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Influence of Barley Varieties on Wort Quality and Performance

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ABSTRACT: Wort from the barley varieties (*Hordeum vulgare*) Pallas, Fero, and Archer grown on the same location were investigated for their influence on oxidative stability and volatile profile during wort processing. Barley varieties had a small influence on radical formation, thiol-removing capacity, and volatile profile. Wort boiling with and without hops had a large influence on these same parameters. Potentially antioxidative thiols were oxidized in sweet wort, but reduction of thiols using tris(2-carboxyethyl)phosphine hydrochloride revealed that Archer wort had a significantly larger content of total thiols than Pallas and Fero. Oxidized thiols resulted in gel proteins and longer filtration time for Archer wort. Our study shows that wort processing to a large extent will eliminate variations in volatile profile and thiol levels in wort which otherwise might arise from different barley varieties.

KEYWORDS: *barley variety, wort, thiols, oxidative stability, volatiles, boiling*

INTRODUCTION

Raw materials as well as oxidative reactions in the initial stages of beer brewing can have a large influence on quality and storage stability of the final beer.[1] Recently, a correlation was found between the oxidative stability of boiled wort and the oxidative stability of the corresponding beers measured by electron spin resonance (ESR) spectroscopy, [2] underlining the importance of wort quality in relation to beer quality. How the barley variety influences the oxidative stability of the wort remains uncertain; however, antioxidative potentials have been observed to vary between different barley varieties.[3] Detailed investigations of barley and malt in relation to beer stability is preferably studied using wort, as the fermentation process strongly influences the overall oxidative stability as well as the volatile profile, making it difficult to isolate the effects of barley and malt.

Protein thiols have been suggested to have antioxidative properties in beer,[4,5] and thiols have been quantified in beer exhibiting a high correlation to oxidative stability measured by ESR spectroscopy.[6] The thiols present in beer either come from the malt and are carried through to the beer as heat-stable proteins or smaller peptides or are produced by the yeast during fermentation. Upon oxidation the thiols form disulfide bonds which in the initial stages of brewing leads to protein complexes, also called gel proteins. High amounts of gel proteins in the mash have proved to result in longer filtration times,[7−9] which is unwanted by the brewers. In a recent study it was found that sweet wort contained compounds able to oxidize cysteine when it was added to sweet wort, and this capacity was named the thiol-removing capacity.[10] It was further found that increasing malt roasting resulted in a decrease in the thiol-removing capacity and that pilsner malt had a smaller thiol-removing capacity than pale ale malt. These observations were ascribed to the presence of a thiol-oxidising enzyme previously described by Bamforth et al.[21] How wort boiling and hopping influence on thiol content and thiol-removing capacity remains unknown but is important for understanding of the role of thiols and how they are preserved throughout the brewing process.

Often pilsner malt is chosen based on its malting and brewing performance with the purpose of increasing output. The idea of choosing a barley variety based on its contribution to the flavor profile or flavor stability of the final beer has received much less attention, and it is not clear to which extent the barley variety can be used to influence beer flavor and oxidative stability of wort and beer.

The aim of this study was to investigate how the barley varieties, wort boiling, and hopping influence the oxidative stability of wort as well as the volatile profile. It remains unclear how or if it is possible to select a certain malting barley variety that influences the oxidative stability of the final beer. Often such comparisons between different raw materials are difficult to make as the growing and malting conditions are difficult to standardize. In this study three two-row barley varieties (*Hordeum vulgare*) Pallas, Fero, and Archer were grown on the same location and harvested and malted simultaneously in order to minimize the effects of different handling. Furthermore, in an attempt to clarify reactions of thiols during the initial stages of the brewing process this investigation was also carried out with the purpose of investigating how the thiol-removing capacity responded to cysteine as well as glutathione.

METHODS

Chemicals. Acetonitrile, glutathione, 4-methyl-1-pentanol, tris (2-carboxyethyl)phosphine hydrochloride (TCEP), and 2,2,6,6-tetramethylpiperidine-1-oxy (TEMPO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tris(hydroxymethyl)amino methane (tris), ThioGlo 1 fluorescent thiol reagent, and trifluoroacetic acid (TFA,

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>99.8%) were obtained from Merck (Darmstadt, Germany). Bradford Bio-Rad Protein Assay Reagent was obtained from Bio-Rad Laboratories (Hercules, CA, USA). Bovine Serum Albumin (BSA) standard of 2.0 mg/mL was obtained from Thermo Fisher Scientific Inc. (Rockford, IL, USA). Megazyme assay kit for determination of β-glucans was purchased from Megazyme International Ireland (Wicklow, Ireland). Ethanol (96%) was obtained from Kemetyl (Køge, Denmark). All chemicals were of analytical grade or highest possible purity. Water was purified through a Milli-Q water purification system (Millipore, Billerica, USA).

Malt. The three two-row barley varieties (*H. vulgare*) Pallas, Fero, and Archer were grown on the same location "Fuglebjerggaard" at Zealand, Denmark and harvested in 2009. The chosen varieties are former commercially grown varieties in Denmark and of renewed interest today for organic farming. To be sure to overcome dormancy, the barley was malted simultaneously in the spring of 2010 with steeping for 2 days and 4 h at 18 °C, germination for 4 days and 18 h at 17 °C, drying for 3 days at 15 °C, and kilning for 24 h at 85 °C. The freshly kilned malt was stored for more than 20 days before mashing.

Mashing. Three individual mashings were carried out according to Analytica EBC 4.5.1 "Extract of Malt: Congress Mash"[11] with a few modifications described by Frederiksen et al.[12] Wort produced from the third mashing was used for wort boiling. Filtration rate, pH, sugar content, and color were measured on the fresh sweet wort carried out in duplicate.

Boiling. Two 300 mL aliquots of each sweet wort were transferred to a 500 mL conical flask. To one flask of each wort was added 2 g of hops (*Humulus lupulus*) (First Gold, leafs, 6% AA (alpha)), and the content of each conical flask was kept boiling in a bath of rape seed oil (140 °C) for 1 h.

