. Anhydrous acetonitrile, glutathione, L-cysteine, 4-methyl-1-pentanol, 2,2,6,6-tetramethylpiperidine-1-oxy (TEMPO), and α-(4-pyridyl-1-oxide)-*N*-*t*-butylnitrone (POBN) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tris(hydroxymethyl) amino methane (tris) and ThioGlo 1 fluorescent thiol reagent were obtained from Merck (Darmstadt, Germany). Water was purified through a Milli-Q water purification system (Millipore, Billerica, USA). Ethanol (96%) was obtained from Kemetyl (Køge, Denmark). 5,5-Dithiobis(2-nitrobenzoic acid) (DTNB) was obtained from Fluka, Stenheim, Germany.

Roasting. Fresh pale ale malt (two-row spring barley (*Hordeum vulgare*), harvest 2009) was purchased from Weyermann, Bamberg, Germany through Maltbazaren, Copenhagen, Denmark. The malt was received less than five days after kilning and was roasted the following day. Malt was roasted by distributing it in a single layer on a cloth on a baking plate and heating it in an oven at 125 °C, 135 °C, 145 °C, 160 °C, or 190 °C for 50 min.

Mashing. Mashing was carried out on the 6 different malts (1 nonroasted and 5 roasted) on the first and second day after roasting, according to Analytica EBC 4.5.1 "Extract of Malt: Congress Mash",[11] with the modifications described by Frederiksen et al.[3] The 6 malts were mashed individually and named as illustrated in Table 1. Sweet wort was frozen at −80 °C.

Color. Sweet wort color was determined spectrophotometrically on a Cintra 40 Spectrofotometer (GBC, Melbourne, Australia) according to Analytica EBC 8.3 "Color":[11]

$$C = 25 \cdot f \cdot \mathrm{A430} \quad (1)$$

where C is the color in EBC units, f is the dilution factor, A430 is the absorbance at 430 nm, and 25 is a multiplication factor.

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Table 1. Characteristics of the 6 Sweet Worts Applied in This Study[a]

malt	roasting temp. (°C)	wort pH	wort °Bx	wort color (EBC)
1		5.82 ± 0.04	13.460 ± 0.000	8 ± 1
2	125	5.69 ± 0.05	13.462 ± 0.001	15 ± 1
3	135	5.58 ± 0.03	13.462 ± 0.002	33 ± 2
4	145	5.52 ± 0.02	13.460 ± 0.006	44 ± 4
5	160	5.38 ± 0.01	13.425 ± 0.000	68 ± 2
6	190	5.10 ± 0.10	13.380 ± 0.004	100 ± 10

[a]This included the roasting temperature, at which malt was roasted for 50 min, wort pH, wort sugar content measured in degree brix (°Bx), and wort color measured in European Brewery Convention (EBC) color units. Averages and standard deviations are based on two individual mashes.

Sugar Content (°Brix). °Brix values were determined using a refractometer (Analytic Jena, Jena, Germany).

Heat Treatment of Sweet Wort. Sweet wort was thawed in a 5 °C refrigerator overnight. An aliquot of 32 mL was transferred to a 250 mL blue cap flask and incubated in a 40 °C water bath. To avoid microbial growth, 100 μL of chloramphenicol (stock 30 mg/L ethanol) and 100 μL ampicilin (stock 100 mg/mL H_2O) were added to the sweet wort. The blue cap flask was closed with a cap to avoid evaporation, and a magnetic stirrer ensured constant stirring at 300 rpm.

Sampling for ESR measurements was carried out at 0, 1/2, 1, 2, 4, 6, 8, and 10 h. At each sampling, 6× 0.5 mL were distributed to safety-lock Eppendorf tubes, and all reactions were terminated by quick-freezing the tubes in liquid nitrogen. Samples were stored at −80 °C until analyzed.

Sampling for volatile analysis was carried out at 0 and 10 h. At regular intervals, the lid was led to match the ESR sampling process and to ensure that oxygen was present at all times.

Electron Spin Resonance (ESR). Ethanol and α-(4-pyridyl-1-oxide)-*N*-*t*-butylnitrone (POBN) were added to sweet wort samples in final concentrations of 5% vol. and 40 mM, respectively, according to the method described by Frederiksen et al.[3] The sweet wort samples were subsequently incubated at 60 °C for 90 min. ESR spectra were recorded with a Miniscope MS 200 X-band spectrometer (Magnettech, Berlin, Germany) using 50 μL micropipets as sample cells (Brand GMBH, Wertheim, Germany). Spectra were recorded at room temperature using the following settings: microwave power, 10 mW; sweep width, 49,82 G; sweep time, 30 s; steps, 4096; number of passes, 6; modulation, 100 mG; and microwave attenuation, 5 mW. The amplitudes of the spectra were determined and are reported as the height of the first doublet. ESR spectra of an aqueous 2 μM 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) solution were recorded each day to compensate for day-to-day variation.

