Research Article

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Technological influence on sensory stability and antioxidant activity of beers measured by ORAC and FRAP

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Abstract

BACKGROUND: Many studies have confirmed a wide variation in the phenolic content and antioxidant activity of beers. However, when commercial beers are studied, there is usually no information available on the brewing technology applied. In this study, technological parameters were varied systematically to influence the antioxidant content of beer with a view to improving its flavor stability. High-throughput assays, ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) were investigated as fast analytical methods to evaluate the influence of brewing technology on antioxidant activity.

RESULTS: Beers (n = 12) were brewed with systematic technological variations (malt modification, hopping regime) to influence the antioxidant potential. A late hop addition resulted in significantly higher phenolic content (high-performance liquid chromatography with diode-array detection – HPLC-DAD) and antioxidant activity. Raw protein content and malt modification significantly influenced phenolic content and the antioxidant activity of beers hopped at the beginning of wort boiling. Samples were stored under forced and natural conditions and were evaluated by a sensory panel. The decline of bitter iso-α-acids as an analytical marker for oxidative aging was significantly lower in beers brewed from malts with high raw protein content. These samples also had higher antioxidant activity values. Panelists gave higher ratings for beer quality to aged beers with a late hop addition. However, late hopping resulted in enhanced hoppy aroma attributes and therefore an altered aroma profile.

CONCLUSIONS: Both antioxidant capacity methods were well suited as fast methods to evaluate brewing raw material and technological influence on antioxidant activity. The appropriate choice of barley malt and the malting regime could be promising tools to enhance the antioxidant activity of traditionally hopped beers.

Supporting information may be found in the online version of this article.

Keywords: beer; antioxidant; barley; malt; hops; stability

INTRODUCTION

Beer is the most frequently consumed alcoholic beverage in the world, with an increasing export rate. However, higher export rates entail longer transport and storage times, which lead to higher expectations concerning long-term storage stability. Beer flavor is subject to changes during storage, both by non-oxidative and oxidative processes. Oxidative reactions due to the entry of oxygen lead to the rapid formation of off-flavors in beer.[1] Antioxidants in beer have therefore been of great interest to brewers due to their beneficial role in beer storage stability.[2–4] The antioxidant compounds of beer are heterogeneous, belonging to completely different chemical substance groups: Sulfur dioxide,[3,5,6] thiols,[7] Maillard reaction products,[8,9] hop-derived bitter compounds like α-acids[10–12] and phenolic substances[13–17] from both barley malt and hops. Both brewing raw materials are rich in antioxidants. Barley (*Hordeum vulgare* L.) contains a variety of phenolic compounds: Benzoic and cinnamic acid derivatives, hordatines, lignans and flavonoids.[13,18–21] During malting, the phenolic content of grain extracts increases due to the enzymatic release of phenolic acids from cell-wall polysaccharides and due to the enhanced extractability of the malted grain.[22–24] As recent studies have shown, the antioxidant activity of malt is correlated with the antioxidant activity of the raw barley, which is influenced by genotype and growth factors.[25,26] Technological malting parameters are varied as a tool to adjust malt quality parameters. The phenolic content of malted barley depends on the malting regime, with higher levels of malt modification resulting in a higher content of

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free phenolic acids.[27] There is a myriad of antioxidant compounds derived from hops (*Humulus lupulus* L.): Resinous compounds like α-acids or prenylated chalcones and non-resin phenolic compounds like phenolic acids or flavonoids.[13,28–30] Hop is either added at the beginning of wort boiling to achieve the desired bitterness or at the end of boiling or during whirlpool rest to impart a hoppy aroma to the final beer.[30] A hopping regime with a late hop addition has been described as beneficial for oxidative beer stability.[4,10] The higher concentration of unisomerized α-acids in late hopped beers leads to a precipitation of potentially pro-oxidant transition metal ions and thus improves oxidative beer stability.[10] In a recent study, Wietstock *et al.* found reduced iron concentration in worts after the addition of hop CO_2-extract.[31] A modified hop dosage was found to improve oxidative beer stability and resulted in lower contents in staling aldehydes after storage compared with a single hop dosage at the beginning of wort boiling.[31] The positive effect of hopping was mainly attributed to the antioxidant properties of hop α- and β-acids.[31] Apart from resinous compounds, hop phenolic compounds have been discussed as potential antioxidants by a number of authors.[32–34]

Even though positive effects of phenolic substances have been reported,[33,34] their role in beer flavor stability is still controversial. Flavor changes during storage include the loss of bitterness or the development of a harsh lingering bitter taste as well as the development of a typical aged flavor. The stale beer flavor is characterized by the appearance of sweet, cardboard, and bread-like flavor notes.[35] The overall stale flavor of beer is caused by a number of different compounds, but the vital role of staling aldehydes was emphasized by a number of studies.[35–37] Staling aldehydes arise from the oxidation of fatty acids, the Strecker degradation of amino acids, the release of bound aldehydes during beer storage, the Maillard reaction, melanoidin catalyzed oxidation of higher alcohols, and the degradation of bitter acids.[35] The degradation of bitter iso-α-acids was described as an important analytical marker for oxidative beer aging by a number of authors.[38–42] Oxidative degradation reactions play a central role in the loss of bitter acids during beer storage.[41] In the presence of oxygen, iso-α-acids are oxidized to hydroperoxy- and hydroxyl-allo-iso-α-acids, regardless of their *cis-* / *trans-* configuration.[39] In a study by Karabín *et al.* (2014), decline in iso-α-acids correlated with the decline in antioxidant potential during beer storage.[38] The degradation of iso-α-acids was therefore used in this study as a marker for oxidative beer aging apart from changes in sensory attributes. The phenolic content and antioxidant activity of beer is greatly influenced by raw material choice and the brewing process. Accordingly, a wide variation in phenolic profile and antioxidant activity across commercial beer samples has been shown by a number of research studies.[17,43–45] However, when commercial beer samples are studied, there is usually no detailed information available on raw materials and brewing process.

Until now, antioxidant activity in brewing science has mainly been assessed by electron spin resonance (ESR) spectroscopy-spin trapping of radicals formed during a 60 °C forcing test.[3–6,10] High-throughput antioxidant activity assays are usually classified depending on the underlying reaction mechanisms. Radical or reactive oxygen species are reduced by the antioxidant either by the transfer of an electron or a hydrogen atom and are thus classified as electron transfer reactions and hydrogen atom transfer reactions, respectively.[46,47] Assays based on electron transfer mechanisms include the ferric reducing antioxidant power (FRAP)-assay, which is based on the reduction of a complex of iron (III) to iron (II).[48] As the pro-oxidant activity of reducing compounds is based on the reduction of transition metal ions, these assays might give an insight into the pro-oxidant potential of reducing compounds.[1] The oxygen radical absorbance capacity (ORAC)-assay is based on the scavenging of peroxy radicals by the transfer of hydrogen atoms.[49] Peroxy radicals are reactive intermediates in the oxidation of organic compounds by reactive oxygen species.[1] Thus the peroxy radical scavenging activity of antioxidants might be an interesting criterion for their role in beer flavor stability. We therefore chose the antioxidant capacity assays ORAC and FRAP, which are based on different reaction mechanisms. In this study we thus evaluated the impact of technological parameters on the phenolic composition and antioxidant activity of bright lager beer with the aim of improving flavor stability. In the pilot-scale brewing trials, the raw materials, barley malt, and hop products, as well as the hopping regime, were systematically varied. The influence of barley malt on antioxidant activity and storage stability of beer has not yet been thoroughly investigated. Barley malt samples differing in raw protein content and in the technological malting parameters (resulting in differences in malt modification) were therefore chosen to ensure a wide variation in malt-derived substances in the resultant beers.