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

$$C = 25 \cdot f \cdot A_{430} \quad (1)$$

where C is the color in EBC units, f is the dilution factor, A_{430} is the absorbance at 430 nm, and 25 is a multiplication factor. Samples were filtered through a 0.45 μm filter prior to analysis.

Sugar Content (°Brix). Sugar content in °Brix was determined using a refractometer (Analytic Jena, Jena, Germany).

β-Glucan Determination in Malt. β-Glucan concentrations in malt in replicates were determined spectrophotometrically at 510 nm after digestion with the Megazyme assay kit according to AACC 32-23.[13]

Electron Spin Resonance (ESR) Spectroscopy. Wort samples were thawed and filtered (Mini Sart, 0.45 μm), and ethanol and α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN) spin trap were added to 5% v/v and 40 mM, respectively. Wort was incubated for 90 min at 60 °C before ESR spectra were recorded with a Miniscope MS 200 X-band spectrometer (Magnettech, Berlin, Germany) using 50 μL micropipets as sample cells. Spectra were recorded at room temperature using the following settings: microwave power, 10 mW; B_0 field, 3363 G; sweep width, 50 G; sweep time, 30 s; steps, 4096; number of passes, 6; modulation, 1 G; attenuation, 5 mW. Amplitudes of the spectra were measured and are reported as the height of the second doublet. The response of the ESR equipment was tested using an aqueous TEMPO solution (2 μM). Samples were analyzed in triplicate.

Determination of Thiol-Removing Capacity. Thiol levels were determined according to Lund and Andersen 2011[6] using both cysteine and glutathione as standards. Briefly, thiol levels in wort were determined using the thiol-selective reagent ThioGlo 1 which yields a fluorescent adduct after reaction with a thiol group (excitation wavelength, λ_{ex} = 384 nm, emission wavelength, λ_{em} = 513 nm). Analysis was carried out in microtiter plates after cysteine (wort diluted 40 times) or glutathione (wort diluted 10 times) were added to the wort at concentrations between 0 and 20 μM.

In our previous study[10] we found that sweet wort contains compounds able to oxidize cysteine and glutathione upon addition to the sweet wort. The amount of thiols that can be added to the wort before the thiol−ThioGlo 1 adducts appear is defined as the thiol-removing capacity.

Glutathione−ThioGlo 1 adducts exhibit a much stronger fluorescent response than cysteine−ThioGlo 1, which is seen in Figure 2. This feature was detailed by Hoff et al.[14] where it was found that thiols with a neighboring free amino group, which is the case for cysteine, result in ThioGlo 1 adducts with reduced fluorescence intensity. This difference in fluorescence intensity does not influence the interpretation of the thiol-removing capacity nor the fact that the thiol-removing capacity is more reactive toward cysteine than toward glutathione.

Thiol Determination Using External Standard Curve. The thiol-removing capacity makes it impossible to apply the standard addition procedure for thiol quantification in wort, so thiols can only be quantified using an external standard curve. Consequently, quantification and comparison can only be carried out on light worts of similar color and therefore with a similar matrix. A standard curve was prepared in buffer between 0 and 12 μM, and sweet, boiled, and hopped wort samples were quantified relative to this standard curve. Samples were undiluted.

Thiol Determination after Reduction with TCEP. The external standard curve was prepared in the range of 0−20 μM in 0.25 mM tris buffer (pH = 7.5). TCEP (tris(2-carboxyethyl)phosphine hydrochloride) was added to the wort to 1.92 mM (final concentration), and the mixture was incubated for 5 min. Thiol concentration was determined as described above. Any possible interaction between TCEP and the NEM (*N*-ethyl maleimide) group of ThioGlo 1[15] was controlled by background correction with a blank sample of ThioGlo 1 and TCEP at appropriate concentrations.

Volatile Profile. Head space analysis was carried out in triplicate using 5 mL of wort and 0.25 mL of 4-methyl-1-pentanol (5 mg L^{-1}) as the internal standard. Volatile compounds were collected on traps containing 250 mg of Tenax-TA with mesh size 60/80 and a density of 0.37 g mL^{-1} (Buchem bv, Apeldoorn, The Netherlands). Samples were equilibrated to 37 ± 1 °C in a circulating water bath and then purged with nitrogen (75 mL min^{-1}) for 30 min.

Trapped volatiles were desorbed using an automatic thermal desorption unit (ATD 400, Perkin-Elmer, Norwalk, USA). Primary desorption was carried out by heating the trap to 250 °C with a flow (60 mL min^{-1}) of carrier gas (H_2) for 15.0 min. The stripped volatiles were trapped in a Tenax TA cold trap (30 mg held at 5 °C), which was subsequently maintained at 300 °C for 4 min (secondary desorption, outlet split 1:10). This allowed for rapid transfer of volatiles to 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, CA) through a heated (225 °C) transfer line. Separation of volatiles was carried out on a DB-Wax capillary column 30 m long × 0.25 mm internal diameter, 0.25 μm film thickness. Column pressure was held constant at 2.4 psi, resulting in an initial flow rate of approximately 1.2 mL min^{-1} using hydrogen as carrier gas. The column temperature program was as follows: 10 min at 40 °C, from 40 to 240 °C at 8 °C min^{-1}, and finally 5 min at 240 °C. The mass spectrometer was operating in the electron ionization mode at 70 eV. Mass-to-charge ratios between 15 and 300 were scanned. Volatile compounds were 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, CA) was used for data analysis. Concentrations are presented as relative areas calculated as peak area of the volatile compound divided by the peak area of internal standard. One chromatogram of sweet Fero wort (abbreviated, F1s) was removed from the data set due to excessive amounts of water on the trap.