Transition Metals (Fe and Cu). Sweet wort samples were acid digested in a microwave oven using the solvents and temperature program detailed in Wyrzykowska et al. [12] The multielemental composition of sample digests were subsequently analyzed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Perkin-Elmer 3300, United States) with the spectrometer equipped with an octopole reaction cell for interference removal (Agilent 7500ce, Manchester, UK) following the instrumental settings listed in Hansen et al.[13] Samples were determined in duplicate.

Thiol Levels Determined Using Fluorescent Reagent ThioGlo 1. Thiol levels were determined according to the standard addition procedure described by Lund and Andersen[7] based on the thiol reacting fluorescent reagent ThioGlo 1. Glutathione was replaced with cysteine for the standard addition curve, and analyses were carried out using microtiter plates where cysteine was added in concentrations between 0 and 20 μM to sweet wort diluted 40 times. Each standard addition curve was made in triplicate on each plate, and the average represents a single analysis. For each sweet wort sample, the single analysis was carried out in independent triplicates.

Thiol Levels Determined Using Ellman's Reagent, DTNB. The thiol level in one sweet wort sample (9.8 EBC) was determined spectrophotometrically after derivatization by Ellman's reagent, DTNB. The experiment was carried out based on the method described in Jongberg et al.,[14] and a standard curve of cysteine and a standard addition curve of cysteine and sweet wort diluted 40 times were constructed. Analysis was carried out by mixing 250 μL of sample, 1.00 mL of 0.25 M tris buffer (pH 7.5), and 250 μL of 10 mM DTNB dissolved in 0.25 M TRIS buffer (pH 7.5). The mixture was protected against light and allowed to react for exactly 30 min, and absorbance was measured at 412 nm. Blanks without DTNB and blanks without sample were prepared, and the blank values were subtracted from the sample values.

Volatiles. Headspace analysis was carried out in triplicate using 5 mL of sweet wort and 1.00 mL of 4-methyl-1-pentanol (50 mg L^{-1}) as internal standard. The volatile compounds were collected on a Tenax-TA trap (Buchem bv, Apeldoorn, The Netherlands). Samples were equilibrated to 30 ± 1 °C in a circulating water bath and then purged with nitrogen (75 mL·min^{-1}) for 20 min. Separation and detection were carried out on a gas chromatograph−mass spectrometer (GC-MS 7890A GC-system interfaced with a 5975C VL MSD with Triple-Axis detector from Agilent Technologies, Palo Alto, California) using a polar wax GC-column. Details on equipment and temperature program are found in Deza-Durand and Petersen.[15] Volatile compounds were tentatively identified by probability based matching of their mass spectra with those of a commercial database (Wiley275.L, HP product no. G1035A). The software program, MSDChemstation (version E.02.00, Agilent Technologies, Palo Alto, California), was used for data analysis. Concentrations were calculated as peak area of the volatile compound divided by the peak area of internal standard.

To increase the sensitivity for detection of (*E*)-2-nonenal, selected ion monitoring (SIM) was applied using ions 55 and 83, and a standard curve was created for quantification. Limit of quantification and detection were calculated from the average signal-to-noise ratio of two peaks from beer spiked with 0.8 μg·L^{-1} (*E*)-2-nonenal resulting in a quantification limit of 0.25 μg·L^{-1} and a detection limit of 0.07 μg·L^{-1} with a signal-to-noise ratio of 10 and 3, respectively.

Multivariate Data Analysis. Multivariate data analysis was applied to GC-MS data to describe the change in volatile profile of heat treated sweet worts over 10 h using principal component analysis (PCA) and to determine the correlation of the volatile profile to EBC color using partial least squares (PLS) regression analysis. PCA and PLS regression analyses were performed using Latentix software (LatentiXTM 2.0, Latent5, Copenhagen, Denmark, www.latentix.com). Analyses were carried out on the relative areas calculated as peak area of the volatile compound divided by the peak area of internal standard (4-methyl-1-pentanol). Data were auto scaled and cross-validated.

RESULTS AND DISCUSSION

Sweet Wort Characteristics. Differently roasted malts were prepared by heating a pale ale malt for 50 min at 5 different temperatures. Duplicate congress mashings were carried out for each of the six malts (including the pale ale malt), and the pH, sugar content, and color of the fresh sweet worts were determined (Table 1). Sweet wort color was highly correlated to malt roasting temperature (R^2 = 0.99) and was accordingly used as a marker of the roasting level. pH was negatively correlated to sweet wort color (R^2 = 0.99) in agreement with previous findings.[5] The sugar content in the sweet worts was lower for malt roasted at the two highest temperatures (sweet worts 5 and 6) than for the four malts roasted at lower temperatures (sweet worts 1 to 4). This is most likely due to increased enzyme inactivation as well as starch denaturation. All malts were made from the same batch of pale ale malt, and the observed differences can therefore be directly associated with their different levels of roasting.