MATERIAL AND METHODS

Chemicals and reagents

For chromatographic analyses and standard solutions, high-performance liquid chromatography (HPLC)-grade methanol and acetonitrile (both from VWR, Fontenay-sous-Bois, France), HPLC-grade water (for all HPLC-experiments), distilled water, 99.9% ethanol (Sigma-Aldrich, Steinheim, Germany), and 100% acetic acid (Merck, Darmstadt, Germany) were used. Phenolic standard substances included *p*-hydroxy benzoic acid (99%, pHB), 3,4-dihydroxy benzoic acid (97%, protocatechuic acid, prot), *p*-coumaric acid (98%, p-cou), *o*-coumaric acid (97%), catechin hydrate (98%, cat), vanillic acid (97%, van), ferulic acid (99%, fer), and sinapic acid (99%, sin), obtained from Sigma-Aldrich, and rutin (97+ %, rut), obtained from Acros Organics, Geel, Belgium. Cell-free amino acid mixture – 13 C, 15 N was purchased from Sigma-Aldrich. Buffer solutions were prepared using sodium phosphate dibasic dihydrate (99.5%, Sigma-Aldrich), potassium dihydrogen phosphate (99.5%, Merck), and sodium acetate (water-free, AppliChem GmbH, Darmstadt, Germany). For antioxidant activity analyses, iron (III)-chloride (97%), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ, 99%, Fluka), concentrated hydrochloric acid (37%), 2,2′-azobis (2-methyl-propionamidine) dihydrochloride (AAPH, 97%), and fluorescein sodium salt (98.5–100.5%, Fluka) from Sigma-Aldrich were used. Antioxidant activity analyses were calibrated with (+/−)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox, 97%, from Sigma-Aldrich).

Brewing trials

An overview of the systematic brewing trials and standard quality parameters of the sample beers is given in Table 1. Barley malt raw material (variety Cervinia, Saatzucht Breun, Herzogenaurach, Germany) with differences in raw protein content and degrees of modification was milled on a two-roller mill. The degree of protein modification was measured according to MEBAK (Mitteleuropäische Brautechnische Analysenkommission, collection of analytical methods for brewing science) [50] as the quotient of protein content solubilized by isothermal mashing (65 °C,

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Table 1. Overview of the systematic brewing trials with details on raw materials and hopping technology (hop products: CEX: CO_2 extract, Pe: type 90 pellets) and standard quality parameters

	Malt parameters				Hopping regime					
Sample #	Malt #	Malt raw protein content (%)	Amount of malt/brewing trial (kg)	Degree of protein modification (%)	Hop product and amount of α-acids added (beginning of wort boiling)	Whirlpool hopping	Alcohol (% Vol.)	Extract (g L^{-1})	Color (EBC)	pH
1	1	11.2	5.4	43.4	CEX (2.9 ± 0.04 g)	-	5.13	21.8	8.0	4.6
2			5.4		Pe (2.9 ± 0.006 g)	-	4.84	24.7	7.5	4.5
3			5.4		Pe (2.9 ± 0.006 g)	Pe, 2.5 g L^{-1}	5.23	23.0	8.5	4.7
4	2	11.0	5.4	42.6	CEX (2.9 ± 0.04 g)	-	4.81	25.9	5.5	4.7
5			5.4		Pe (2.9 ± 0.006 g)	-	4.36	25.1	5.5	4.5
6			5.4		Pe (2.9 ± 0.006 g)	Pe, 2.5 g L^{-1}	4.96	27.8	7.0	4.7
7	3	9.7	5.4	41.3	CEX (2.9 ± 0.04 g)	-	5.03	22.1	5.0	4.4
8			5.4		Pe (2.9 ± 0.006 g)	-	4.94	21.4	5.5	4.5
9			5.4		Pe (2.9 ± 0.006 g)	Pe, 2.5 g L^{-1}	5.05	22.3	6.0	4.6
10	4	10.1	5.4	42.5	CEX (2.9 ± 0.04 g)	-	5.04	20.0	6.0	4.4
11			5.4		Pe (2,9 ± 0.006 g)	-	4.95	19.9	6.0	4.7
12			5.4		Pe (2.9 ± 0.006 g)	Pe, 2.5 g L^{-1}	5.37	21.1	6.5	4.7

1 h) and malt raw protein content. All sample beers were brewed in duplicate at the pilot-scale (60 L) brewing plant at the Forschungsbrauerei Weihenstephan. The amount of milled malt was calculated to achieve an original gravity of 11.5%. Worts were prepared from 5.4 kg malt using an infusion mashing regime with the following steps: mashing-in temperature: 62 °C, 62 °C, rest for 30 min, heating-up to 72 °C at a heating rate of 2 °C/min, 72 °C, rest for 30 min, heating-up to 78 °C at a heating rate of 1.2 °C/min, enzyme deactivation rest at 78 °C for 10 min. Worts were lautered after a lauter rest of 20 min. Lautering was finished when a 38 L of kettle full wort was obtained. At the beginning of wort boiling, worts were hopped with either CO_2 extract (37.5% α-acids) or type 90 pellets (5.7% α-acids) of the Hallertauer tradition hop variety, Hopsteiner, Mainburg, Germany. Hop dosage at the beginning of wort boiling was calculated to achieve 25 bitter units. Worts were boiled for 60 min. For some brewing trials, hops were also added during whirlpool rest (2.5 g L^{-1} pellets type 90) without a reduction in the quantity of hops added at the beginning of wort boiling. After a whirlpool rest of 10 min, the wort was cooled and yeast was added (Saflager W-34/70 by Fermentis, rehydrated in first wort) to a viable cell concentration of $15*10^6$ cells mL^{-1}. Worts were fermented for 5 days at 12 °C until an extract concentration of 3.5% w/w was reached. Afterwards, the young beers were matured at 16 °C until a total diacetyl value of < 0.1 mg L^{-1} (MEBAK 2.21.5.1[51]) was reached. The beers were stored at 0 °C for 2 weeks and filtered (3 filter layers, K150, Pall Corporation). All beers were filled under CO_2 conditions in 0.33 L longneck bottles and stored at 4 °C. The analytical values of beer samples are given as the average of duplicate brewing trials (i.e. two technological replicates).

