

T. Kunz, N. O. Brandt, T. Seewald and F.-J. Methner

# Carbohydrates Addition during Brewing – Effects on Oxidative Processes and Formation of Specific Ageing Compounds

Carbohydrates are very important in human nutrition and play an important role in the food / beverage industry. Various breweries use fermentable and non-fermentable carbohydrates for the creation of beer-specialties to increase e.g. the beers palate fullness. It is well known that carbohydrates are involved in several processes and mechanisms like Strecker degradation, Maillard reaction, oxidative processes and fermentation performance etc. during brewing which are mainly responsible for the final beer taste and the overall beer instability.

The aim of the study was to investigate the influences of fermentable (glucose, fructose, maltose, sucrose) and non-fermentable (isomaltulose) carbohydrate additions during wort boiling on the oxidative wort / beer stability and the formation of specific ageing components during storage. Especially the formation of ageing compounds like 2-/3-methylbutanal (MB) as additional indicators of oxidative processes [5, 36, 53, 57] were evaluated in wort and beer using GC-MS. These data were compared with the EAP- and Tmax-values measured by EPR-spectroscopy, the SO<sub>2</sub>-consumption rate as well as the reduction potentials of the used carbohydrates against Fe<sup>3+</sup> (optimized Chapon method [49]) to get a deeper insight in the carbohydrate influences on oxidative processes in wort and beer.

Descriptors: carbohydrates, reduction potential, oxidative stability, staling aldehydes, sulphur dioxide

In correlation to an increased reduction potential, the carbohydrate additions of fructose and isomaltulose have caused a significant acceleration of oxidative processes during wort boiling as indicated by an acceleration of the prooxidative acting radical generation via the Fenton-/Haber-Weiss reaction system, a higher increase in colour and a stronger formation of specific ageing components. On the other hand, glucose and sucrose showed a negligible effect in comparison to the control brew without carbohydrate addition. Altogether the results have shown that the carbohydrates (especially fructose and isomaltulose) which lead to a high reduction potential against Fe<sup>3+</sup> in the wort and beer matrix are responsible for a strong consumption rate of SO<sub>2</sub> in the final beer and cause the fasted release of specific ageing components. In this context our previous investigations have to be taken in consideration [35, 39, 41] which proposed that specific formed reductive acting structures like reductones/enediols are able to reduce metal ions like Fe<sup>3+</sup> very fast. On this way the carbohydrates can accelerate the oxygen activation per electron transfer and prooxidative acting radical generation by the Fenton/Haber-Weiss reaction system resulting in a lower oxidative wort / beer stability. On the other hand, carbohydrates, especially low molecular weight sugars, raise the osmotic pressure on yeast during fermentation leading to higher SO<sub>2</sub>-production which acts as antioxidant by scavenging ROS and could bind aldehydes in reversible carbonyl complexes

[4, 37, 47, 67]. In summary, the results give a clear advice that the carbohydrates like fructose and isomaltulose with a high reducing potential in wort matrix are responsible for an acceleration of oxidative processes, should be added direct before fermentation. This application avoids the negative effect on oxidative processes during wort boiling and the positive influence on SO<sub>2</sub>-formation during fermentation is utilized for a partial compensation of the faster SO<sub>2</sub>-consumption rate during storage.

## 1 Introduction

For the creation of beer-specialties and the improvement of beers palate fullness fermentable and non-fermentable carbohydrates can be added during or at the end of wort boiling, prior fermentation or to the final beer. Non-fermentable low molecular weight carbohydrates like the disaccharide isomaltulose as well as oligosaccharides have an influence on the palate fullness [47]. Nowadays there are also many beer-mixed beverages containing a large amount of carbohydrates.

Beside the direct influences on extract, viscosity, palate fullness, sweetness, etc it is generally known that carbohydrates are involved in many reactions during the brewing process, such as formation of Strecker degradation products, Maillard reaction, oxidative processes, fermentation, etc. which all have a huge impact on the beer flavour and flavour instability.

Established literature [7, 8, 18, 36, 37, 54, 57] indicates that the beer stale-flavours result from the formation of unsaturated, volatile carbonyl compounds, e.g. 2-methylbutanal (MB), 3-MB, phenylacetaldehyde, benzaldehyde, 2-furfural (2-F), hydroxymethylfurfural,

## Authors

Dipl.-Ing. Thomas Kunz\*, MSC Niklas Ole Brandt, Dipl.-Ing. Torsten Seewald, Prof. Frank-Jürgen Methner, Technische Universität Berlin, Institut für Lebensmitteltechnologie und Lebensmittelchemie, Fachgebiet Brauwesen, Berlin, Germany; \*corresponding author: thomas.kunz@tu-berlin.de

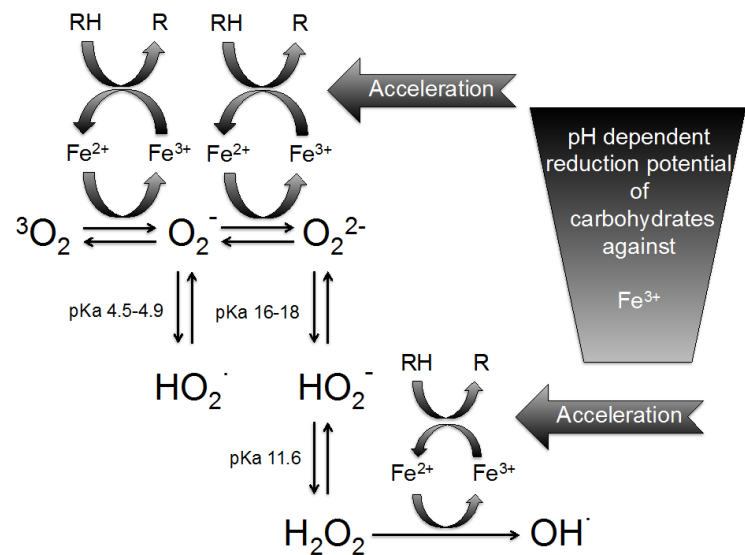
and trans-2-nonenal. Among the pathways, known to be involved in the formation of these carbonyls, are the Strecker degradation of amino-acids, the melanoidin-mediated oxidation of higher alcohols, the autoxidation of unsaturated fatty acids, and the aldol condensation of short-chain aldehydes.

Based on findings from different research studies using Electron Paramagnetic Resonance (EPR) spectroscopy [2, 3, 20–24, 29, 30, 37, 40, 42, 45, 62, 64, 66], another possible pathway for beer deterioration was proposed in recent studies [67]. For this kind of reaction mechanisms free radicals are formed by oxygen activation and Fenton type reactions during beer ageing which, in turn, can initiate a series of radical reactions that are responsible for the generation of ageing components by direct oxidative degradation of amino acids via hydroxyl and ethoxy radical attack.

The general prooxidative acting generation of reactive oxygen species (ROS, e.g.  $\cdot\text{OH}$  in wort and beer matrices via electron transfer to atmospheric oxygen or the Fenton-/Haber-Weiss reaction system primarily dependent on the availability of the reactants, catalysts, pH and temperature [6, 22, 23, 30, 37, 62]. The resulting oxidative reactions can be suppressed by antioxidants which are present in the raw materials added artificially or generated during fermentation such as sulphur dioxide ( $\text{SO}_2$ ).