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Oxidative Stability of Sweet Worts. The oxidative stability of the sweet worts was examined by incubating them at 40 °C with access to atmospheric oxygen for 10 h and then testing the radical forming ability of samples collected at different times. The ability of radical formation was examined by quantification of the amount of radicals measured by ESR spectroscopy generated after heating the samples at 60 °C for 90 min with added spin trap, POBN, and 5% ethanol.[4] The amplitude of the first doublet of each ESR spectrum recorded during the heat treatment was used as a measure of the amount of radicals generated (Figure 1A). The results showed that the

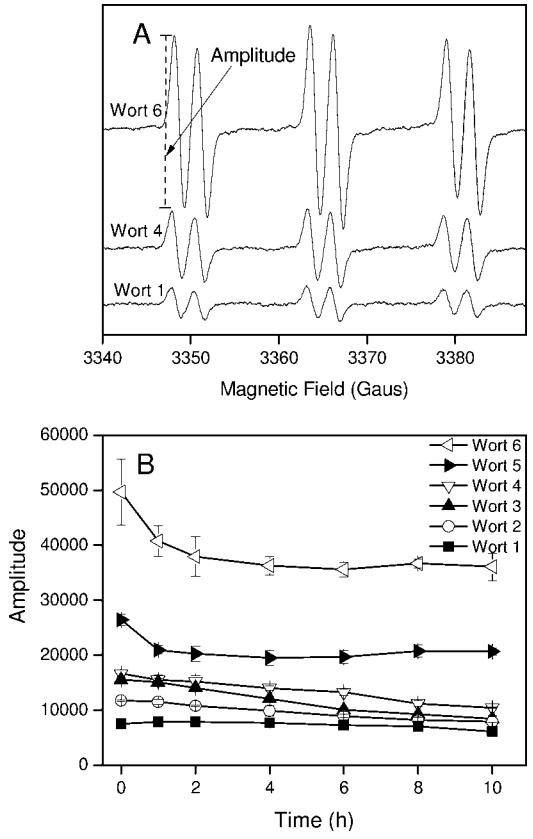

Figure 1. (A) ESR spectra of POBN spin adducts formed in fresh sweet worts 1, 4, and 6 after 90 min of incubation at 60 °C with 5% ethanol and 40 mM POBN spin trap. (B) The time dependence of the different radical formation capabilities of sweet worts during incubation at 40 °C. The capability of radical formation was evaluated by ESR spectroscopy by heating collected samples 90 min at 60 °C with added POBN and 5% ethanol. Results are shown as mean values, and standard deviations are given as error bars (n = 3).

formation of radicals in dark sweet worts was higher than that in lighter sweet worts (Figure 1B), which is consistent with previous observations.[2] The ability of radical formation decreased in the darkest sweet worts, 5 and 6, within the first hour of storage at 40 °C but still had a constant and high level during the following 9 h of incubation. The lighter sweet worts, 1, 2, 3, and 4, showed a lower but more constant decrease in radical formation over the 10 h; however, the difference in radical formation from 0 to 10 h of heat treatment decreased from sweet wort 4 to sweet wort 1 where the radical formation in sweet wort 1 was almost constant throughout the 10 h (Figure 1B). These results show that dark sweet worts, 5 and 6, are more oxidatively unstable than lighter sweet worts, and even though the storage at 40 °C had a larger influence on the ability of dark sweet worts to generate radicals compared to light sweet worts, they still had a high capacity for generation of radicals after 10 h at 40 °C.

The presence of Fe and Cu may promote metal-catalyzed oxidation and thereby affect the radical formation and the oxidative stability.[4] The transition metals, Fe and Cu, were therefore quantified in each of the six sweet worts. Malt roasting was found to have a large influence on Fe and Cu contents in the sweet worts but in different ways. The Cu concentration decreased from the lighter sweet wort 1 to the darker sweet worts 4, 5, and 6 (Figure 2). The concentrations

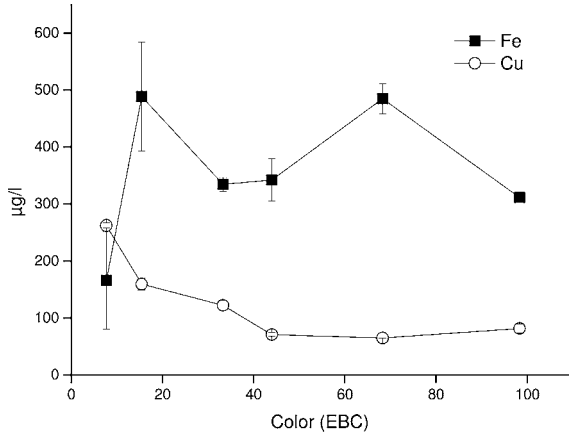

Figure 2. Fe and Cu content in sweet worts 1 to 6 plotted against color (EBC). Results are shown as mean values, and standard deviations are given as error bars (n = 2).