Standard analyses

All sample beers were analyzed for standard quality parameters according to MEBAK[51]: pH (2.13), extract and alcohol (2.9.6.3), free amino nitrogen (FAN) (2.6.4.1), color (2.12.2), total nitrogen (total N) (Kjeldahl, 2.6.1.1), total polyphenols (2.16.1), and total anthocyanogens (2.16.2).

Chromatographic analysis of beer constituents

Hop α- and iso-α-acids were analyzed by HPLC according to MEBAK 2.17.3[51] in duplicates. Nineteen amino acids were analyzed by HPLC-MS/MS as described by Sonntag *et al.* (2010),[52] in triplicates, after the dilution of beer samples with water (1/10, v/v). All diluted samples were stored at −18 °C until analysis. Before analysis, samples were diluted with acetonitrile (1/2, v/v), passed through a membrane filter (Macherey Nagel, Düren, Germany, 0.2 µm) and then spiked with internal standard solution (Cell Free Amino Acid Mixture – 13 C, 15 N, 20 mM, 1/1000, v/v). Amino acids were separated on a Xbridge amide column (3.5 µm, 2*150 mm, Waters, Eschborn, Germany) equipped with an amide security guard column (Waters) by gradient elution with (A) 5% (v/v) acetonitrile (7.5 mM ammonium acetate, pH 3) and (B) 95% (v/v) acetonitrile (7.5 mM ammonium acetate, pH 3) with the following gradient steps at 50 °C column temperature and a flow of 0.4 mL min^{-1}: 0–1 min: 95% A, 2–5 min: 90% A, 10 min: 70% A, 11–15 min: 60% A, 16–19 min: 95% A. The HPLC-system consisted of an Agilent 1200 series system (Agilent, Waldbronn, Germany) with a HiP-ALS SL autosampler, a 1200 series bin pump module, a 1200 series degasser, and a 1100 series colcom column oven and was coupled to Triple Quad 4500 MS (SCIEX, Darmstadt). Amino acids were analyzed in positive ion mode by electron spray ionization (ESI), with the following operation parameters: ion spray voltage: 5500 V, curtain gas: 35 psi, nebulizer gas: 55 psi, heater gas: 65 psi, medium CAD, turbogas temperature: 450 °C with MRM-scan mode. Amino acids were quantified using isotopically labeled 13 C, 15 N standards.

Phenolic compounds were analyzed by high-performance liquid chromatography with diode-array detection HPLC-DAD after a solid-phase extraction work-up procedure as described by Garcia *et al.* (2004)[53] and Dvorakova *et al.* (2007)[54] with modifications; 50 g of the beers were adjusted to pH = 2 and then sucked through SPE (solid phase extraction)-cartridges at approximately 1 drop s^{-1} (Chromabond EASY, 500 mg, 6 mL, Macherey Nagel, Düren, Germany, preconditioned with 5 mL Methanol and 10 mL acetic acid (1% aq, v/v)) using a Chromabond tubing adapter, Macherey Nagel, Düren, Germany. SPE cartridges were washed with 30 mL

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Table 2. Concentration of polyphenols, hop bitter acids, and antioxidant activity of the sample beers. Different letters indicate significant differences between sample beers grouped according to the hopping regime used (ANOVA, post hoc test: Student t-test, $P \leq 0.05$, n = 8)

Sample #/ group	Malt #	Total polyphenols (mg L^{-1})	Anthocyano-gens (mg L^{-1})	Total iso-α-acids (mg L^{-1})	Total α-acids (mg L^{-1})	FRAP (TE, µM)	ORAC (TE, µM)
CEX		**140.6 ± 17.65, b**	**38.4 ± 7.93, b**	**23.4 ± 3.3, a**	**1.23 ± 0.52, b**	**924 ± 81.9, b**	**7069 ± 1525.2, b**
1	1	128.5 ± 16.26	40.0 ± 9.9	21 ± 3,9	0.94 ± 0.08	947 ± 112.1	8216 ± 853.2
4	2	155.0 ± 7.07	43.0 ± 7.07	24 ± 0.0	1.72 ± 0.57	965 ± 55.2	8243 ± 1171.4
7	3	152.0 ± 22.63	41.5 ± 6.36	21 ± 1.1	0.73 ± 0.21	923 ± 76.9	6142 ± 890.3
10	4	127.0 ± 4.24	29.0 ± 2.83	27 ± 0.9	1.55 ± 0.40	862 ± 86.0	5674 ± 1288.4
Pe		**159.4 ± 14.33, b**	**46.0 ± 10.90, b**	**17.3 ± 1.7, b**	**0.79 ± 0.38, b**	**963 ± 87.5, b**	**7240 ± 1141.3, b**
2	1	153.0 ± 25.46	49.0 ± 12.73	16 ± 0.8	1.19 ± 0.50	1059 ± 65.2	8307 ± 1459.9
5	2	168.0 ± 5.66	53.0 ± 2.83	17 ± 3.3	0.98 ± 0.13	959 ± 33.3	7441 ± 607.7
8	3	166.0 ± 15.56	49.5 ± 12.02	17 ± 0.7	0.55 ± 0.20	945 ± 104.7	6623 ± 670.14
11	4	150.5 ± 6.36	32.5 ± 3.54	19 ± 0.9	0.46 ± 0.03	889 ± 67.4	5938 ± 958.2
Pe, WP		**220.6 ± 29.02, a**	**75.0 ± 10.35, a**	**19.8 ± 2.9, b**	**2.20 ± 0.85, a**	**1241 ± 61.0, a**	**9291 ± 1019.9, a**
3	1	193.0 ± 9.9	73.0 ± 15.56	15 ± 0.3	1.18 ± 0.01	1254 ± 91.4	10 060 ± 866.0
6	2	240.0 ± 26.87	84.5 ± 0.71	23 ± 1.4	2.88 ± 1.01	1271 ± 73.3	10 148 ± 1266.4
9	3	219.5 ± 50.2	79.0 ± 4.24	20 ± 0.2	2.09 ± 0.32	1241 ± 62.1	8911 ± 887.8
12	4	230.0 ± 9.9	63.5 ± 2.12	21 ± 1.0	2.66 ± 0.68	1200 ± 88.3	8044 ± 1172.9

acetic acid (1% aq, v/v) and sucked dry. Phenolic compounds were eluted with 8 mL methanol and made up to 10 mL. All the samples were worked up and analyzed in triplicate and passed through a membrane filter (Macherey Nagel, 0.45 µm) prior to measurement after storage at −18 °C. Samples were separated on a Luna C18-HPLC column (5 µm, 250*4.6 mm, phenomenex®, Aschaffenburg, Germany) equipped with a C18 security guard column (4*30 mm, phenomenex®) using a Waters e2695 system coupled with a Waters 2998 photodiode array detector and Empower® 3 Software (Waters, Eschborn, Germany). Separation was achieved by gradient elution with A (1% acetic acid aq, v/v) and B (1% acetic acid in methanol, v/v) using the following steps: 0 min: 98% A, 30 min: 85% A, 37 min: 90% A, 55 min: 70% A, 90 min: 30% A and a flow of 1 mL min^{-1}. Phenolic compounds were identified by their retention time and ultaviolet (UV) spectra in comparison with standard compounds. Individual substances were quantified by external standard calibration at four wavelengths: *p*-Hydroxybenzoic, protocatechuic, vanillic acid (265 nm), catechin (280 nm), ferulic, *p*-coumaric, sinapic acid (310 nm), and rutin (360 nm). External standard calibration was achieved by working up mixtures of standard compounds spiked to a phosphate buffer solution (pH = 2) by solid phase extraction as described above. Adjustments due to matrix effects were made according to recovery rates determined by standard addition to a commercial lager beer sample.