In general, all raw materials which are used for brewing (malt, hops, water, yeast, adjuncts) influence the flavour stability and the staling potential of a resultant beer. Barley includes many precursors, enzymes, antioxidative and prooxidative substances that are involved in a multifaceted interplay during the production of malt and beer. The antioxidative activity of malt can result from polyphenols and melanoidins. However, there is a debate about whether intermediates of the Maillard reaction and Maillard reaction products such as melanoidins have pro- or antioxidative character [1, 10, 14, 18, 25, 41, 52, 58, 60]. Several authors [10, 25, 60] claim that reductones and melanoidins have high antioxidative capacities. On the other hand, according to *Bravo et al.* [7, 8],  $\alpha$ -dicarbonyls, intermediates in the Maillard reaction, can markedly enhance the formation of specific aldehydes during beer storage, and blockage of  $\alpha$ -dicarbonyls yielded a significantly lower accumulation of these aldehydes. Other authors [14, 25, 27, 41, 52, 58] discovered a correlation between the content of Maillard reaction products in special malt, caramel beers, stout and an increase of radicals in the Fenton reaction assay resulting in an acceleration of prooxidative action. In this context *Kunz et al.* [41, 52] described a reaction mechanism of specific intermediate Maillard reaction products with reductone/enediol structure on prooxidative processes caused by their reduction potential against oxidised metal ions, especially  $\text{Fe}^{3+}$ , resulting in an acceleration of oxygen activation and the generation of highly reactive oxygen species (ROS) such as  $\cdot\text{OH}$  radicals.

Analogous to specific Maillard reaction products, typical reductone/enediol corresponding structures are generated by the pH dependent open chain structures of carbohydrates during wort boiling. In this context previous studies [35, 39, 43, 44] with carbohydrates (glucose, maltose, sucrose, fructose, isomaltulose) addition prior wort boiling, have shown, that in correlation to the detected pH depending reduction properties of carbohydrates against the metal ion  $\text{Fe}^{3+}$  an acceleration of oxidative processes during wort boil-



**Fig. 1** Proposed mechanism for the acceleration in oxygen activation and radical generation (Fenton reaction system) caused by the reduction potential of carbohydrates against oxidized metal ions, especially  $\text{Fe}^{3+}$  ([39]/considering [2, 11, 15, 21, 23, 24])

ing is observable. According to *Kunz et al.* [39, 43, 44] is the pH dependent reduction potential of carbohydrates against oxidized catalytically acting metal ions like  $\text{Fe}^{3+}$  responsible for accelerated oxygen activation per electron transfer in presence of oxygen and the accelerated radical generation over the Fenton reaction system as shown in figure 1.

In this connection the carbohydrates isomaltulose and fructose could be characterized by the highest reduction potential at pH range of wort and the most negative influences on oxidative wort stability. In direct comparison, the carbohydrates maltose > glucose > sucrose showed significantly lower influences on oxidative processes during wort boiling.

On the other hand earlier studies [47, 54] have also shown that the carbohydrate addition prior fermentation leads to an increase of the osmotic pressure resulting in a stronger  $\text{SO}_2$ -formation during fermentation. This fact should have a positive effect on the oxidative beer stability because sulphur dioxide can act as an significant antioxidant in the beer matrices by scavenging ROS and masking stale flavour by binding staling aldehydes as sulphite carbonyl complexes [4, 9, 15, 28, 45–48, 50, 55, 61–63].

In consideration of the opposing effects on oxidative processes it was of special interest to investigate the influences of different fermentable and commonly used non-fermentable carbohydrates added before wort boiling on the final oxidative beer stability and the formation of specific ageing components during storage. The aim of the study was to investigate the influences of fermentable (glucose, fructose, maltose, sucrose) and non-fermentable (isomaltulose) carbohydrate additions during wort boiling on the oxidative wort / beer stability and the formation of specific ageing components during storage. The focus was to elucidate the influences of carbohydrate addition on generation of reactive oxygen species (ROS) via EPR-spectroscopy and formation of specific ageing compounds (2-/3-MB/2-F) during beer production and storage using Gas Chromatography with Mass Spectrometry (GC-MS). Furthermore standard wort and beer analyses according to

MEBAK [56] were carried out to get additional information about the carbohydrate influences on extract, alcohol, colour, SO<sub>2</sub>-content and SO<sub>2</sub>-consumption during storage.

## 2 Materials and methods

### 2.1 Model trials

To get a first insight in the pro- and antioxidative properties of the used carbohydrates in the brewing trials a pre-trial with a model solution was carried out to determine their general impact on oxygen activation and radical generation in a Fenton reaction system at beer pH.

#### Used carbohydrates in model and brewing trials:

Palatinose™ (isomaltulose) ≥ 98 %, C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>; M = 342,30 g/mol  
Südzucker AG, Mannheim, Germany, www.suedzucker.de, CAS 13718-94-0

Fructose ≥ 99 %, C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>; M = 180.16 g/mol

Südzucker AG, Mannheim, Germany, www.suedzucker.de; CAS 57-48-7

Sucrose ≥ 99.7 %, C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>; M = 342.30 g/mol

Südzucker AG, 68165 Mannheim, Germany, www.suedzucker.de; CAS 57-50-1

Glucose monohydrate ≥ 99 %, C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> × H<sub>2</sub>O; M = 198.17 g/mol

Agrana Fruit Germany GmbH, Konstanz, Germany, www.agrana.de; CAS 14431-43-7

D-(+)-maltose monohydrate Type II ≥ 95 %; C<sub>12</sub>H<sub>22</sub>O<sub>11</sub> × H<sub>2</sub>O; M = 360.31 g/mol

Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, www.sigmaaldrich.com; CAS 6363-53-7

### 2.2 Wort boiling pre-trials – lab scale

For the laboratory boiling pre-trial, a kettle-full wort was taken out of the Pilsner type brewing process (5 hL two-unit stainless-steel brewery, Meyer-Brau KG “Schnuckenbräu”, Hünzingen) at begin of boiling into a 10 L KEG and cooled down. This wort with 11 % w/w extract was diluted to 9 % w/w and with the dilution water the same six different sugars as in the first trial (calculated on 5 % w/w extract) and 0.025 mg Fe<sup>2+</sup>/L were added. Of each 1 L sample, 400 mL were taken away and 600 mL were boiled for 1 h under reflux.

### 2.3 Semi technical brewing trials – research brewery

Beers with 3% carbohydrate addition (Glucose monohydrate, Fructose, D-(+)-maltose monohydrate type II, Sucrose, Isomaltulose) and a control brew (water addition) were brewed.

Therefore, 11 °P kettle full wort was produced and cooled down. For every trial 40 kg wort was taken away into the mash tun kettle of the pilot brewery (TU Berlin) and heated up to 80 °C. 5 L of the six different sugar solutions with softened tap water were added to achieve an extract-rise of 3 % w/w.