of Fe were generally higher, than the levels of Cu, except in sweet wort 1, which had a considerably lower level of Fe than the other sweet worts. Since the same pale ale malt was used for all malt roastings, the total metal content introduced into the mashings were the same in the six mashes. The variations observed in Fe and Cu contents in the sweet worts caused by the roastings must therefore be explained by different abilities of the malt solids to bind metals. Supporting this explanation, Frederiksen et al.[3] found that Fe and Cu added during mashing was trapped by insoluble malt solids resulting in an only slight increase in Fe and Cu content in the sweet wort after filtration. Roasting of the pale ale malt therefore reduces the ability of the malt solids to bind Fe but improves the ability to bind Cu. The fact that radical signal intensity in sweet wort increases with increasing malt roasting temperature where as the concentration of Cu decreases and Fe remains somewhat constant suggests that other components in the dark sweet worts also affect the oxidative stability.

Thiols in Sweet Wort. Protein-thiols have been suggested to have antioxidative activity in beer,[6] and their presence in sweet wort was investigated for their possible influence on the oxidative stability in the present study. Quantification of thiols in the sweet worts was attempted by using the standard addition procedure described by Lund and Andersen[7] and an external standard curve procedure, both of which are based on the thiol reacting fluorescent reagent ThioGlo 1. However, the external standard curve procedure was rejected due to the large variation in color of the sweet wort samples resulting in reduced excitation of ThioGlo 1 in dark samples compared to that of the uncolored standards and thereby resulting in inaccurate quantification of thiols. The standard addition

procedure was therefore tested in order to solve this problem; however, the sweet worts did not give rise to fluorescence emission typical for thiol-ThioGlo 1 derivatives. Upon addition of cysteine, the fluorescence emission was only observed when cysteine was added in concentrations higher than 8 μM (Figure 3). Dilution of the sweet wort resulted in fluorescence

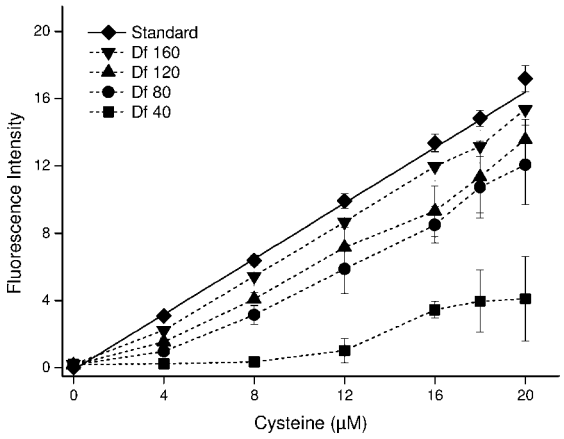

Figure 3. Thiol determination based on derivatization with the fluorescent reagent ThioGlo 1: standard addition curves of sweet pilsner wort (6.4 EBC) with cysteine added at concentrations between 0 and 20 μM. Wort was diluted with buffer between 40 and 160 times (Df 40−160). The standard curve of cysteine in buffer is illustrated with a regression line (standard). Results are shown as mean values, and standard deviations are given as error bars (n = 3).

responses, which approached the behavior of cysteine added to a pure buffer solution (Figure 3). The results demonstrate that the sweet wort contained compounds able to oxidize the cysteine added to the sweet wort. This effect will be referred to as thiol-removing capacity. The smallest dilution factor of 40 was chosen in order to minimize background fluorescence of the sweet wort sample as previously described for beer.[7] Glutathione was also oxidized when added to the sweet wort. However, it seemed to be less sensitive to oxidation than cysteine as a larger concentration of sweet wort was required to inhibit the emission of ThioGlo 1 derivatives (data not shown). Muller[16] found that addition of glutathione to a 65 °C mash under aerobic conditions resulted in an accelerated oxidation of glutathione compared to a simulated mash carried out on a solution of glutathione alone. The simulated mashing of glutathione carried out under anaerobic conditions inhibited all oxidation of glutathione. The results from the current study strongly support the theory about constituents of malt enhancing oxidation of not only glutathione but also cysteine. However, in previous studies, thiols have been detected in light-colored sweet worts.[16−18] In these studies, Ellman's reagent, DTNB, was applied for thiol determination. To verify that the current results were not caused by the different choice of thiol derivatizing agent, the standard addition procedure was tested on a sweet wort using the DTNB method. As shown in Figure 4 the same thiol-removing capacity was detected, which excludes that the difference in derivatization agent is the reason for the difference in results. Stephenson et al.[18] found that the presence of oxygen had a negative influence on the content of thiols in sweet wort based on aerobic and anaerobic mashing trials, and Jin et al.[17] detected thiols in sweet wort produced on an industrial scale, likely to be carried out under anaerobic conditions. The EBC congress mash method, applied