Antioxidant activity analyses

Antioxidant activity of sample beers was determined using 96-well plates and the Synergy™ H4 (Biotek, Bad Friedrichshall, Germany) micro-plate reader. Both the FRAP and ORAC assays used a Trolox stock solution of 4 mM in ethanol. The stock solution was diluted to the desired concentration with distilled water (FRAP) or phosphate buffer, 10 mM, pH = 7.4 (PB, ORAC). To determine the ferric reducing antioxidant power (FRAP) according to Jimenez-Alvarez *et al.* (2008),[55] degassed beers were diluted in distilled water. Antioxidant activity was measured in quadruples relative to a trolox standard calibration (100, 200, 300, 400, 500µM). A FRAP reagent was prepared with an acetate buffer solution (300 mM, pH = 3.6), TPTZ (10 mM, in 400 mM HCl), and 20 mM aqueous iron(III)-chloride solution (10/1/1, v/v/v); 25 µL diluted samples and standards were pipetted into 96-well plates and after the addition of 250 µL FRAP reagent, the mixture was agitated and then incubated for 8 min at 25 °C prior to measuring absorbance at 593 nm. Oxygen radical absorbance capacity (ORAC) was measured relative to a trolox standard calibration (12.5, 25, 50, 100, 200 µM in phosphate buffer, 10 mM, pH = 7.4 (PB)) as described by Spreng *et al.* (2018).[56] Degassed beer samples were diluted in a phosphate buffer solution; 25 µL diluted samples were pipetted into the 96-well plates and 150 µL of fluorescein were added. The plate was agitated and then incubated at 37 °C for 30 min. Fluorescence was measured in three cycles (f_0, every 90 s, excitation: 485 nm, emission: 520 nm) prior to the addition of 25 µL AAPH (240 mM, in PB). Fluorescence was measured in 57 cycles (f_i, every 90 s, excitation: 485 nm, emission: 520 nm). The area under the fluorescence decay curve (AUC) was calculated as described by Ou *et al.* (2001)[49] as: $AUC = 1 + f_1/f_0 + \ldots + f_{57}/f_0$. Net AUC was calculated as: $AUC_{net} = AUC_{Trolox/Sample} - AUC_{Blank}$. Trolox-equivalents (µmol trolox/L) were obtained from the Trolox standard calibration curve. The antioxidant activity of the two technological replicates was measured in quadruples. Antioxidant activity values as reported in Table 2 are the mean values of both technological replicates for each variation of raw materials and the hopping regime.

Forced and natural ageing experiments and sensory analysis

Samples were evaluated by 6–10 trained panelists in a fresh, force-aged, and naturally aged state. Beers were naturally aged for 5 months at 25 °C in the dark. Beers were force aged according to MEBAK Sensorik 4.5.2.8:[57] 1 day of agitation (100/min), 4 days storage at 40 °C in the dark. Overall beer quality was assessed with the DLG (Deutsche Landwirtschaftsgesellschaft)-score (MEBAK Sensorik 4.5.2.1[57]) whereas aging intensity was evaluated using the Eichhorn aging scale (MEBAK Sensorik 4.5.2.8[57] The DLG scale ranges from 1 (severe flavor defects) to 4 (still pure) and 5 (pure flavor). The aging scale rates the aging intensity from 1 (fresh) to 4 (strongly aged flavor). Profiles of hoppy aroma notes (fruity, flowery, citrusy, green / grassy, hoppy / spicy) were assessed on a five-point scale (0: not detected – 4: very strong) according to MEBAK Sensorik 3.2.2.[57]

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Table 3. The influence of the hopping regime on the content of phenolic substances in the sample beers. Values are reported for each beer ± standard deviation, n.d.: not detected. Different letters indicate significant differences between sample beers grouped according to the hopping regime used (ANOVA, post hoc test: Student t-test, $P \leq 0.05$, n = 8)