Multivariate Data Analysis. Multivariate data analysis was applied to GC-MS data to evaluate the variation between the barley varieties as well as the influence of boiling with and without hops using principal component analysis (PCA). PCA is a multivariate projection method designed to extract and visually display the systematic variation in the data matrix of the volatile compounds, making it possible to include many statistical variables at the time. The aim of

Table 1. Characteristics of Wort Produced from Pallas, Fero, and Archer Malt[a]

	Archer	Fero	Pallas
β-glucan in malt (g/100g)	0.82 ± 0.00^{A}	1.73 ± 0.03^{B}	1.81 ± 0.00^{C}
filtr. time (SW) (min)	42 ± 5.7^{A}	21.5 ± 2.1^{B}	16 ± 2.8^{C}
°Brix (SW)	13.39 ± 0.09^{A}	13.44 ± 0.01^{A}	13.45 ± 0.00^{A}
°Brix (BW)	13.44 ± 0.02^{A}	13.44 ± 0.02^{A}	13.45 ± 0.00^{A}
°Brix (BHW)	13.44 ± 0.00^{A}	13.45 ± 0.00^{A}	13.45 ± 0.00^{A}
pH (SW)	5.94 ± 0.01^{D}	6.01 ± 0.00^{B}	5.98 ± 0.01^{C}
pH (BW)	5.97 ± 0.02^{C,D}	5.85 ± 0.01^{E}	6.08 ± 0.00^{A}
pH (BHW)	5.50 ± 0.00^{H}	5.57 ± 0.01^{G}	5.66 ± 0.01^{F}
EBC color (SW)	5.15 ± 0.08^{E}	4.35 ± 0.08^{F}	3.91 ± 0.05^{G}
EBC color (BW)	6.15 ± 0.10^{C}	6.15 ± 0.08^{C}	5.53 ± 0.05^{D}
EBC color (BHW)	9.30 ± 0.16^{B}	9.98 ± 0.03^{A}	9.25 ± 0.19^{B}
thiols (SW)	0.06 ± 0.01^{F}	−0.03 ± 0.01^{F}	0.01 ± 0.04^{F}
thiols (BW)	0.77 ± 0.06^{D}	1.24 ± 0.07^{A}	0.91 ± 0.02^{C}
thiols (BHW)	1.05 ± 0.01^{B}	0.61 ± 0.0^{E}	0.74 ± 0.07^{D}

[a]Sweet wort (SW), boiled wort (BW), and boiled hopped wort (BHW). Values are given as means ± standard deviations based on independent duplicates. A, B, C, D, E, F indicate the samples statistical difference, within each type of analysis, and the levels bearing different letters are significantly different ($p < 0.05$).

the PCA algorithm is to determine the latent factors or principal components (PCs) in the data set which describe most variation. On the basis of vector algebra the algorithm calculates and compresses the data material into scores (samples) and loadings (volatiles) for PCs which are plotted in the score plot and loadings plot, respectively. The position of a sample in the score plot expresses the pattern of the volatile profile, so those samples with similar scores reflect the same volatile profile.[16] PCA was performed using Latentix software (LatentiXTM 2.0, Latent5, Copenhagen, Denmark, www.latentix.com). Analyses were carried out on the relative peak areas, and data were autoscaled and cross validated.

Statistical Data Analyses. Statistical analysis was carried out as one-way ANOVA using the software JMP 9, SAS Institute, Inc., USA. Some day to day variance was observed from thiol quantification, day was included in the model as a random effect, and the LSD (least significant difference) value was determined

RESULTS

Sweet Wort Characteristics. Three barley varieties (*H. vulgare*) Pallas, Fero, and Archer were chosen for analysis in this study. Pallas is an old conventional barley variety used today in organic farming, while Fero and Archer are former grown varieties which also may have relevant properties in relation to organic farming. For the current study all three barley varieties were grown at the same location and harvested and malted simultaneously in order to obtain malts where the differences were primarily due to the selected variety. With this set up, it is possible to compare the effect of the variety on the influence on volatile profile and oxidative stability in the initial stages of brewing. The malts were mashed using the EBC congress mash procedure,[11] and the sweet worts were boiled simultaneously either with or without hops for 1 h. The extracts (°Brix) in the three worts were very similar and not influenced by boiling and hopping (Table 1). The barley varieties had a small but significant influence on sweet wort pH, and boiling resulted in a small increase in wort pH for all varieties being significant for Fero and Archer. Boiling with hops resulted in a significant decrease in pH for the three varieties, presumably due to introduction of alpha acids. The amount of hops applied in the current study was overdosed in order to detect increased effects compared to commercially produced wort. Sweet wort color was significantly influenced by the barley variety with Archer being the darkest at 5.15 ± 0.08 EBC and Pallas the lightest at 3.91 ± 0.05 EBC. The boiling process resulted in a significant increase in EBC color for all worts from all three barley varieties, and at the same time the boiling also eliminated some of the color differences between the barley varieties found in the sweet worts. However, boiled wort from Pallas remained significantly lighter than the other two. The increase in EBC color is most likely caused by heat-induced Maillard reactions, and boiling with hops resulted in a further increase in EBC color, eliminating the varietal differences. Filtration time varied between the three varieties and, in particular, gave Archer a long filtration time of 42 ± 5.7 min compared to Fero and Pallas with filtration times of 21.5 ± 2.1 and 16 ± 2.8 min, respectively. The content of β-glucan in Archer malt of 0.82 g/100 g ± 0.00 was significantly lower than in Fero and Pallas with 1.73 g/100 g ± 0.03 and 1.81 g/100 g ± 0.00, respectively.