Heating the kettle from 80 °C to boiling temperature took 15 min. At beginning of the 60 min. boiling the wort was hopped with 1.3 g

hop CO<sub>2</sub>-extract Hallertauer Magnum (46.6% α-acids, Hopsteiner). The hop addition was kept low to make sure that the hops' influence on the oxidative wort stability [34] cannot distort small differences between the used carbohydrates. Whirlpool rest was hold for 20 min before the wort was cooled down by means of a plate heat exchanger and pitched with 15 × 10<sup>6</sup> yeast-cells per mL (pure bred RH-yeast). The fermentation temperature was 12 °C and up to 14 °C for the last two of ten fermentation days. After fermentation the beer was transferred into KEGs. A one-day diacetyl-rest at room temperature followed. The green beers were stored at 1 °C for two and a half weeks. After maturation the beers were dispensed with a mixture of 30 % tetra-iso-hop and 10 % iso-hop to achieve a lager beer comparable bitterness. The hopped beer was filtered through a three-stage cartridge membrane filter (Donaldson).

All six filtered beers were bottled with a manual bottle-filling unit under CO<sub>2</sub>-atmosphere.

The beers were forced-aged (28 °C/10 weeks) to get a deeper insight in the decrease of the oxidative stability during storage. At the beginning and end of storage the ageing compounds were determined via SAFE GC-MS. Furthermore, forcing tests with altering 60 °C/0 °C were carried out over 15 warm days.

In an additional trial 3 % isomaltulose was added into the same wort late during the whirlpool rest. After cooling a wort EPR measurement was carried out.

### 2.4 Wort analyses according to MEBAK [56]

These were as follows: extract (2.9.6.3); density (2.9.2.3); viscosity (2.25.3); pH-value (2.13); colour (2.12.2); T-value (EPR) (2.15.3); total nitrogen (2.6.1.1); MgSO<sub>4</sub>-precipitable nitrogen (2.6.3.1).

### 2.5 Beer analyses according to MEBAK [56]

These were as follows: apparent extract (2.9.2.3); original extract (2.9.6.3); final degree of attenuation (2.8.4); alcohol (2.9.6.3); density (2.9.2.3); viscosity (2.25.3); pH-value (2.13); colour (2.12.2); EAP and T<sub>max</sub>-value (EPR) (2.15.3); turbidity (2.14.1.2); total nitrogen (2.6.1.1); sulphur dioxide (SO<sub>2</sub>) (2.21.8.3); free amino nitrogen (FAN) (2.6.4.1.1); total polyphenols (2.16.1); bitterness units (2.17.1); forcing test 60 °C/0 °C (2.14.2.1).

### 2.6 Reduction potential – optimised chapon method [12, 49]

The Chapon method [12] describes the reduction potential of beverages and reduction substances in solutions against Fe<sup>3+</sup>. The method's principle relies on the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> which reacts with 2,2'-bipyridyl to form a red coloured complex with its absorbance at 510 nm.

#### Preparation of solutions and measurement conditions for the optimised Chapon method [49]:

Solution A was prepared by dissolving 150 mg NH<sub>4</sub>Fe(III)(SO<sub>4</sub>)<sub>2</sub> × 12 H<sub>2</sub>O in approx. 25 mL bi-distilled water and 0.2 mL concentrated

H<sub>2</sub>SO<sub>4</sub>. After the salt was completely dissolved, the volume was made up to 50 mL by adding bi-distilled water.

Solution B was prepared by dissolving 50 mg 2,2'-bipyridyl in approx. 45 mL bi-distilled water and 4 mL 0.1 N H<sub>2</sub>SO<sub>4</sub>. After the salt was completely dissolved, the volume was made up to 50 mL by adding bi-distilled water.

Both solutions are mixed shortly before starting the measurement.

The carbohydrates (0.2 M) were dissolved in acetate buffer (1 M, pH 4.5) and the measurement was carried out for 1 h at 60 °C.

## 2.7 EPR methods used: determination of the endogenous antioxidative potential (EAP value), T<sub>600</sub>-determination [45, 56]

The determination of the "Endogenous Antioxidative Potential" (EAP value) was carried out according to MEBAK 2.15.3 [56]. The determination of the "Endogenous Antioxidative Potential" (EAP value) is based on the detection of the radical generation during accelerated beer aging (60 °C). Initially, the radical generation can be delayed or prevented by the endogenous anti-oxidative activity of beer. After the consumption of antioxidants, the EPR signal increases when spin-trap adducts, mainly hydroxyethyl radicals, are generated. The intersection of the two linear slopes, which evolve due to the delay, gives a relative measure of the beer stability. The time at the intersection is defined as the EAP value respectively the former used lag-time (Fig. 2) The T<sub>600</sub> value is defined as the EPR signal intensity measured after 600 min of reaction time (forced aging at 60 °C) and qualitatively indicates the amount of radicals that are generated (Fig. 2). This radical generation is affected by the content of metal ions, pH value, polyphenols, proteins, intermediate Maillard reaction products, etc.

For buffer solution trials, the used carbohydrates (0.175 M) were dissolved in a phosphate buffer (0.2 M, pH 4.5) with 5 % EtOH content and 300 ppb (FeSO<sub>4</sub>)<sub>7</sub> H<sub>2</sub>O. The EPR-measurement was carried out for 500 min at 60 °C for the determination of the T<sub>400</sub>-value.

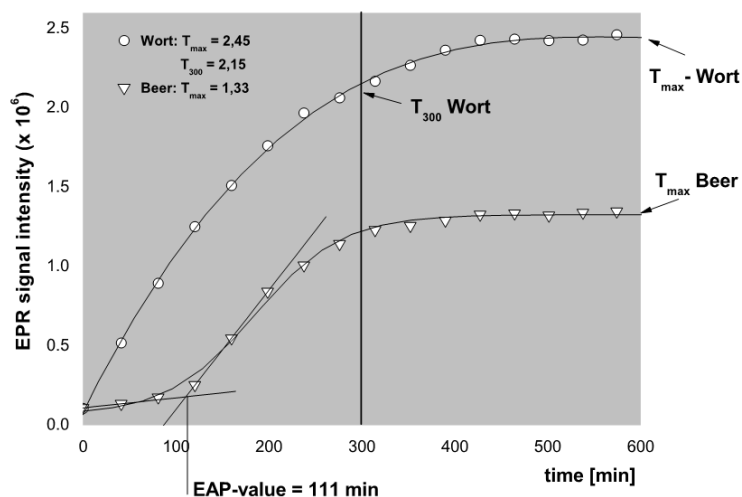


Fig. 2 Determination of T<sub>max</sub> and EAP-value [56]

In case of wort measurements, the defrosted wort samples were heated up to 40 °C/10 min. and centrifuged for 10 min (6000 rpm /5403 G) at 20 °C. For each sample 16.2 mg POBN was diluted in 100 µL of double distilled water. For the preparation of EPR samples 700 µL of ethanol and 10 mL wort sample were filled into 16 mL vials. At the beginning of the measurement 100 µL of the POBN-solution was added and the samples were shaken twice and placed in an auto sampler.