Sample/ group	pHB (mg L^{-1})	Prot (mg L^{-1})	Van (mg L^{-1})	p-Cou (mg L^{-1})	Fer (mg L^{-1})	Sin (mg L^{-1})	Cat (mg L^{-1})	Rut (mg L^{-1})	Sum (mg L^{-1})
CEX	**0.18 ± 0.03b**	**0.08 ± 0.01c**	**0.85 ± 0.11b**	**0.74 ± 0.14a, b**	**1.98 ± 0.60**†	**0.36 ± 0.04b**	**3.24 ± 0.64c**	**n. d.**	**7.44 ± 1.32c**
1	0.21 ± 0.03	0.07 ± 0.01	0.87 ± 0.09	0.89 ± 0.05	1.85 ± 0.16	0.36 ± 0.07	3.02 ± 0.40	n. d.	7.26 ± 0.76
4	0.17 ± 0.01	0.08 ± 0.01	0.87 ± 0.05	0.80 ± 0.04	2.91 ± 0.17	0.42 ± 0.02	4.09 ± 0.17	n. d.	9.34 ± 0.38
7	0.15 ± 0.01	0.11 ± 0.03	0.75 ± 0.02	0.55 ± 0.01	1.47 ± 0.1	0.33 ± 0.04	3.17 ± 0.68	n. d.	6.52 ± 0.83
10	0.20 ± 0.02	0.07 ± 0.01	0.94 ± 0.15	0.73 ± 0.06	1.74 ± 0.16	0.34 ± 0.04	2.74 ± 0.16	n. d.	6.77 ± 0.44
Pe	**0.21 ± 0.03b**	**0.13 ± 0.02b**	**0.88 ± 0.08b**	**0.62 ± 0.21b**	**1.78 ± 0.34**†	**0.40 ± 0.03a, b**	**4.13 ± 0.60b**	**1.96 ± 0.17b**	**10.3 ± 0.66b**
2	0.21 ± 0.03	0.10 ± 0.01	0.93 ± 0.08	1.03 ± 0.02	1.55 ± 0.31	0.42 ± 0.04	4.00 ± 0.71	1.98 ± 0.01	11.28 ± 0.17
5	0.18 ± 0.01	0.15 ± 0.03	0.83 ± 0.02	0.60 ± 0.12	2.21 ± 0.28	0.38 ± 0.01	4.25 ± 0.25	2.00 ± 0.25	10.61 ± 0.29
8	0.20 ± 0.01	0.14 ± 0.02	0.79 ± 0.04	0.59 ± 0.03	1.65 ± 0.11	0.42 ± 0.02	4.65 ± 0.31	1.83 ± 0.10	10.28 ± 0.50
11	0.24 ± 0.01	0.11 ± 0.01	0.95 ± 0.03	0.64 ± 0.07	1.71 ± 0.07	0.36 ± 0.06	3.63 ± 0.61	2.02 ± 0.17	9.66 ± 0.66
Pe, WP	**0.27 ± 0.04a**	**0.17 ± 0.04a**	**1.01 ± 0.09a**	**0.94 ± 0.16a**	**2.32 ± 0.63**†	**0.42 ± 0.04a**	**5.79 ± 0.76a**	**5.04 ± 0.31a**	**16.0 ± 1.47a**
3	0.25 ± 0.01	0.13 ± 0.01	0.91 ± 0.07	1.14 ± 0.08	2.17 ± 0.07	0.36 ± 0.11	4.78 ± 0.76	4.83 ± 0.23	14.63 ± 1.05
6	0.25 ± 0.01	0.17 ± 0.04	1.06 ± 0.05	1.02 ± 0.04	3.32 ± 0.13	0.43 ± 0.02	6.54 ± 0.60	5.18 ± 0.39	17.98 ± 1.17
9	0.25 ± 0.02	0.22 ± 0.01	0.95 ± 0.05	0.76 ± 0.08	1.87 ± 0.09	0.43 ± 0.02	6.02 ± 0.32	4.91 ± 0.50	15.41 ± 0.64
12	0.33 ± 0.02	0.16 ± 0.02	1.10 ± 0.04	0.86 ± 0.03	1.91 ± 0.05	0.46 ± 0.04	5.83 ± 0.42	5.23 ± 0.50	15.89 ± 1.04

†values were not normally distributed.

Statistical evaluation

All statistical evaluations were performed using SAS JMP® 12.2.0 (64 bit) software. The analytical values of the sample beers are presented as mean ± standard deviation of both brewing trial duplicates and multiple analytical determinations. Data were standardized to z-scores by autoscaling before multivariate correlations. Pearson r correlation coefficients were calculated between beer composition data and antioxidant activity values. Only correlation coefficients corresponding to *P* values below 0.01 were reported in the text. For the comparison of groups, all technological replicates (n = 2 for each combination of barley malt and hopping regime) were taken into account. Analytical data were checked for normal distribution using the Shapiro–Wilk test. Analysis of variance (ANOVA) was used to register significant differences between grouped samples. If the null hypothesis of ANOVA was rejected, i.e. group means were different, each pair of experimental groups was tested for significant differences using the Student t-test. A *P* value below 0.05 was considered significant.

As sensory data with a fixed scale were not normally distributed, differences between groups of beers were analyzed by the non-parametric Kruskal–Wallis rank sum test. Groups were compared using the Steel–Dwass test as a method for post-hoc analysis for non-parametric multiple comparison. A *P* value below 0.05 was considered significant.

RESULTS AND DISCUSSION

Chemical composition and antioxidant activity

As expected, the use of different hopping regimes resulted in variations in chemical composition and antioxidant activity of the beers (Table 2). Due to the high load of hops added at the beginning of whirlpool-rest (2.5 g L^{-1}), higher values of total polyphenols, anthocyanogens, unisomerized α-acids, total N and FAN were observed. Higher total N and FAN levels were observed for beers brewed from malt 1 and 2 (Table 4). Accordingly, higher EBC color values (5.5–8.5 as compared to 5.0–6.5) were observed for beers brewed from malt 1 and 2 (Table 1). Hop addition in the whirlpool also led to a higher content of unisomerized α-acids (1.18–2.88 mg L^{-1}) compared to beers hopped at the beginning of wort boiling (0.55–1.72 mg L^{-1}). Total polyphenol and total anthocyanogen content of the beers ranged from 127 to 240 mg L^{-1} and 29 to 84.5 mg L^{-1}, respectively. Phenolic compounds are known as especially important antioxidants,[14–16,58] so individual free phenolic compounds were determined by HPLC (Table 3). Ferulic acid was found to be the most abundant phenolic acid in the sample beers; its concentration ranged from 1.47–3.32 mg L^{-1}. Apart from ferulic acid, vanillic and *p*-coumaric acids were present in high concentrations of 0.75–1.10 mg L^{-1} and 0.55–1.14 mg L^{-1}. This is in accordance with findings of other authors.[16,54,59,60] Other determined phenolic acids included the benzoic acid derivatives *p*-hydroxybenzoic (0.15–0.33 mg L^{-1}) and protocatechuic acid (0.07–0.22 mg L^{-1}) as well as the cinnamic acid derivative sinapic acid (0.33–0.46 mg L^{-1}). Apart from phenolic acids, the flavanol (+)-catechin (2.74–6.54 mg L^{-1}) and the flavonol glycoside rutin (< LOD-5.23 mg L^{-1}) were determined. Beers hopped with polyphenol-free CO_2 extract did not contain rutin, marking this flavonol-glycoside as a hop-derived compound.[29,34,61] The (+)-catechin and rutin content was in accordance with literature values;[54,62] however, higher levels were detected in whirlpool-hopped beers. Besides resinous hop compounds and phenolics, sulfite has been known to be an effective antioxidant in beer.[6] Sulfur dioxide delays the onset of radical generation measured by ESR and thus induces a lag phase in forced aging experiments.[6] A correlation between beer flavor stability and the length of the lag phase was described.[3] Sulfur dioxide is a reducing compound formed by the yeast during fermentation. However, variation of the SO_2 content was not the aim of this study and fermentation conditions were kept constant for all sample beers.