Thiol Concentrations and Thiol Removing Capacity. *Thiol Concentrations.* In previous studies reduced thiols have been detected in wort.[17−19] In the current study the nonreduced thiol content was found to be close to zero (Table 1) and low compared to what has previously been reported in beer.[6] The fact that almost no thiols are present in the wort is most likely explained by application of the EBC congress mash procedure where the mash is exposed to atmospheric air throughout the mashing process as well as during filtration. The fact that thiols previously have been shown to be present in reduced form in wort underlines that the EBC congress mash method is not suitable for studies of thiols. Our observation is in agreement with the findings of Stephenson et al.,[19] who showed that aerobic EBC congress mashing reduced thiol levels considerably compared to EBC congress mashing performed in an anaerobic chamber. Even though the thiol concentrations in the present study are very low, it seems that boiling (with or without hops) actually increase the thiol concentrations. This could be due to unfolding of proteins during boiling, leaving the thiol groups more susceptible to reaction with the ThioGlo 1 reagent, or inactivation of the thiol-oxidizing enzyme.

To quantify the amount of oxidized thiols in the wort, the disulfide bonds were reduced to free thiols using the reducing agent TCEP (tris(2-carboxyethyl)phosphine). It was found that Archer had a larger amount of reducible thiols compared to Pallas and Fero and that boiling and hopping of the wort did not influence the amount of reducible thiols significantly

(Figure 1). These results show that the amount of reducible and potentially antioxidative thiols in wort is rather large (ca. 60−110 μM), and as the thiols most likely became oxidized due to the aerobic mashing conditions, optimization of the mashing process is likely to have a positive influence on prevention of thiol oxidation. It is not certain that all thiols were on their reduced form before mashing; however, reduced thiols have previously been detected in wort where they were also found to be sensitive toward oxidation.[19] The fact that thiol oxidation occurs during the mashing process therefore seems to be the most reasonable explanation.

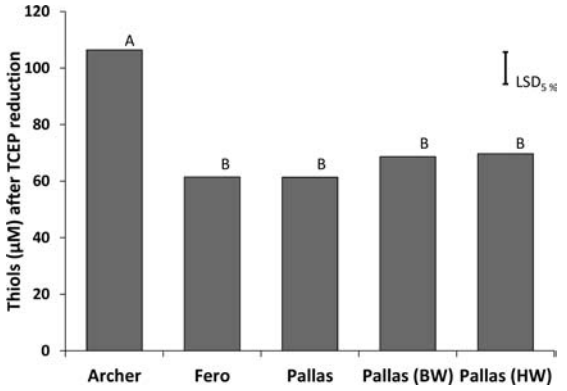

Figure 1. Thiol concentrations in wort samples treated with the thiol reducing agent TCEP. Due to a day to day variation between the measurements, LSD (least significant difference) value is presented. Values are given as means (n = 3), and the LSD value is presented by an error bar. Letters indicate the statistical difference of samples, and the levels bearing different letters are significantly different (p < 0.05).

Sweet Archer wort had the longest filtration time (Table 1) along with the largest amount of reducible thiols (Figure 1). Formation of gel proteins, caused by thiol oxidation and generation of disulfide bonds between protein and peptide thiols during mashing, can result in a viscous layer leading to increased filtration times.[7−9] Basically all thiols were found to be oxidized in the current study. As sweet Archer wort was found to contain more reducible thiols, more disulfide bonds are likely to have formed, possibly explaining the longer filtration time. Also, the amount of β-glucans, present in the malt at the point of filtration, is known for its negative influence on filtration time.[20] However, as Archer malt had the lowest content of β-glucan (Table 1) compared to the other malts the possible influence of β-glucan can be neglected. Therefore, keeping thiols in their reduced form is important for preservation of their possible antioxidative potential as well as to keep a short filtration time, stressing the importance of carrying out the mashing production under oxygen-controlled conditions.

Thiol-Removing Capacity. In a recent study we found that compounds present in sweet wort were able to oxidize cysteine, and this ability to oxidize thiols was referred to as thiol-removing capacity.[10] In the current study it was investigated how barley varieties, wort boiling, and wort hopping influenced the thiol-removing capacity as well as how the thiol-removing capacity affected cysteine compared to glutathione. Wort boiling seemingly reduced the thiol-removing capacity completely when adding glutathione to the wort. However, a small amount of thiol-removing capacity remained after boiling when cysteine was added (Figure 2). All three barley varieties showed the same tendency (Figure 3), and results are only presented for Pallas wort in Figure 2. Bamforth et al.[21] found a heat-stable enzyme, present in fresh malt, capable of oxidizing thiol groups (cysteine, glutathione, and dithiothreitol), resulting in disulfide cross-linking of proteins. This enzyme retained approximately 70% of its activity when heated to 70 °C, which is the mashing off temperature in the current study. After heating at 100 °C for 30 min, approximately 40% of the thiol-oxidizing activity

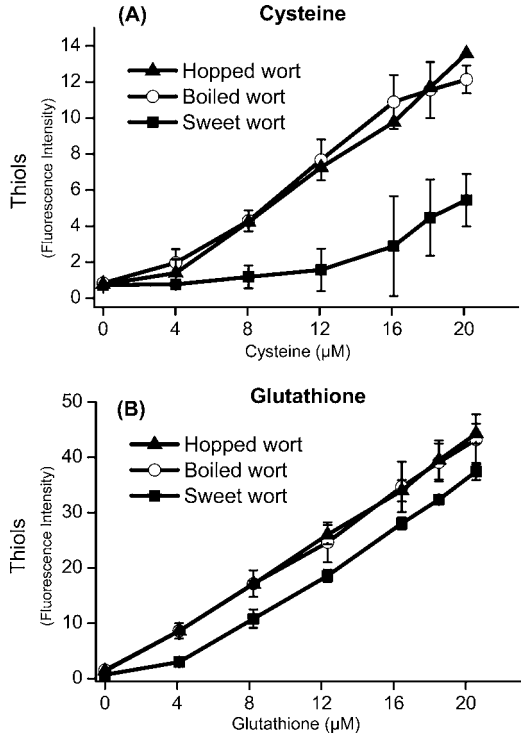

Figure 2. Addition of thiols to the worts. (A) Fluorescent response from Pallas wort (sweet, boiled, and boiled and hopped) with added cysteine in concentrations between 0 and 20 μM. Wort was diluted 40 times. (B) Fluorescent response from Pallas wort (sweet, boiled, and boiled and hopped) with glutathione added in concentrations between 0 and 20 μM. Wort was diluted 10 times. Values are given as means (n = 3), and standard deviations are shown by error bars.