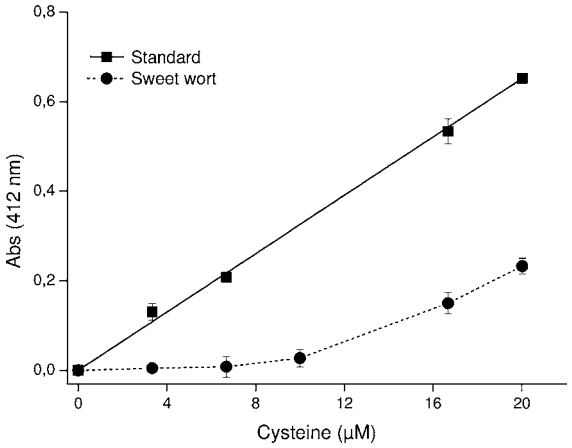

Figure 4. Thiol determination based on derivatization with DTNB: standard curve of cysteine and standard addition curve of sweet pilsner wort (9.8 EBC). Results are shown as mean values, and standard deviations are given as error bars (n = 2).

in the current study, included aerobic conditions during both mashing and filtration. Therefore, an analysis of thiol-removing capacity in sweet wort produced on an industrial scale was also carried out; however, the same thiol-removing capacity was detected (data not shown). This suggests that access to oxygen during mashing does not explain the presence of the thiol-removing capacity.

Malt roasting proved to influence the thiol-removing capacity in sweet wort, which decreased with an increasing level of roasting (Figure 5). Sweet wort 1 (not roasted) had thiol-

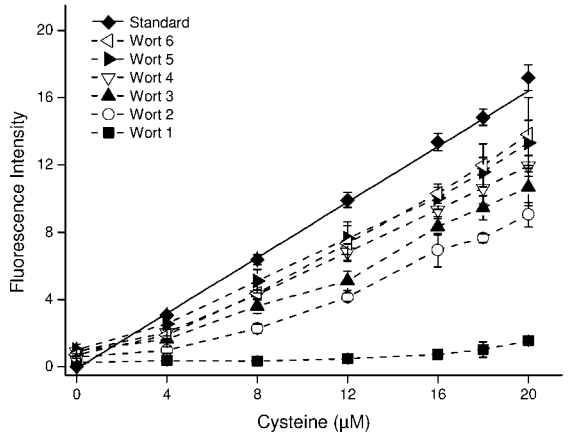

Figure 5. Thiol determination based on derivatization with the fluorescent reagent ThioGlo 1: standard addition curves of sweet wort 1 to 6 with cysteine added at concentrations between 0 and 20 μM. Worts were diluted 40 times, and the standard curve of cysteine in buffer is illustrated with a regression line (standard). Results are shown as mean values, and standard deviations are given as error bars (n = 3).

removing capacity enabling it to consume more than 15 μM of cysteine when diluted 40 times. A large part of the ability to oxidize cysteine was lost when malt was slightly roasted (sweet wort 2, malt roasted at 125 °C for 50 min). Further roasting of the malt lowered the thiol-removing capacity in the sweet wort until a roasting temperature of 160 °C (sweet wort 5). The difference between roasting at 160 and 190 °C was very small indicating that further roasting would not affect the thiol-removing capacity. Bamforth et al.[19] found a heat stable

enzyme, present in fresh malt, capable of oxidizing thiol groups (cysteine, glutathione, and dithiothreitol) resulting in disulfide cross-linking. The enzyme is inactivated by autoclaving but retained approximately 70% of its activity after heating to 70 °C. Analyses in the current study were carried out on fresh malt with a mash out temperature of 70 °C, and according to Bamforth et al.,[19] the enzyme was therefore likely to be present in the sweet wort. Hence, the decrease in thiol-removing capacity from sweet wort 2 to 6 could be explained by increasing enzyme inactivation with increasing roasting temperature. If this is the case, the large difference in thiol-removing capacity between sweet wort 1 and 2 may reflect the lack of roasting of sweet wort 1 and thereby the lack of inactivation of the thiol oxidizing enzyme.