Relationships between antioxidant activity values and beer constituents were evaluated by multivariate correlations. The ORAC value correlated positively ($P \leq 0.01$) with FAN, total N, and total anthocyanogens, and with the *p*-coumaric acid content. The FRAP value of the beers correlated positively ($P \leq 0.01$) with total

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Table 4. The content of total nitrogen, FAN and aromatic amino acids in the sample beers. Values are reported for each beer ± standard deviation (n = 6), n.d.: not detected. Different letters indicate significant differences between sample beers grouped according to the hopping regime used (ANOVA, post hoc test: Student t-test, $P \leq 0.05$, n = 8, nsd: not significantly different)

Sample #/ group	Total N (mg L^{-1})	FAN (mg L^{-1})	Trp (mg L^{-1})	Tyr (mg L^{-1})	Phe (mg L^{-1})
CEX	**834 ± 146.3 nsd.**	**97 ± 37.4 a, b**	**26.7 ± 8.78 b**	**49.3 ± 20.89b**	**49.2 ± 29.3nsd.**
1	1003 ± 42.4	124 ± 5.0	32.6 ± 1.12	64.9 ± 2.16	71.0 ± 1.50
4	916 ± 46.7	144 ± 7.8	35.4 ± 2.07	70.3 ± 2.98	80.0 ± 3.20
7	687 ± 21.9	64 ± 0.7	15.4 ± 4.52	23.3 ± 6.32	14.8 ± 3.99
10	732 ± 101.1	80 ± 21.2	23.2 ± 0.48	38.0 ± 4.67	29.77 ± 6.84
Pe	**740 ± 97.0 nsd.**	**80 ± 22.3 b**	**22.8 ± 9.77 b**	**40.5 ± 20.47 b**	**38.8 ± 24.65 nsd.**
2	810 ± 195.9	75 ± 42.4	30.7 ± 0.42	57.0 ± 0.43	55.8 ± 0.62
5	751 ± 37.5	104 ± 10.6	29.0 ± 2.14	54.7 ± 4.79	59.9 ± 4.19
8	654 ± 1.4	69 ± 12.7	13.4 ± 8.24	17.9 ± 10.9	10.3 ± 7.15
11	747 ± 36.8	96 ± 21.9	22.1 ± 9.04	40.6 ± 18.8	37.7 ± 20.98
Pe, WP	**864 ± 118.7 nsd.**	**124 ± 26.5 a**	**35.5 ± 5.40 a**	**80.7 ± 15.25 a**	**68.2 ± 19.23 nsd.**
3	1004 ± 19.1	137 ± 22.6	32.6 ± 3.51	67.5 ± 9.87	70.1 ± 6.75
6	924 ± 29.7	149 ± 0.7	39.4 ± 2.67	80.6 ± 4.23	90.0 ± 3.27
9	729 ± 14.1	103 ± 17.7	30.6 ± 5.09	74.6 ± 12.85	44.4 ± 6.60
12	800 ± 80.6	106 ± 21.2	38.6 ± 4.12	98.3 ± 11.63	66.99 ± 14.10

polyphenols, total anthocyanogens and catechin content. The FRAP values correlated significantly ($P \leq 0.01$) with ORAC values. Antioxidant activity in both assays correlated significantly ($P \leq 0.01$) with the sum of phenolic compounds determined by HPLC. Phenolic compounds have widely been described as potent antioxidants;[14–16,58] correlations with antioxidant activity values were therefore expected. A recent study by Spreng and Hofmann (2018)[56] investigated antioxidants in pilsner-type beer by activity-guided fractionation. Apart from numerous phenolic compounds, aromatic amino acids (tryptophane and phenylalanine) were described as antioxidants.[56] This might explain the correlation between free amino nitrogen content and antioxidant activity. All beers were analyzed for their aromatic amino acid content by high-performance liquid chromatography–mass spectrometry (HPLC-MS/MS) (Table 4). We found significant correlations ($P < 0.01$) between the sum of amino acids and the ORAC values of the sample beers. Aromatic amino acid (phenylalanine, tyrosine, tryptophane) content correlated significantly ($P < 0.01$) with the ORAC values; tyrosine content correlated significantly ($P < 0.01$) with FRAP values.

Influence of hop products and hopping regime on phenolic content and antioxidant potential

The influence of the hopping regime on antioxidant activity was investigated first (Table 2). No significant differences were observed between the beers hopped at the beginning of wort boiling with CO_2 extract or type 90 pellets, even though hop phenolic ingress is higher for pellet dosage.[63] However, with an early hop dosage and therefore longer hop boiling times there is also a greater depletion of hop phenolic compounds.[13,64] The whirlpool-hopped beers had a significantly higher antioxidant potential (Table 2). This could be explained by the higher ingress of hop phenolic compounds due to the large amount of hops added (2.5 g L^{-1}). A beneficial effect of a higher hop addition on beer antioxidant activity has been described by other authors.[10,34,63] Late hop addition resulted in significantly higher levels of total polyphenols, anthocyanogens, and unisomerized α-acids (Table 2), which are potent antioxidants.[4,10,14–16] Apart from higher total polyphenol values, the concentration of individual phenolic compounds in the beers with a second hop addition in the whirlpool was significantly ($P = 0.05$) higher than in beers without a second hop addition for all substances measured except for ferulic acid (Table 3). The sum of individual phenolic compounds of whirlpool-hopped beers was 15.98 ± 1.47 mg L^{-1} compared to 7.44 ± 1.32 mg L^{-1} and 10.34 ± 0.66 mg L^{-1} for the beers hopped at beginning of boiling with CO_2 extract and type 90 pellets, respectively. The rutin, catechin, and protocatechuic acid concentrations were significantly different between all three hopping regimes. The aromatic amino acids tryptophane and tyrosine were present in significantly higher concentrations in the whirlpool-hopped sample beers (Table 4) and could thus contribute to the higher antioxidant potential.[56]

Influence of barley malt on phenolic content and antioxidant potential

Since the whirlpool-hopped beers had a significantly higher antioxidant potential in both assays, the influence of barley malt was investigated using only the beers hopped at the beginning of wort boiling with either CEX (hop CO_2-extract) or Pe (hop pellets type 90), without the further addition of hops in the whirlpool (Fig. 1a). Beers brewed from malts 1 and 2 (high raw protein content) had significantly higher antioxidant potential in the ORAC assay than beers brewed from malts 3 and 4 (low raw protein content). However, there were no significant differences between FRAP values. This result indicates the major influence of barley malt on the antioxidant activity in the ORAC assay of beers hopped at the beginning of wort boiling. The ORAC assay measures the antioxidant activity of a hydrophilic sample against the peroxyl radical in a hydrogen atom transfer reaction.[49] The peroxyl radical is an intermediate in the oxidative degradation of organic compounds and the formation of stale flavor compounds.[1] Beers with a high potential to scavenge the peroxy radical might therefore have a higher stability. The FRAP assay, on the other hand, measures the reducing activity against iron (III) in a single electron transfer reaction.[46] The potential to reduce transition metal ions is essential for a pro-oxidative reaction in the reductive oxygen activation.[1] The pro-oxidant potential of reducing Maillard reaction compounds in beer aging has been described by other