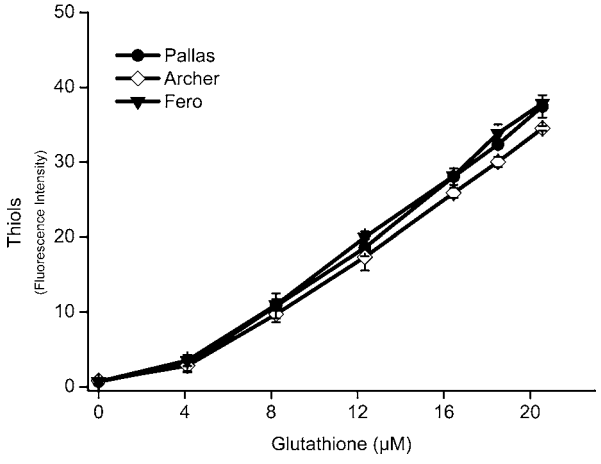

Figure 3. Fluorescent response from Pallas, Fero, and Archer sweet wort with glutathione added in concentrations between 0 and 20 μM. Wort was diluted 10 times. Values are given as means (n = 3), and standard deviations are shown by error bars.

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remained and it was suggested that the remaining thiol-oxidizing activity was caused either by a very heat-stable enzyme or by nonenzymatic oxidation.[21] The results from the current study correlate with previous findings of the thiol-removing capacity being heat sensitive.[10,21] The occurrence of both enzymatic and nonenzymatic thiol oxidation in wort was also suggested in previous studies[10,19,21] though, whether the thiol oxidation occurring after wort boiling is explained by enzymatic or nonenzymatic oxidation remains uncertain.

The thiol-removing capacity caused a greater loss of cysteine than glutathione. In order to keep the results within the same concentration range, wort was diluted 40 times when using cysteine but only 10 times when using glutathione (Figure 2). As thiol adducts are detected by addition of 8 μM glutathione and addition of 16 μM cysteine a rough estimate makes the thiol-removing capacity at least 8 times more efficient toward cysteine than toward glutathione when including the dilution factor. In line with this, Bamforth et al.[21] found that even though glutathione displayed a much higher affinity for the enzyme than cysteine, cysteine was oxidized approximately 5 times more rapidly. The influence of barley variety was also investigated, but no effect was found on the thiol-removing capacity (Figure 3). The same result was found using cysteine (data not shown). The current study further showed that addition of hops did not systematically influence detection of thiols in the boiled wort (Table 1), and the potential antioxidative effect of the hops did not seem to influence the thiol-removing capacity when adding either cysteine or glutathione (Figure 2). In a previous study[10] a variation in thiol-removing capacity was found between two different malts (origin unknown), but in that study, barley, malting, and storage conditions were very different and not standardized as it was in the current study. This indicates that malting and storage conditions may have a larger influence on the thiol-removing capacity than the barley variety itself. It is known that freshly kilned malt should be stored for more than 20 days to improve filtration rates.[22] Bamforth et al.[21] explained this improvement of the malt quality during storage by inactivation of the thiol-oxidizing enzymes, supporting that storage time and conditions influence the thiol-removing capacity.

Radical Intensity Measured by ESR Spectroscopy. The radical-forming ability of sweet wort, boiled wort, and hopped wort made from Archer, Fero, and Pallas malt was determined by electron spin resonance (ESR) spectroscopy. Quantification was carried out by detection of radicals generated after heating samples at 60 °C for 90 min in the presence of the spin trap, POBN, and 5% ethanol. The amplitude of the second doublet of each ESR spectrum, recorded during heat treatment, was used as a measure of the amount of radicals generated (Figure 4A). Wort boiling without hops resulted in increased radical formation compared to sweet wort (Figure 4B), whereas radical formation of boiled/hopped wort and sweet wort was not statistically different. The increase in radical intensity during boiling and the antioxidative effect of hops supports the findings of Wietstock et al.[23] The barley varieties showed small effects on radical intensity though Fero was significantly more sensitive to boiling than Archer and Pallas.

Volatile Profiles. The volatile profile of sweet wort, boiled wort, and hopped wort was determined with the purpose of clarifying whether the barley variety or the boiling or hopping of the corresponding worts had an influence on the volatile profile. Hops contribute with a large amount of volatiles influencing wort aroma; however, the focus of this study

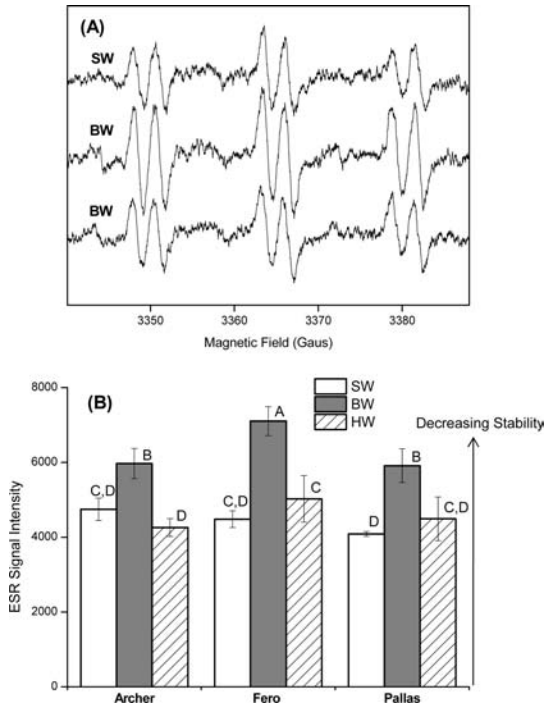

Figure 4. (A) ESR spectra of wort made from Pallas malt (sweet wort (SW), boiled wort (BW), and hopped wort (HW)) analyzed after 90 min of incubation at 60 °C with POBN (40 mM) and ethanol (5%). (B) Radical signal intensities measured by ESR spectroscopy of wort produced from Pallas, Fero, and Archer malt (sweet wort (SW), boiled wort (BW), and hopped wort (HW)). Values are given as means (n = 3). Letters indicate the statistical difference of samples, and levels bearing different letters are significantly different ($p < 0.05$).