Also, nonenzymatic oxidation of thiols has been suggested to occur in sweet wort,[18,19] and the decrease in thiol-removing capacity in malt roasted at temperatures above 125 °C might also be influenced by nonenzymatic reactions. Samaras et al.[20] found that the total amount of phenolic compounds in wort decrease during the roasting of malt indicating that the phenols are oxidized and likely to produce more quinones. Thiol reactive quinones are typically generated through oxidation of phenols and have been found to react with thiols in both wine[21] and in myofibrillar proteins[22] forming thiol-quinone adducts. However, if the quinones were to be responsible for the removal of thiols in wort by forming thiol-quinone adducts, the thiol-removing capacity should increase with increasing roasting due to the formation of increased amounts of quinone; hence, this explanation is rejected. The Maillard reaction products formed during roasting may also be excluded as main contributors to the thiol-removing capacity as these compounds also increase in quantity with increasing roasting.

Volatile Compounds in Sweet Wort. Analysis of volatile compounds was carried out on fresh sweet wort and on sweet wort heat treated at 40 °C for 10 h. The results showed that many volatiles in the darker worts decreased in concentration after 10 h of heat treatment, whereas the concentration in the light sweet worts remained constant. A gas chromatogram and an example of the loss in volatiles are shown for sweet wort 6 in Figure 6. PCA was performed (Figure 7), based on the 49 volatile compounds listed in Table 2. Principal component (PC) 1 explains the variation caused by malt roasting with

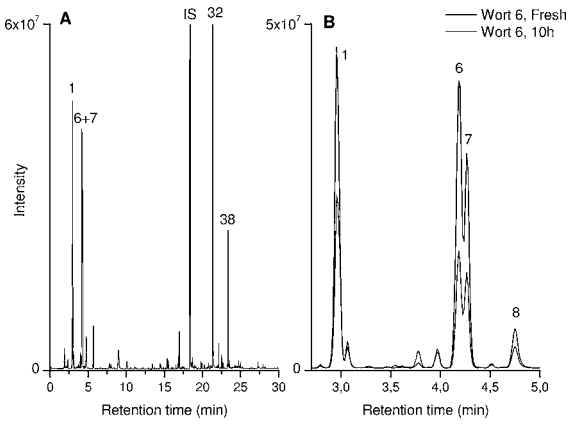

Figure 6. (A) Gas chromatogram of fresh sweet wort 6. (B) Gas chromatogram for fresh and heat treated (10 h) sweet wort 6 presented from retention time 2.5 to 5.0 min. Numbers refer to the compounds in Table 2. Internal standard (IS) is 4-methyl-1-pentanol.

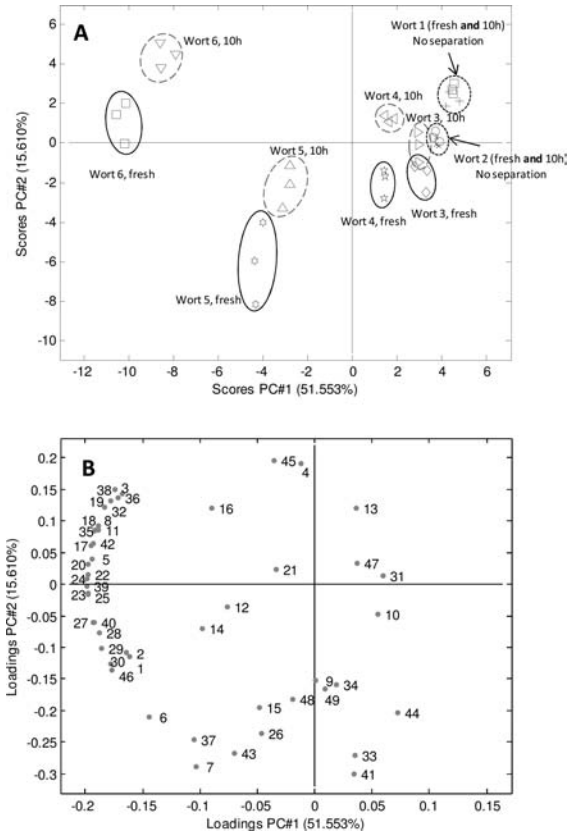

Figure 7. (A) Principal component analysis (PCA) score plot of volatile compounds in both fresh and heat treated sweet worts 1 to 6. The dotted oval encloses both fresh sweet worts and sweet worts heat treated at 40 °C for 10 h. The solid oval encloses fresh sweet worts. The dashed oval encloses sweet worts heat treated at 40 °C for 10 h. (B) Loadings plot. Each volatile compound is represented by a number corresponding to that in Table 2. Each volatile profile was determined in triplicate.

increasing concentrations of Maillard reaction products going from right to left. PC 2 explains the variation between heat treated and fresh sweet worts. The PCA score plot illustrates that sweet worts 1 and 2 were completely unaffected by 10 h of heat treatment (no separation between the fresh and heat treated sweet worts in Figure 7A). Sweet wort 3 showed a minor loss in volatile compounds, while heat treatment of sweet worts 4, 5, and 6 resulted in a larger loss of volatile compounds.