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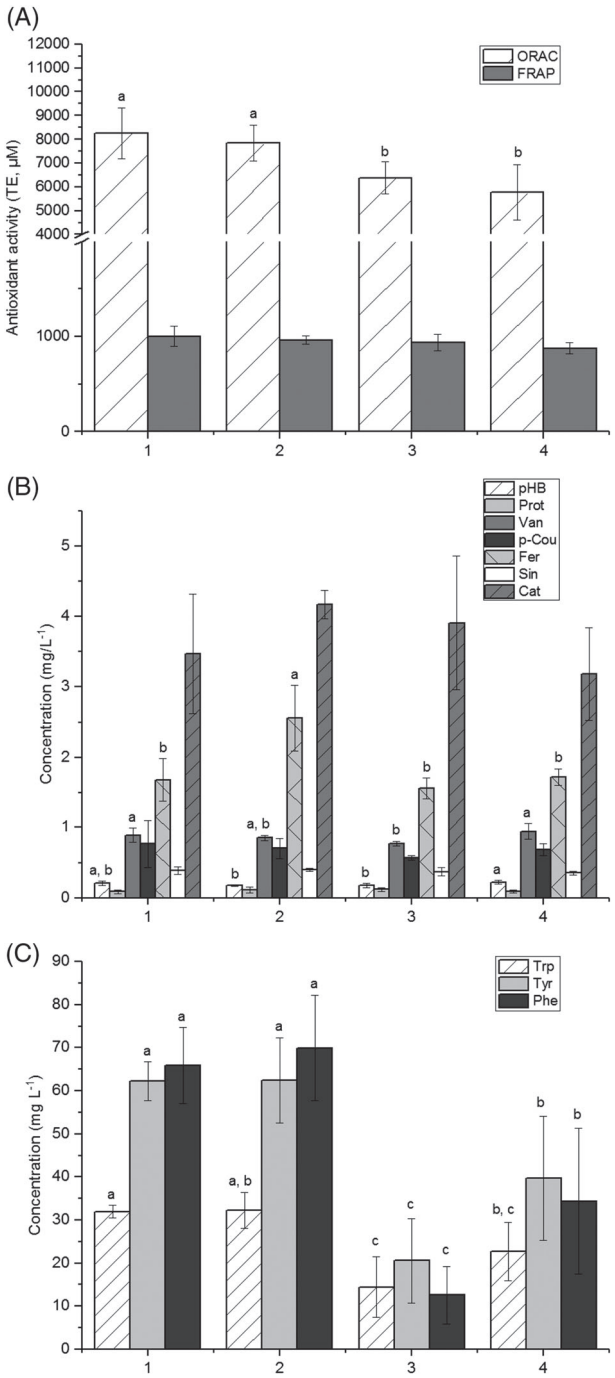

Figure 1. Influence of the four different malts on the antioxidant capacity (a), the concentration of individual phenolic substances (b), and aromatic amino acids (c). Only sample beers hopped at the beginning of wort boiling are included. Significant differences are indicated by different letters (ANOVA, post hoc test: Student t-test, $P < 0.05$, n = 4).

authors.[65,66] Thus the FRAP assay could give insight into the potential pro-oxidant reaction of reducing compounds. The results in this study showed that, even though the FRAP value correlated with the ORAC value, differences were observed for malt-derived antioxidants. Beers brewed from malts 1 and 2 had significantly higher ORAC values than beers brewed from malts 3 and 4. However, FRAP values were not significantly different. Due to the interesting antioxidant properties of phenolic compounds,[14–16,58] the influence of the different barley malts on individual phenolic content in beer was investigated (Fig. 1b). There were no significant differences in total polyphenols determined by the EBC method between the malt samples. However, *p*-hydroxybenzoic and vanillic acid content were significantly different between the samples. Malts 1 and 4 (higher degree of protein modification than malts 2 and 3) resulted in higher contents of *p*-hydroxybenzoic and vanillic acid. The cinnamic acid derivatives *p*-coumaric and ferulic acid, which are known as powerful antioxidants,[16] were present in higher concentrations in beers brewed from malt samples 1 and 2 (high raw protein content). Ferulic acid was present in significantly higher concentrations in beers brewed from malt sample 2. Apart from the original barley sample, the phenolic content of the individual sample beers was also influenced by the degree of malt modification. Beers brewed from malts 1 and 2 also had higher total nitrogen content (751–1004 mg L^{-1} compared to 654–800 mg L^{-1}). Cai *et al.* (2015) described a correlation between *p*-coumaric and ferulic acid content and soluble nitrogen in their study of 68 barley genotypes.[67] Apart from phenolic compounds, beers brewed from malts 1 and 2 had higher total N and FAN content (Table 4). As described above, the ORAC values of the beers correlated significantly with both FAN and total N content ($P < 0.01$). Aromatic amino acids, in particular, might exert antioxidant activity in beers.[56] The aromatic amino acid content in the sample beers was relatively high: 15–90 mg L^{-1} phenyl alanine, 18–98 mg L^{-1} tyrosine and 13–39 mg L^{-1} tryptophane compared to the sum of phenolic compounds (Table 4). These compounds might therefore contribute significantly to the beer's antioxidant activity, even if their individual activity values are lower than those of phenolic compounds.[56] The aromatic amino acid content was significantly higher in beers brewed from malt samples with high raw protein content (Fig. 1c). Apart from that, higher content of reducing Maillard reaction compounds might explain the higher ORAC values of beers brewed from malts with higher raw protein content. Maillard reaction compounds formed from amino acids and reducing sugars are also responsible for beer color. Higher color values were observed for beers brewed from malts 1 and 2 (Table 1). However, the effect of higher ORAC values on beer flavor stability needs to be further investigated, in particular because higher concentrations in amino acids might lead to higher concentrations of the respective Strecker aldehydes in aged beers.[36] An appropriate choice of barley raw material and the malting regime might be a promising tool to enhance oxidative beer stability. The antioxidant compounds derived from barley malt should therefore be further researched.

Raw material influence on sensory properties of fresh and aged beer

The sensory quality of fresh beers was rated as high, with higher ratings given to whirlpool-hopped beers. The Kruskal–Wallis rank sum test was used to rank groups of samples according to their sensory attributes. Higher score mean values indicated higher ratings of the respective sensory attribute by the panelists (Table 5). Groups were then compared by the Steel–Dwass test to detect significant differences. The influence of the three different hopping regimes was first investigated. The force-aged whirlpool-hopped beers were rated significantly higher in the DLG scores than the beers hopped at the beginning of wort boiling, and their aging scores were rated significantly lower ($P \leq 0.05$, Table 5). The same was observed for the samples aged naturally for 5 months at 25 °C. But the naturally aged beers hopped at the beginning of wort boiling with pellets type 90 were rated with significantly lower

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Table 5. Statistical evaluation of the total DLG scores and ageing scores of sample beers grouped by hopping regime: frequencies and score means by Wilcoxon/Kruskal–Wallis test and *P* values for non-parametric pairwise comparison by Steel–Dwass ($P < 0.05$ was considered significantly different, n = 8 technological replicates)