Beer samples were degassed in an ultrasonic bath for 20 min (max. 20 °C). For each sample 8.4 mg POBN was diluted in 50 µL of double distilled water (3.5 3.6 mMol POBN). For the preparation of EPR samples 150 µL of ethanol and 12 mL beer sample were filled into 16 mL vials. At the beginning of the measurement 50 µL of the POBN-solution was added and the samples were shaken twice and placed in an auto sampler.

Instruments: EPR spectra were obtained with an X-band Spectrometer, ESP-300, cavity type Bruker 4108 TMH, No. 8603 and E-Scan, Bruker, Rheinstetten, Germany. EPR-parameter for pretrial model solution, wort and beer analyses: centre field ca. 3475 G; attenuation 0 dB; sweep width 14 G; receiver gain 3.99 x 10<sup>3</sup>; resolution 512, mod. amplitude 1.47 G; modulation frequency 86 kHz; conversion time 10 ms; time constant 41 ms; scans beer 25, scans wort 30.

## 2.8 SO<sub>2</sub> determination using continuous flow analysis (CFA) [38, 56]

The determination of SO<sub>2</sub> was carried out by Continuous Flow Analysis (CFA) (cp. MEBAK 2.21.8.3) under an optimized procedure using a Teflon® membrane [38]. SO<sub>2</sub> is released from beer at high temperatures as a gas and dialyzed by a Teflon membrane into a formaldehyde solution. P-rosaniline is added and the molecule binds with the sulphur dioxide-formaldehyde complex at a temperature of 45 °C forming a red coloured complex, which then can be determined colour metrically at 560 nm. The evaluation was done with a CFA-software under consideration of peak heights and a calibration line.

## 2.9 Calculation of SO<sub>2</sub>-consumption rate

The SO<sub>2</sub>-consumption rate was determined via correlation coefficients between detected SO<sub>2</sub>-values and its associated EAP-values under consideration of all different measurements. As a result for every beer a linear fitting was received. The reciprocals of the slopes of these linear fittings were calculated and multiplied with 1000. The unit of the SO<sub>2</sub>-consumption rate is µg/min\*L.

## 2.10 Ageing compound analysis in beer by GC-MS and Solvent Assisted Flavour Evaporation (SAFE) [16]

After filtration and addition of 1 µg pentanal, the beer samples were extracted with 150 mL ether to remove the non-volatile material. The unified extracts were distilled under high vacuum by means of a SAFE apparatus. After drying over Na<sub>2</sub>SO<sub>4</sub>, the distillate was concentrated to 5 mL. The concentrated distillate was applied in 20:1 split mode to a gas chromatograph (6890 Agilent, 0.6 mL/

min He). The staling aldehydes were evaluated by an MSD 5973 mass spectrometer (Agilent Technologies, Waldbronn).

Instruments were as follows: GC/MS, gas chromatograph 6890 (Agilent Technologies, Waldbronn); capillary column VF-5 MS; 60 m x 0.25 mm, 0.25  $\mu$ m film thickness (Varian, Darmstadt); cold injection system CIS 4 (Gerstel, Mühlheim).

### 2.11 Osmolarity

Osmotic pressure was determined with OsmoLAB One/16S by a thermistor-tip that measures the refrigeration of the samples. For starting a measurement, the device has to be cooled down for about 12 min. The OsmoLAB must be calibrated with a two-point calibration. Therefore 300 mOsmol and 850 mOsmol are measured for three times. All three measurements of this standard solution must be in a standard variance of less than 1 %.

For measuring samples 50  $\mu$ L are filled into a sample tube and are measured for three times. The results of osmolarity are expressed as mOsmol and refer to litre.

## 3 Results and Discussion

In consideration of the described reaction mechanisms pre-trials with different carbohydrate model solutions were carried out to get a first overview in the pro- and antioxidative properties of the used carbohydrates in the brewing trials.

In the first step the optimised Chapon method [12, 35, 39, 49] was used to ascertain the different reduction potential of the carbohydrates against iron ions with oxidation step 3+.

The results in figure 3 A demonstrate the strongest reduction potential (method 2.6) of the reducing carbohydrate buffer solutions in the order of isomaltulose > fructose before the other carbohydrates follow with a clear lower reduction potential. Thereby

it is notable that the “non-reducing carbohydrate” sucrose has a higher reduction potential against  $\text{Fe}^{3+}$  than glucose. The significant loss of the reduction properties of the generally known “reduction carbohydrate” glucose at low pH can be explained by a change in the equilibrium between the cyclic hemiacetal form, without a free aldehyde group, and the open chain aldehyde structure. At low pH, the formation of the open chain aldehyde structure of glucose is inhibited. Contradictory to this, at low pH, fructose has a higher ability to generate the open chain structure resulting in much stronger reducing properties. The increasing reduction potential of the “non-reduction sugar” sucrose at low pH can be explained by the invert sugar’s acid hydrolysed formation and the appearance of the strong reduction potential of fructose [39, 41].

In direct comparison the results of EPR-measurements in figure 3 B (method 2.7) verify an earlier described [39] direct correlation between an increased reduction potential of carbohydrates and the general influences on oxygen activation and radical generation in a Fenton reaction system at beer pH. Analogous to the detected reduction properties of the used carbohydrates (Fig. 3 A), a higher reduction potential is responsible for a stronger increase in the prooxidative acting radical generation ( $T_{400}$ -value) in model buffer solution. In this context the carbohydrates isomaltulose and fructose are characterized by the highest radical generation, whereas glucose and sucrose show comparable significantly lower influences on oxidative processes as indicated by the radical generation.

In summary the results suggest a carbohydrate-dependent acceleration of oxidative processes caused by the Fenton/Haber-Weiss reaction system and oxygen activation per electron transfer resulting in an increasing formation of reactive oxygen species (e.g.  $\text{HO}^{\bullet}$ ).

To get a first overview into the influences of different carbohydrates (glucose, fructose, maltose, sucrose, isomaltulose) on oxidative wort stability and the formation of specific aging components during wort boiling different pre trials with 5% carbohydrate addition in kettle full wort (80 °C) were carried out in lab scales as described in method 2.2.