concerns mainly the barley varieties, and only the fate of the compounds present in sweet and boiled wort were investigated and followed through to the hopped wort. Compounds coming exclusively from the hops were not included in analysis. Through headspace analysis 24 volatile compounds were identified in sweet and boiled wort (Table 2), and to investigate whether there was a difference between the volatile profiles of the barley varieties along with the boiling and hopping principal component analysis (PCA) was performed with scores and loadings presented in Figure 5. No volatile secondary oxidation products were detected; however, nondetectable precursors for oxidation may have been generated, resulting in release of off-flavors during storage of a final beer.[1] From the score plot it was found that sweet Archer wort differed from sweet Pallas wort and sweet Fero wort. After wort boiling, the differences between Archer and Pallas were leveled out while Fero was found to differ in volatile profile from the two others. Addition of hops masked the differences between the barley varieties; however, when excluding boiled wort from the score plot, Fero remained different from Pallas and Archer (data not shown), meaning that hops did not eliminate the differences completely as it appears in the present plot. When looking more specifically at the volatile compounds sweet Archer wort differentiated from sweet Fero wort and sweet Pallas wort by having a significantly higher concentration of 2-methyl-1-propanol, benzaldehyde, and limonene and a tendency for a higher concentration of phenylacetaldehyde and 2-pentanone. Furthermore, Archer had a significantly lower concentration of hexanal and a tendency for a lower concentration of 2-methyl-1-butanol and 2-ethyl-1-hexanol compared to sweet Fero and

Table 2. Volatile Compounds Included in the PCA Plot (Figure 5)[a]

no.	name	target ion	no.	name	target ion
1	propanal	29	13	3-methyl-1-butanol	55
2	2-methylpropanal	43	14	pentanol	42
3	butanal	44	15	1-hexanol	56
4	3-methylbutanal	44	16	3-ethyl-cyclobutanone	41
5	2-pentanone	43	17	furfural	96
6	2-methyl-butanal	57	18	2-ethyl-1-hexanol	57
7	2,4-dimethyl-3-pentanone	43	19	benzaldehyde	77
8	hexanal	56	20	2-methylpropanoic acid	43
9	2-methyl-1-propanol	43	21	phenylacetaldehyde	91
10	1-butanol	56	22	2,5-dimethyl-benzaldehyde	133
11	2-methyl-1-butanol	57	23	3-methyl-2-hexen-1-ol	71
12	limonene	68	24	phenol	94

[a]Compounds are identified in sweet, boiled, and boiled and hopped wort, though volatiles specific for the hops are not included. Target ion (Tgt) is used for identification, and the number of each compound corresponds to the location in the loadings plot (Figure 5B).

Pallas wort. After boiling the volatile profiles had changed and Fero differentiated from Pallas and Archer by having a significantly larger concentration of furfural and a tendency for a larger concentration of phenylacetaldehyde, 2-ethylhexanol, and 1-butanol. After boiling with hops Fero remained different from Archer and Pallas with a significantly larger content of furfural and a tendency for a larger content of phenylacetaldehyde and 1-butanol; however, again, this is not seen in Figure 5 as the contribution from the hops mask this tendency in the presented PCA plot. Furfural and phenylacetaldehyde are typical heat-induced compounds, and their formation correlates with the larger increase in color detected in boiled and hopped Fero wort compared to boiled and hopped Archer and Pallas wort. The results of volatile analysis show that the barley variety does influence the volatile profile, and barley may be selected with the purpose of influencing the volatile profile of the wort and possibly the final beer. Interestingly, boiling was found to eliminate some differences while introducing others unique to each barley variety, showing that also the processing influences and changes flavor composition.

It was previously found that no volatile compounds were evaporated from light sweet wort left at 40 °C for 10 h, whereas volatile compounds were found to evaporate from sweet worts made from malt roasted to more than 33 EBC.[10] In the current study boiling resulted in loss of many volatiles, even for light worts, which is exemplified in Figure 6 for the compounds showing the largest loss: hexanal, limonene, pentanol, and benzaldehyde. Few compounds increased in concentration during boiling, and some were present in approximately equal concentration before and after boiling, most likely due to formation during boiling. Furfural, phenol, propanal, and the Strecker aldehyde 2-methylpropanal were among the main compounds formed during boiling (Figure 7). Wort boiling also resulted in generation of Strecker aldehydes, and the contribution of Strecker aldehydes from hops is very limited. Development of 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde is shown in Figure 8. The antioxidative activities from hops did not seem to inhibit Strecker aldehyde production during wort boiling though; Wietstock et al.[23] found hops to have an inhibiting effect on generation of the Strecker aldehydes and staling compounds, 2-methylbutanal and 3-methylbutanal, during beer storage. Boiling with hops resulted in increased concentrations of most compounds present in sweet worts. Hops have a broad and complex volatile profile, and many compounds present in the wort are also present in hops. The compounds, which in this study are found to be unique for the malt, are isoamylalcohol, phenylacetaldehyde, 2,5-dimethylbenzaldehyde, propanal, hexanol, and 2- and 3- methylbutanal (data not shown).