	DLG fresh	DLG force aged	DLG nat. aged	Aging score fresh	Aging score force aged	Aging score nat. aged
Hopping regime	**score mean**	**score mean**	**score mean**	**score mean**	**score mean**	**score mean**
CEX	9.1	6.4	6.9	13.3	17.1	19.0
Pe	8.3	10.4	10.3	15.6	14.0	13.0
Pe, WP	18.2	18.5	18.3	7.3	5.6	4.9
Category - category	***P* value**	***P* value**	***P* value**	***P* value**	***P* value**	***P* value**
Pe – CEX	0.9708	0.4435	0.4442	0.7306	0.6601	0.0462*
CEX – Pe, WP	0.0292*	0.0041*	0.0127*	0.1603	0.0050*	0.0077*
Pe – Pe, WP	0.0149*	0.0362*	0.0272*	0.0643	0.0360*	0.0041*

aging scores than the beers hopped with CO_2 extract. The beneficial effects of higher hop dosages on beer flavor stability and carbonyl content have been researched by other authors;[10,34,63] however, the masking effects of hop aroma compounds were not taken into account. In this study, the hop aroma attributes fruity, flowery, citrusy, green / grassy, and hoppy / spicy were evaluated by the tasters. All hop aroma attributes were rated significantly higher for fresh and naturally aged whirlpool-hopped beers (Table S1). In force-aged whirlpool-hopped beers, all hoppy aroma attributes except 'fruity' were rated significantly higher. For the attribute 'citrusy', significant differences were only found between the force-aged samples hopped at the beginning of wort boiling with CO_2 extract and the whirlpool-hopped samples. Potential masking effects of hop aroma compounds might thus play a role in the higher ratings given to aged whirlpool-hopped sample beers by the panelists. Saison *et al.* investigated the contribution of staling aldehydes to the aged flavor of beer.[37] These authors described a significant masking effect of fresh flavor notes on threshold values of various staling aldehydes.[37] The masking effects of hop aroma compounds on the threshold values of staling aldehydes should be further researched to gain a better insight into the aging behavior of strongly hopped beers. The influence of the malt samples on beer flavor stability was assessed for the beers hopped at the beginning of wort boiling. There were no significant differences between the four malt samples in the DLG or the aging-scores for fresh, force-aged and naturally aged beers (Table S2). As decline in bitter iso-α-acids is a useful beer aging indicator,[38–41] the description of the bitter impression of the aged samples was investigated separately. No significant differences between the hopping regimes were observed in the bitter aging scores for the fresh and force-aged beers. The DLG bitter score rates not only the intensity of aging of the bitterness but also the overall quality of the perceived bitterness. The DLG bitter scores of the fresh, force-aged, and naturally aged beers with a second hop addition in the whirlpool were rated higher than beers hopped only at the beginning of wort boiling (Kruskal–Wallis rank sum test). However, a pairwise comparison of the groups with the Steel–Dwass test did not register significant differences. We found no significant differences in overall bitterness quality (DLG score) or bitter aging score for force-aged and naturally aged samples brewed from different malts. Apart from the sensory description of bitterness, we measured the decline in bitter iso-alpha-acids in the naturally aged beers (Table 6) as an analytical marker for oxidative beer aging.[38,39] No significant differences between the hopping regimes were found for the absolute decline in iso-α-acids.

Table 6. Absolute and relative decline of iso-α-acids during natural ageing of sample beers and influence of the different malts on bitter acid degradation. Different letters indicate significant differences between groups of sample beers (ANOVA, post hoc test: Student t-test, $P = 0.05$, n = 6)

Group/sample #	Decline in iso-α-acids (mg L^{-1})	Relative decline in iso-α-acids (%)
Malt 1	3.73 ± 1.22, c	20.6
1	3.53 ± 1.11	16.8
2	4.71 ± 0.98	29.6
3	2.41 ± 0.08	15.6
Malt 2	4.78 ± 0.49, b	23.0
4	4.56 ± 0.37	18.8
5	4.53 ± 0.62	26.8
6	5.26 ± 0.06	23.3
Malt 3	5.21 ± 0.46, b	26.9
7	4.89 ± 0.58	23.3
8	5.08 ± 0.12	29.1
9	5.65 ± 0.32	28.4
Malt 4	7.65 ± 1.1, a	34.4
10	8.82 ± 0.58	32.2
11	7.27 ± 0.11	38.8
12	6.85 ± 1.17	32.4

This confirmed results by Mikyška *et al.* (2011), who found no significant influence of hopping regimes on beer bitterness depletion in a 6 months' storage experiment.[63] However, other authors described the stability of iso-α-acids in a forced aging experiment to depend on hop polyphenol dosage.[34] In our experiments, significant differences ($P = 0.05$) were found between the beers brewed from the four different malts (Table 6). For malt 1 the decline in iso-α-acids was significantly lower than for malt samples 2–4; the decline of iso-α-acids in samples brewed from malt sample 4 was significantly higher than for malt samples 1–3. This could be explained by a protecting effect of malt-derived antioxidants. Beers brewed from malts 1 and 2 had significantly higher ORAC values than those brewed from malts 3 and 4 (Fig. 1a). However, no significant differences in the sensory rating (DLG-scores, aging scores) of fresh, force-aged and naturally aged sample beers brewed from the different malts were observed.

In this study, a possible relationship with antioxidant nitrogen compounds (especially aromatic amino acids[56]) was found. However, the potential of amino acids as precursors for aged flavor compounds has to be taken into account when evaluating their

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potential as antioxidants.[1,35] The oxidative degradation of amino acids to the respective Strecker aldehydes was described in a recent study.[36] The Strecker degradation product benzaldehyde is formed from phenyl alanine and plays a role in the stale flavor of beer.[35,36] Increasing the content of aromatic amino acids as antioxidants in beer might therefore also lead to increased staling aldehyde content.

CONCLUSIONS

In this study we systematically varied the chemical composition of sample beers by varying the choice of raw materials and brewing technology with a view to promoting higher antioxidant activity. We used high-throughput antioxidant capacity assays based on different reaction principles: ORAC and FRAP. Late hop addition resulted in a high ingress of total polyphenols and individual phenolic compounds. This explained the significantly higher antioxidant activity of these beers in both the FRAP and ORAC assays. A high dosage of hops late in the hot process area of the brewhouse was beneficial for sensory stability. However, the aroma profile of the beer was altered due to the higher hop aroma ingress, which is not always desirable for pale lager beers. The antioxidant activity of beers hopped traditionally at the beginning of wort boiling was influenced to a large extent by the choice of barley as a raw material. Beers brewed from malt samples with high raw protein content had significantly higher antioxidant activity in the ORAC assay. In this study, the decline in bitter acids was analyzed as a marker for oxidative degradation of beer during storage. Decline in iso-α-acids was lower in beers brewed from barley malts with high raw protein content; these beers also had significantly higher antioxidant activity in the ORAC assay. However, beside their potential as antioxidants, aromatic amino acids act as precursors to staling aldehydes. The significance of the ORAC assay for beer flavor stability therefore needs to be further validated, taking into account the concentration of staling aldehydes in aged beers.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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