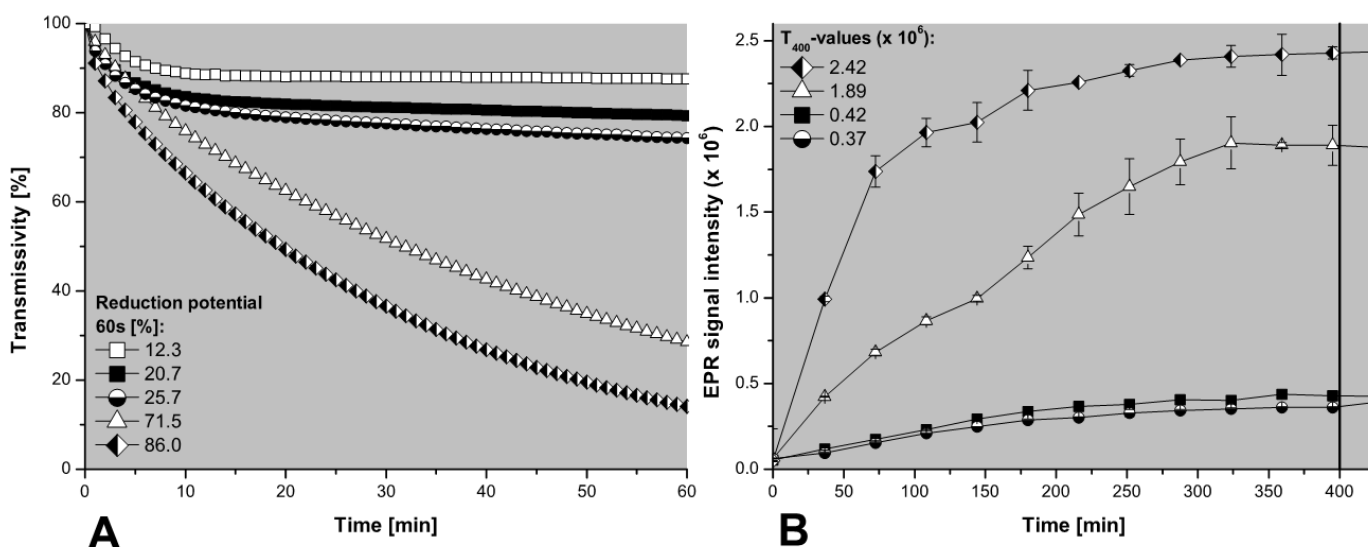
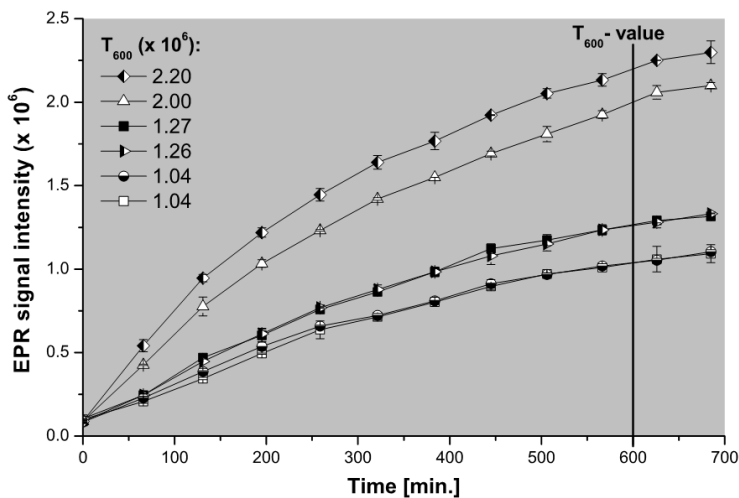
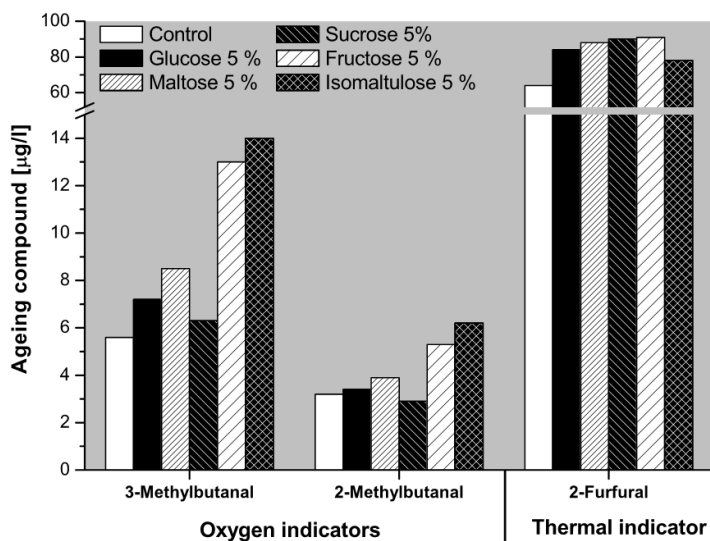


Fig. 3 A: Reduction potential according to optimized Chapon method. B: Buffer solution pre-trials measured using EPR spectrometry (Buffer □, glucose ■, sucrose ●, fructose △, isomaltulose ◆) [35, 39, 41]. Error bars represent  $\pm 1$  standard deviation,  $n = 2$

**Tab. 1** Pitching wort analyses (mean of duplicate) of the pre-trial (1 given by MEBAK [56];  $\pm$  represents maximal  $\pm 1$  standard deviation,  $n = 2$ )

Wort analyses	Unit	Repeatability	Control	Glucose	Fructose	Maltose	Sucrose	Isomaltulose
Extract real	% w/w		9.63	14.15	14.23	14.48	14.20	13.99
Density	g/cm <sup>3</sup>	0.0001 <sup>1</sup>	1.03661	1.05561	1.0559	1.05697	1.05574	1.05497
pH	1	0.022 <sup>1</sup>	5.95	5.88	5.85	5.89	5.92	5.83
Colour	EBC	0.1m <sup>1</sup>	6.1	6.6	7.5	6.8	6.1	7.8
T <sub>600</sub> -value (*106)	EPR s.i.	–	1.04	1.27	2.00	1.26	1.04	2.20
2-Methylbutanal	µg/L	–	3.2	3.4	5.3	3.9	2.9	6.2
3-Methylbutanal	µg/L	–	5.6	7.2	13.0	8.5	6.3	14.0
2-Furfural	µg/L	–	64	84	91	88	90	78

**Fig. 4** Data of EPR-measurement (EAP- and T<sub>max</sub>-values) of pre-trial worts with carbohydrate addition (5%), control □, glucose ■, fructose △, sucrose ●, maltose ▶, isomaltulose ◆. T<sub>max</sub>-value represents the maximal EPR signal intensity. Error bars represent  $\pm 1$  standard deviation,  $n = 2$ **Fig. 5** Ageing components of pre-trial worts with carbohydrate addition (5%) detected after boiling

In table 1 the results of standard wort analyses, EPR measurements and ageing compound detection are summarized.

The extract varies in all worts with carbohydrate addition are in a

range of 14–14.5 %. Whereas the control kettle full wort in logical order to the missing carbohydrate addition has lower extract content of 9.7 %. Furthermore it is obvious, that fructose and isomaltulose addition leads to the highest colour, highest T<sub>max</sub>-value (EPR) and highest amount of the detected Strecker aldehydes (2-MB/3-MB) as shown in figure 4 and 5.

The results indicate a first significant correlation between radical generation, colour and content of typical beer staling aldehydes (Correlations: T<sub>600</sub> – 2-MB:  $r = 0.981$ ;  $y = 0,38 + 2.57 \times 10^{-6} x$ ; T<sub>600</sub> – 3-MB:  $r = 0.986$ ;  $y = -1.16 + 6.99 \times 10^{-6} x$ ; T<sub>600</sub> – Colour:  $r = 0.972$ ;  $y = 4.80 + 1.38 \times 10^{-6} x$ ).

To get a deeper insight on the influences of carbohydrates on oxidative wort and beer stability and the formation of specific ageing components during storage further semi technical trials were carried out. Therefore five brews with 3 % carbohydrate addition (glucose, fructose, maltose, sucrose, isomaltulose) in kettle full wort (80 °C) and a control brew (water addition) were brewed in the TU research brewery as described in methods 2.3.