DISCUSSION

Archer wort needed 42 ± 5.7 min of filtration compared to only 21.5 ± 2.1 and 16 ± 2.8 min for Fero and Pallas. This result could not be explained by the β-glucan determinations in malt where Archer had the lowest content of the three malt samples. Reduction of thiols using TCEP revealed a large difference between the thiol content of the worts, and Archer wort had a significantly larger content of total thiols. All thiols were found to be oxidized, causing generation of gel proteins[7−9] and longer

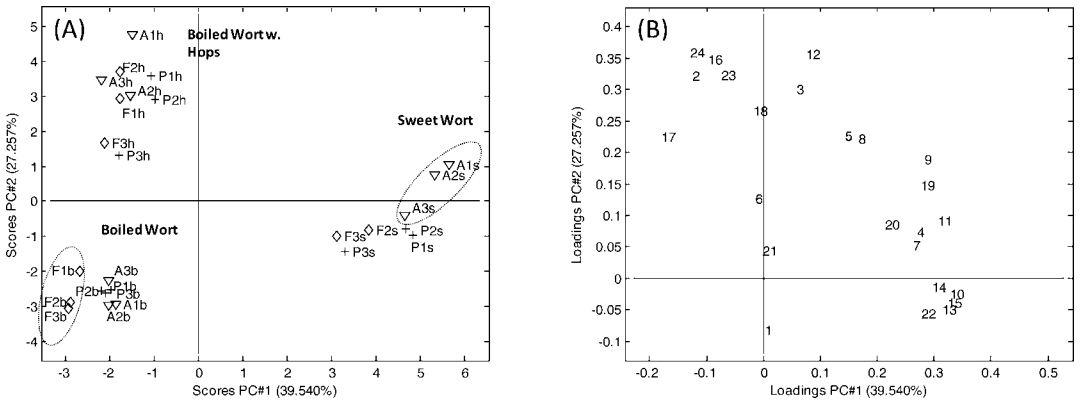

Figure 5. (A) PCA score plot based on the volatile compounds found in sweet, boiled, and hopped Pallas, Fero, and Archer wort. Only the volatiles also detected in sweet and boiled wort have been included for the hopped wort: sweet archer wort (A1s, A2s, A3s), boiled Archer wort (A1b, A2b, A3b), hopped Archer wort (A1h, A2h, A3h); sweet fero (F2s, F3s), boiled fero (F1b, F2b, F3b), hopped Fero (F1h, F2h, F3h); sweet Pallas wort (P1s, P2s, P3s), boiled Pallas wort (P1b, P2b, P3b), hopped Pallas wort (P1h, P2h, P3h). Ovals enclose the wort samples that differentiate from the others. (B) Loadings plot where each volatile compound is represented by a number and identified in Table 2.

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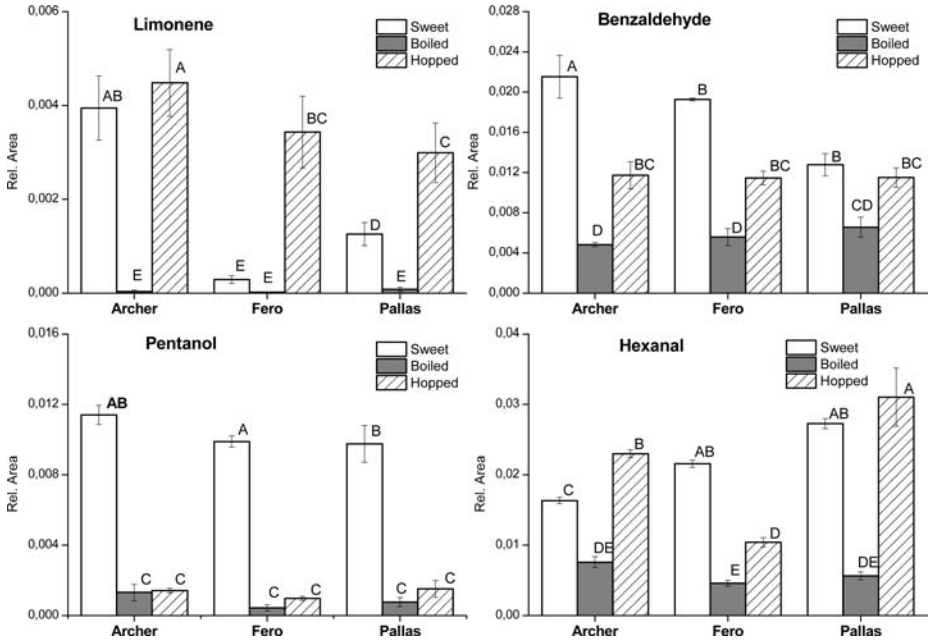

Figure 6. Relative concentrations of hexanal, limonene, benzaldehyde, and pentanol in sweet, boiled, and boiled and hopped Archer, Fero, and Pallas wort. These volatiles are chosen as examples of the loss happening during wort boiling. Values are given as means (n = 3), and standard deviations are shown by error bars. Letters indicate the statistical difference of samples, and levels bearing different letters are significantly different (p < 0.05).