Approximately the same relative loss of volatile compounds was found for sweet worts 4, 5, and 6, and the average loss of the 10 volatile compounds showing the largest loss during heat treatment, compared to the values in fresh sweet wort, is listed in Table 3. This loss shows that the heat induced change of the malt matrix during the roasting process affects the ability of the sweet worts to retain the volatile compounds when exposed to heat and that malt roasting at temperatures between 145 and 190 °C result in the same relative loss of volatiles. Ethyldichloro acetate was added to the sweet worts before sampling and was released from sweet worts 4, 5, and 6 to the same degree as the volatiles from malt, showing that the malt matrix (and the degree of roasting) changes the retentivity of volatiles whether they come from the malt or not.

No volatile oxidation products were released after 10 h of heat treatment of sweet wort, uninfluenced by the degree of roasting. The staling compound (*E*)-2-nonenal, which with the

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Table 2. Volatile Compounds in the 6 Sweet Worts (Heat Treated and Non-Heat Treated) Included in the PCA and PLS Analyses[a]

no.	name	Tgt ion	no.	name	Tgt ion	no.	name	Tgt ion
1	2-methylpropanal	43	18	thiazole	85	35	2-acetylfuran	95
2	2-propanone	43	19	2-methyltetrahydrofuran-3-one	43	36	furfuryl acetate	81
3	ethyl formate	31	20	methylpyrazine	94	37	2-methylpropanoic acid	43
4	ethyl acetate	43	21	octanal	41	38	5-methylfurfural	110
5	2-butanone	43	22	2,6-dimethylpyrazine	108	39	methylpyrazine	94
6	2-methylbutanal	41	23	ethylpyrazine	107	40	2-isoamyl-6-methylpyrazine	108
7	3-methylbutanal	41	24	2,3-dimethylpyrazine	67	41	benzeneacetaldehyde	91
8	3-methyl-2-butanone	43	25	2-ethyl-6-methylpyrazine	121	42	furfuryl alcohol	98
9	toluene	91	26	nonanal	41	43	3-methylbutanoic acid	60
10	3-methyl-pentanal	57	27	2-ethyl-3-methyl-pyrazine	122	44	2-methylbenzaldehyde	91
11	2,3-pentane-dione	43	28	dispirol-nonanone	94	45	ethylbenzaldehyde	133
12	dimethyldisulfide	94	29	ethyl pyrazine	106	46	furfuryl pyrrole	81
13	hexanal	56	30	3-ethyl-2,5-dimethylpyrazine	135	47	2-methyl-2,2-dimethyl-propanoic acid	71
14	2-methyl-1-propanol	43	31	acetic acid	60	48	benzothiazole	135
15	butanol	56	32	furfural	96	49	phenol	94
16	heptanal	70	33	2-ethyl-hexanol	57	-	ethyl-dichloro acetate[b]	29
17	pyrazine	80	34	decanal	57			

[a]The target ion (Tgt) is used for identification, and the number of each compound is based on the order of the retention time on the GC chromatogram. [b]Not included in the PCA model.

Table 3. Relative Loss of the 10 Volatile Compounds, for Sweet Worts 4, 5, and 6, Showing the Largest Loss after 10 h of Heat Treatment at 40 °C Compared to That of Fresh Sweet Wort[a]

no.	name	%	SD
6	2-methylbutanal	56.2	3.3
44	2-methylbenzaldehyde	54.2	38.7
9	toluene	53.2	31.2
10	3-methylbutanal	49.6	4.5
43	3-methylbutanoic acid	45.4	10.2
31	acetic acid	36.9	8.6
37	isobutyric acid	33.6	12.3
13	hexanal	33.2	19.9
36	furfuryl acetate	28.7	7.8
34	decanal	25.7	8.2
	ethyl-dichloro acetate	67.3	7.6

[a]Results are presented as an average loss in percent (%) with standard deviations (SD). Ethyl-dichloro acetate was added to all worts before heat treatment and was also lost during heat treatment.