In table 2 (see p. 84) the results of standard wort analyses as well as the results from EPR-measurements and the analysed ageing components (2-/3-MB) in the pitching wort are summarized. Additionally figure 6 compares the detected colours and the influences of carbohydrates on oxidative wort stability as indicated by the radical generation (T<sub>max</sub>-value /EPR signal intensity).

The results of the standard wort analyses demonstrate a successful and approximately comparable carbohydrate addition because of the control wort extract was nearly 3 % under the carbohydrate added brews.

The pH-values were all in a general typical range for wort pH. With boiling time the pH-values decreased slightly. In case of control brew the pH-value was slightly higher. The total nitrogen contents showed no significant difference between all brews. Thereby it has to be mentioned that the contents of the nitrogen fractions are not calculated to 12 % extract as normally suggested by MEBAK to get a better comparison, owing to 75 % d.m. of the total extract contents are solved malt extracts and app. 25 % d.m. are contributed by added pure carbohydrates without nitrogen fractions. The described dilution factor is also responsible for overall low FAN contents, which has an influence on the fermentation performance resulting in a three days longer fermentation time. With regard to

**Tab. 2 Pitching wort analyses (mean of duplicate) of the different trials (1 given by MEBAK [56]; ± represents maximal ±1 standard deviation, n = 2)**

Wort analyses	Unit	Repeat-ability	Control	Glucose	Fructose	Maltose	Sucrose	Iso-maltulose
Extract real	% w/w		10.85	13.88	14.28	14.22	14.26	14.17
Density	g/cm <sup>3</sup>	0.0001 <sup>1</sup>	1.04168	1.05333	1.05612	1.05585	1.05600	1.05569
pH	1	0.022 <sup>1</sup>	5.63	5.57	5.53	5.57	5.58	5.52
Osmolarity	mOsmol	–	384	656	658	533	540	543
Colour	EBC	0.1m <sup>1</sup>	9.7	9.5	10.8	9.7	9.8	11.2
T <sub>max</sub> -value (·10 <sup>6</sup> )	EPR s.i.	–	2.15	2.12	3.72	2.03	2.06	4.18
Total nitrogen	mg/L	±5.6	671	657	678	676	688	687
Free amino nitrogen	mg/L	±2.7	128.7	142.6	146.2	139.2	145.3	137.1
Total polyphenols	mg/L	±1.8	159.7	159.7	145.1	163.7	162.3	147.9
Bitterness units	EBU	–	7.0	5.1	5.6	5.0	4.6	5.4
Reduction potential	%	±0.62	40.98	40.61	43.01	41.41	41.47	43.10
2-Methyl-butanal	µg/L	±0.31	5.5	6.0	7.0	4.9	5.0	7.5
3-Methyl-butanal	µg/L	±1.06	15.9	19.7	24.2	14.6	14.8	25.3
2-Furfural	µg/L	±10.89	164.9	167.9	164.1	161.3	163.8	181.6

the fermentation characteristics and the influences on the oxidative beer stability also the osmotic pressure were analysed, owing to its contribution to the SO<sub>2</sub>-formation by yeast during fermentation [47, 54].

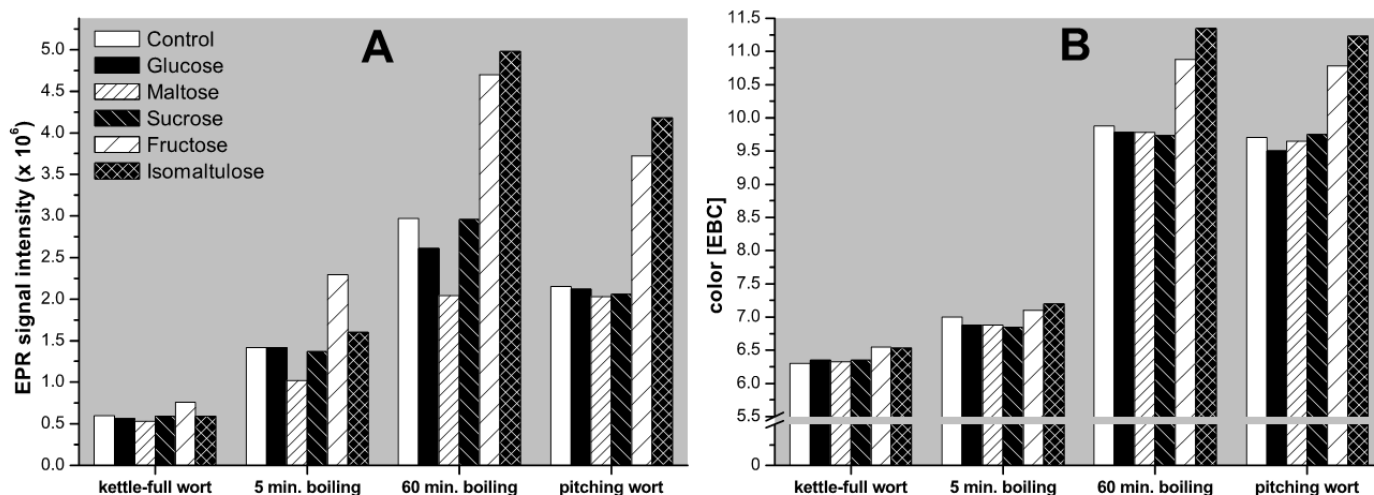
In logical order the control brew displayed the lowest osmolarity, followed by disaccharide added brews (maltose, sucrose, isomaltulose) and as a consequence of particle number per mass the brews with monosaccharides addition (glucose, fructose) showed the highest osmotic pressure in the pitching wort. The polyphenol contents demonstrate only small differences between pitching wort of control and the brews with glucose, maltose or sucrose addition but a slightly approximately 10 % lower amounts in the

wort with fructose or isomaltulose addition. One explanation could be given by a higher amount of polyphenol polymerization occurring from polyphenol oxidation and a subsequent complex formation to higher molecular structures which are able to precipitate or can be separated by centrifugation during sample preparation for analyses.

The possible acceleration of polyphenol oxidation could be connected with the development of the reduction potential, colour and radical generation during wort boiling as shown in figure 6. The brews with added isomaltulose and fructose showed with 43 % reduction potential the highest reduction potential against iron with oxidation step 3+, whereas the other brews have reduction potentials between 40.5 and 41.5 %. Analogical to the results of the model pre-trials

(Fig. 3), the highest reduction potential in the pitching wort was measured at the end of wort boiling with isomaltulose and fructose addition. This fact leads to the lowest oxidative wort stability as indicated by the highest colour and radical generation (T<sub>max</sub>-value) with a significant correlation coefficient (r = 0.94). The other brews with carbohydrate addition (sucrose, glucose, maltose) showed an approximately comparable colour intensity and oxidative wort stability (T<sub>max</sub>-value) in comparison to the pitching wort of control.

In this context the brew with fructose addition demonstrated the highest radical generation already at the beginning of boiling. During the further wort boiling process the results of all brews in general demonstrate a clear decrease in oxidative wort stability.