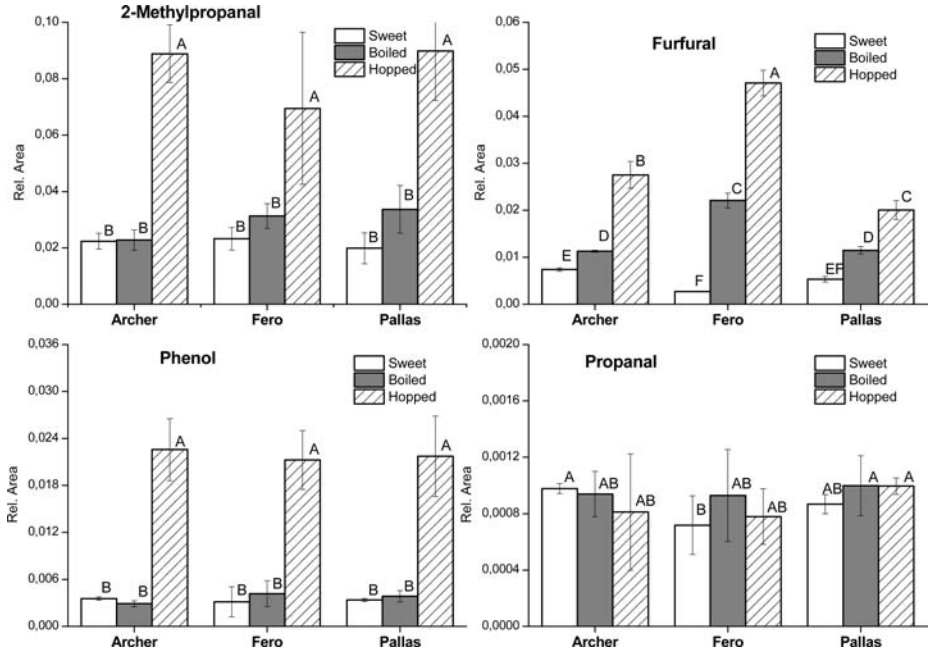

Figure 7. Relative concentration of furfural, 2-methyl propanal, phenol, and propanal in sweet, boiled, and boiled and hopped Archer, Fero, and Pallas wort. These compounds show the largest increase in concentration during boiling. Values are given as means (n = 3), and standard deviations are shown by error bars. Letters indicate the statistical difference of samples, and levels bearing different letters are significantly different (p < 0.05).

filtration time of Archer wort. Therefore, the challenge of keeping thiols in their reduced form during mashing and filtration is not only important to preserve the potential antioxidative capacity of the thiols but also to keep a short filtration time. These results underline the importance of oxygen-controlled processes. Due to the aerobic mashing and filtration all thiols were oxidized, and the differences found between the barley varieties were leveled out. Radical formation was also highly influenced by the processing. Boiling resulted in a large increase in radical formation, whereas boiling with hops prevented this increase in radical formation due presumably to its antioxidative activity as previously described.[23] Barley varieties had little effect on radical formation. Wort from the three barley varieties also had the same thiol-removing capacity, which was not completely eliminated after boiling. The thiol-removing capacity is not present in beer,[6] and as it is still present, to a small extent, in boiled and hopped wort, the remaining activity must somehow become eliminated during the fermentation process.

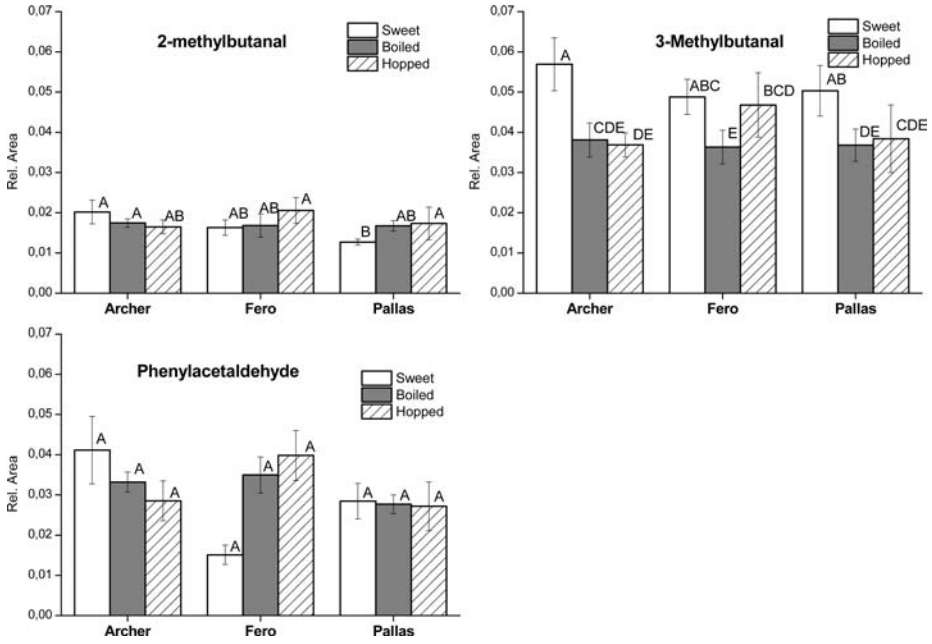

Figure 8. Relative concentration of the Strecker aldehydes 2-methyl-butanal, 3-methylbutanal, and phenylacetaldehyde in sweet, boiled, and boiled and hopped Archer, Fero, and Pallas wort. Values are given as means (n = 3), and standard deviations are shown by error bars. Letters indicate the statistical difference of samples, and levels bearing different letters are significantly different (p < 0.05).

Differences were detected in volatile profile caused by the barley variety however; the volatile profiles were highly influenced by the processing where boiling caused a decrease in many compounds due to evaporation but also an increase in other compounds. Sweet Archer wort differed from sweet Fero and Pallas wort by having a significantly larger concentration of 2-methyl-propanol, benzaldehyde, and limonene as well as a significantly lower concentration of hexanal. Fero wort differed in volatile profile from Archer and Pallas wort after boiling and was found to develop more heat-induced compounds such as furfural and phenylacetaldehyde. This correlated with increased radical intensity of boiled Fero wort measured by ESR spectroscopy. Not surprisingly, boiling caused mainly an increase in heat-induced compounds such as furfural, propanal, phenol, and 2-methylpropanal, and the compounds found most sensitive toward evaporation during boiling were hexanal, limonene, pentanol, and benzaldehyde. Many volatile compounds present in malt are also present in hops. However, the compounds which in this study were found to be unique for the malt are isoamylalcohol, phenylacetaldehyde, 2,5-dimethylbenzaldehyde, propanal, hexanol, and 2- and 3- methylbutanal. On the basis of this investigation of three barley varieties produced under the same conditions we found that when selecting a barley variety with the intension of influencing beer flavor it is important not only to evaluate the sensory properties of the malt and the sweet wort but also to evaluate how the volatile profile is influenced by processing.

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Notes

The authors declare no competing financial interest.

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