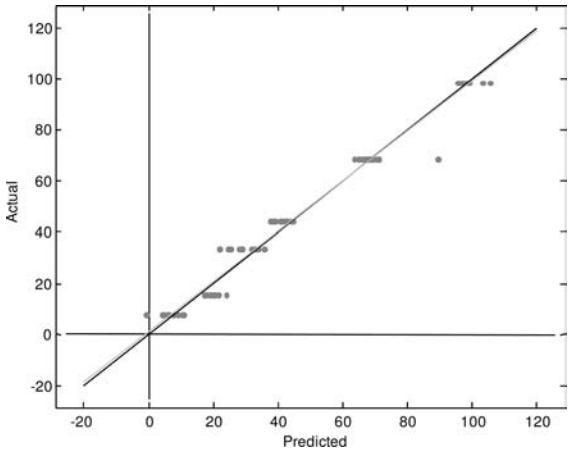

Figure 8. Partial least-squares (PLS) regression analysis of the volatile compounds in sweet worts 1 to 6 versus EBC color based on the first 4 principal components. The coefficient of correlation (r^2) is 0.969, and the root mean square error (RMSE) is 5.6 EBC units. The model is based on the same data set used in Figure 7.

current method could be detected down to 0.07 $\mu g \cdot L^{-1}$, was also not detected. Previous studies have shown that oxidation occurs during wort boiling[23,24] and that even though (*E*)-2-nonenal is not detected in either sweet wort or boiled wort, precursors may have been generated resulting in a release of the staling compound during beer aging,[25,26] as may also be the case for other secondary oxidation products.

The volatile profile of beer is highly influenced by the release of volatile fermentation products. However, the volatile profile of malt[27,28] is very similar to the volatile profile of sweet wort found in this study (Table 2) showing that the mashing process does not change the volatile profile of the sweet wort significantly from the volatile profile of malt. A high correlation between the sampled volatiles and EBC color was found (R^2 = 0.97) using PLS regression analysis (Figure 8). This suggests that the Maillard reaction products produced during malt roasting are the main ones responsible for both color change and the volatile profile of the sweet wort in this study. However, in a recent study color formation and flavor formation at higher EBC values were found to be nonlinear.[29]

Increased Roasting of Malt Results in Less Stable Sweet Worts. This study shows that malt roasting has a larger influence on the oxidative stability of sweet wort and that light and dark sweet worts behave very differently. Light sweet worts were less reactive toward oxidation with low radical intensity and low Fe content, and showed no loss in volatiles when stored at an elevated temperature over an extended period of time. The dark sweet worts were found to be less stable with high radical intensities, high Fe content, and a decreased ability to retain volatiles. It is well known that Maillard reaction compounds are produced during malt roasting; however, whether they act as antioxidants[5,10,20,29] or prooxidants[2,4,30] have been widely discussed. These contradicting results may be caused by the difference in methods applied. While the ESR-based experiments in the present study are based on a complete wort system providing information about the competition

between pro- and antioxidants, antioxidant assays have typically been based on simple model systems measuring the scavenging activity using a semistable radical that has high reactivity toward many types of compounds. Therefore, studies based on these radical scavenging activity assays often result in antioxidative activity, whereas prooxidative activities are neglected. The present study showed that malt roasting, radical intensity, and Fe content are closely linked suggesting that the Maillard reaction compounds act as prooxidants in sweet wort. Reductones are formed during the Maillard reaction and act prooxidatively by driving the Fenton reaction as shown in Figure 9, where Fe(III) is reduced to Fe(II) by the reductones

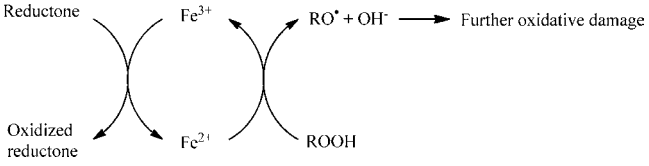

Figure 9. Reaction mechanism describing the prooxidative effect of reductones driving the Fenton reaction in sweet wort by reducing Fe(III) to Fe(II) leading to the oxidation of peroxides and the formation of reactive radical compounds.

leading to oxidation of peroxides and formation of reactive radical compounds which may induce further oxidative damage. This reaction could explain the decrease in oxidative stability of sweet worts produced by highly roasted malts. Contrary to these mechanisms, light sweet worts had a high Cu content and high thiol-removing capacity, whereas dark sweet worts had low Cu content and low thiol-removing capacity. Cu may also work as a catalyst in radical generation, but in the present study, Cu was found not to be correlated to roasting, which suggest that Cu is of minor importance in the oxidative stability of sweet worts. The thiol-removing capacity was gradually inhibited by malt roasting, and this capacity may be explained by the remaining activity of the thiol oxidizing enzyme in the light malts, which is being heat inactivated during roasting.[19] How or if the roasting influences the thiol content is uncertain. Despite the fact that the dark sweet worts had a high radical generation, no oxidation products were found from the analysis of volatiles. However, the dark sweet worts had another important characteristic as they showed a loss of volatiles over time in contrast to the light sweet worts. Therefore, to preserve the volatile profile of sweet wort produced from dark malts, the storage time should be held at a minimum to prevent initiation of oxidative reactions and to limit the loss of volatiles.

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Notes

The authors declare no competing financial interest.

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