**Fig. 6 The correlation (r = 0.94; y = 8.33 + 5.63 · 10<sup>(-7)</sup> x) of EPR-measurement (A, T<sub>max</sub>-values) and wort colours (B) with carbohydrate addition (3 %), detected during the brewing process. T<sub>max</sub>-values represents the maximal EPR signal intensity**

**Tab. 3** Filtered beer analyses (mean of duplicate) of the different trials (1 given by MEBAK [56]);  $\pm$  represents maximal  $\pm 1$  standard deviation,  $n = 2$ )

Beer analyses	Unit	Repeat-ability	Control	Glucose	Fructose	Maltose	Sucrose	Iso-maltulose
Extract apparently	% w/w	0.021	2.25	2.93	3.21	3.08	3.54	5.27
Original extract	°Plato	0.051	10.90	13.96	13.90	14.07	14.11	13.59
Apparent degree of attenuation	% w/w	–	79.39	79.01	76.89	78.12	74.89	61.23
Alcohol content	% v/v	0.031	4.57	5.94	5.76	5.93	5.71	4.51
Density	g/cm <sup>3</sup>	0.00011	1.00694	1.00962	1.01073	1.01020	1.01204	1.01888
pH	1	–	4.58	4.37	4.39	4.47	4.39	4.48
Colour	EBC	0.022m1	7.3	6.8	7.7	7.2	7.0	8.0
T <sub>max</sub> -value (*10 <sup>6</sup> )	EPR s.i.	–	1.35	0.87	0.81	0.74	0.63	1.30
EAP-value	min	–	319	445	562	601	531	283
Total nitrogen	mg/L	$\pm 8.5$	507	501	529	507	512	489
Free amino nitrogen	mg/L	$\pm 2.6$	84.8	83.2	90.2	85.7	90.7	89.5
Total polyphenols	mg/L	$\pm 1.7$	124.3	131.2	131.1	132.3	132.3	112.6
Bitterness units	EBU	$\pm 0.6$	27.5	23.6	23.4	25.1	23.3	25.0
SO <sub>2</sub>	mg/L	$\pm 0.18$	9.9	16.3	19.5	18.2	15.6	12.6
SO <sub>2</sub> consumption rate	$\mu\text{g}/\text{min}\cdot\text{L}$	–	25.35	28.37	33.56	28.30	27.85	39.15
2-Methylbutanal	$\mu\text{g}/\text{L}$	$\pm 0.21$	0.7	1.3	1.2	1.5	1.6	1.5
3-Methylbutanal	$\mu\text{g}/\text{L}$	$\pm 0.26$	1.5	2.8	2.1	4.3	3.0	2.4
2-Furfural	$\mu\text{g}/\text{L}$	$\pm 2.77$	18.5	20.8	20.4	18.0	18.1	17.8

After wort cooling the EPR signal intensities at T<sub>max</sub> were slightly lower, but the isomaltulose wort has overtaken all other brews in decrease of oxidative wort stability followed by the fructose brew.

The low oxidative stability is reflected in a twofold higher radical generation compared to the glucose, maltose and sucrose added wort samples.

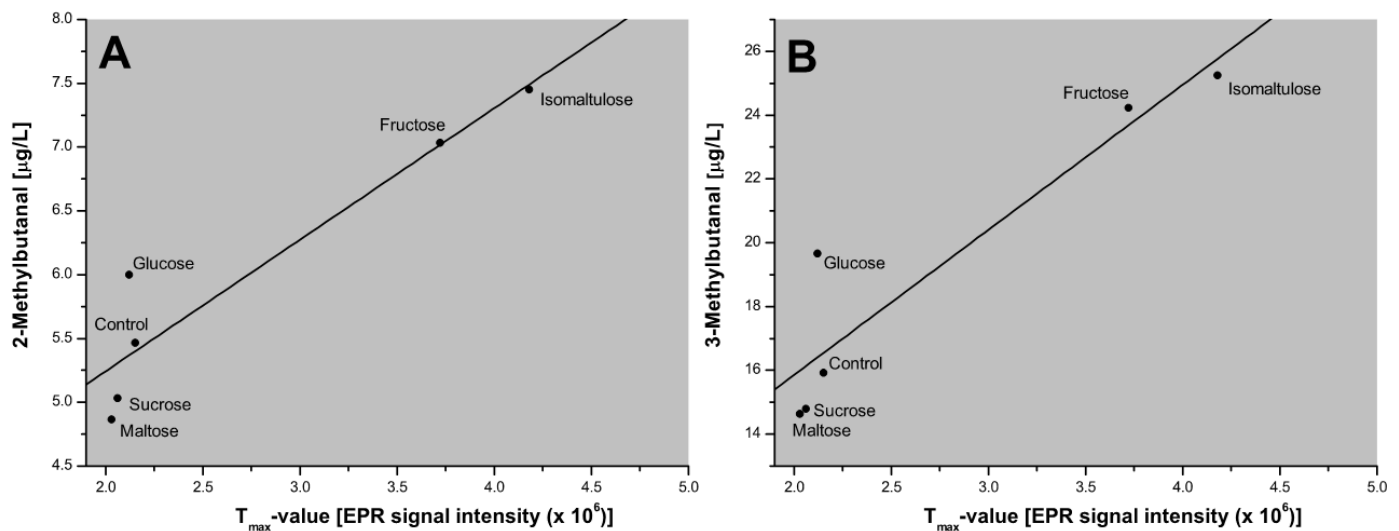
In figure 7, the linear regression between the oxidative wort stability as indicated by the radical generation at the end of wort boiling (T<sub>max</sub>) and the amounts of specific Strecker aldehydes after boiling are shown.

In case of the ageing components 2-/3-MB (Tab. 2) the addition of carbohydrates with stronger reduction potential like isomaltulose and fructose before wort boiling yielded in a significant higher value. All together the T<sub>max</sub>-values and 2-/3-MB contents show a strong linear response of 0.93 and 0.91, respectively. The brews where maltose or sucrose were added lay in a comparable range as the control brew just the glucose addition led to slightly higher contents.

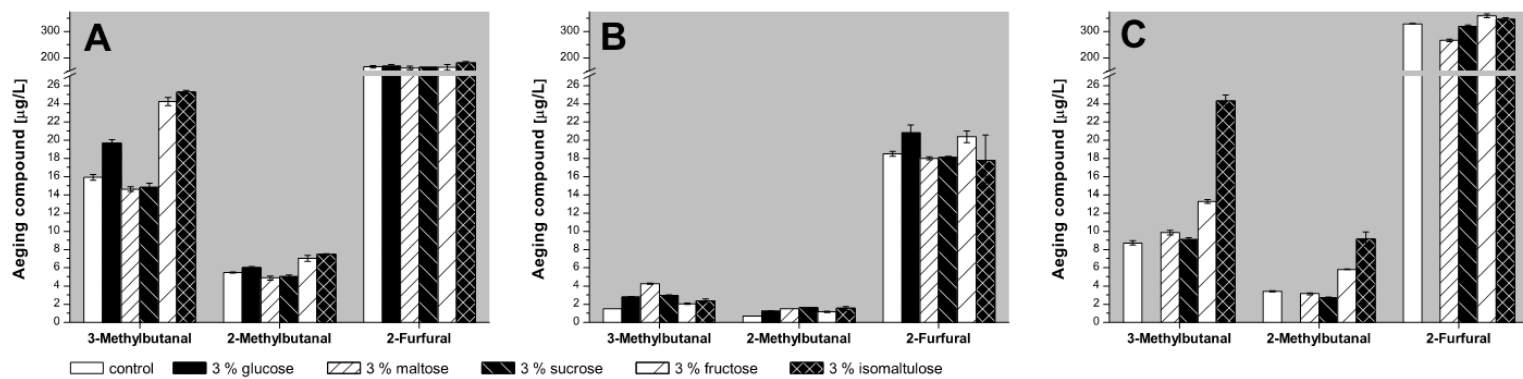
After each wort production and cooling down, the brews were pitched with a homogenized pure bread RH-yeast as described in methods (2.3) as soon as possible.

Table 3 summarizes the results of fresh filtered beer analyses.

As expected the original extract contents of the filtrated beers decreased slightly in comparison to the wort extract during filtration. Even the degrees of attenuation were in a good range between 75 and 79 % w/w with exception



**Fig. 7** Ageing components of worts with carbohydrate addition detected after boiling. A: Correlation of 2-MB with T<sub>max</sub>-value ( $r = 0.93$ ;  $y = 3.18 + 1.03 \cdot 10^{(-6)} x$ ), B: Correlation of 3-MB with T<sub>max</sub>-value ( $r = 0.91$ ;  $y = 6.76 + 4.55 \cdot 10^{(-6)} x$ )



**Fig. 8** Ageing components (oxygen and thermal indicators) in cast worts (A) as well as fresh (B) and aged (C) beers. Error bars represent  $\pm 1$  standard deviation, n = 2

of the brew where isomaltulose was added. In logical order to the non-fermentable carbohydrate properties of isomaltulose the brew was fermented to 61 % w/w. Furthermore, the alcohol contents were between 5.71 and 5.94 % v/v in the brews with fermentable carbohydrate addition. In logical consequence to the lower fermentable extract content, between 4.51 and 4.57 % v/v in control and isomaltulose brew. The pH-values stayed nearly steady in every single brew and lay in a range of two tenth in the different brews whereby the control brew had a slightly higher value with 4.58.

In consequence to the higher wort colours of the brews with isomaltulose and fructose addition also a higher colour in the final beers were detectable. The contents of total nitrogen and free amino nitrogen were approximately comparable in all brews between 490 and 520 mg/L or 83–91 mg/L, respectively.

Also the total polyphenol contents were in a similar range in all brews (124–132 mg/L) with exception of the brew with isomaltulose addition which shows an approximately 1 % lower value. After hopping, in unfiltered beers the final beer bitterness lies between 23.3 and 27.5 EBU whereby approximately 5 to 7 EBU occur from the small hop addition at the beginning of wort boiling.

Analogical to wort analyses, the oxidative beer stability were analysed using EPR-spectroscopy to ascertain the endogenous antioxidative potential (EAP-value) and the amount of prooxidative acting radical generation ( $T_{max}$ -value). The results of the EPR-analyses are demonstrated in direct comparison to the final  $SO_2$ -contents as an important antioxidative acting beer ingredient with significant influences on oxidative stability (Tab. 3).

**Tab. 4** Analyses of aged beers after 10 weeks at 28°C (mean of duplicate; 1 given by MEBAK [56];  $\pm$  represents maximal  $\pm 1$  standard deviation, n = 2)

Beer analyses	Unit	Repeat-ability	Control	Glucose	Fructose	Maltose	Sucrose	Iso-maltulose
Colour	EBC	0.022m1	8.0	7.8	8.7	8.0	7.7	9.8
$T_{max}$ -value (*106)	EPR s.i.	–	1.77	1.16	1.01	0.77	0.71	1.69
EAP-value	min	–	122	288	305	398	346	86
$SO_2$	mg/L	0.51	3.1	7.3	9.2	9.9	7.7	2.6
2-Methylbutanal	µg/L	$\pm 0.78$	3.4	–	5.8	3.1	2.7	9.2
3-Methylbutanal	µg/L	$\pm 0.67$	8.7	–	13.3	9.9	9.1	24.3
2-Furfural	µg/L	$\pm 6.83$	328.9	–	360.2	266.2	318.5	345.8

The EPR-results reveal that the fresh beer with isomaltulose addition shows the lowest oxidative stability as indicated by the lowest EAP-value (283 min) and a strong radical generation ( $T_{max}$ -value  $1.3 \times 10^6$ ) followed by the control beer with a slightly higher EAP-value (319 min) and comparable radical generation ( $T_{max}$ -value  $1.3 \times 10^6$ ). For a better understanding of the influences on oxidative stability during storage it is to point out that the isomaltulose brew leads to a definitely higher  $SO_2$ -content (12.6 mg/L) adverse to the control (9.9 mg/L) but lower oxidative beer stability. Thereby the general higher  $SO_2$ -contents of brews with carbohydrate addition during wort boiling can be explained by an increase of the osmotic pressure resulting in a stronger  $SO_2$ -formation during fermentation [47, 48, 50]. This is also the main reason for the significant higher EAP-values and lower radical generation ( $T_{max}$ -values) of all other brews with fermentable carbohydrate (maltose, fructose, sucrose, glucose) additions in comparison to the isomaltulose brew and control.

In this context it is noticeable that the brew with fructose addition led to the highest  $SO_2$ -content (19.5 mg/L) of all beers but just a comparable or lower EAP-value and relatively high radical generation in relation to the other fermentable carbohydrate additions.

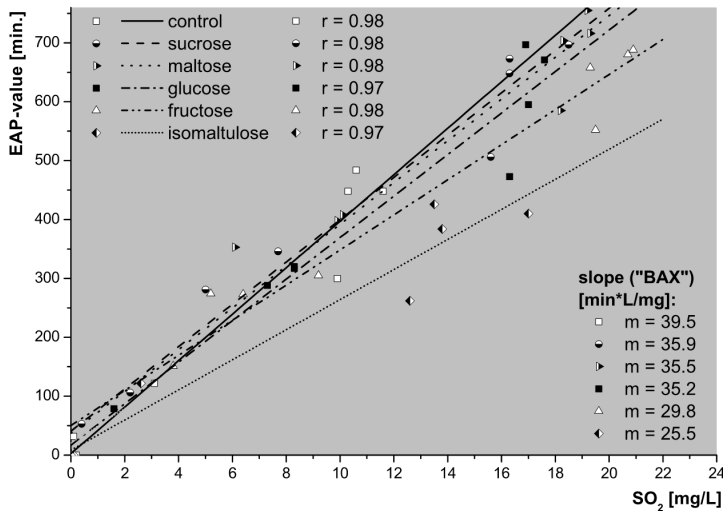
The analysed ageing components were significant degraded during fermentation.

In all fresh beers the values of the Strecker aldehydes 2-/3-MB which are also described as oxidation indicators in the literature [5, 36, 53, 57] and 2-F as thermal indicator stay together with low amounts in a very small range and cannot give a clear information

about differences in the state of oxidative stability. Only the 3-MB amount of the brew with maltose addition was slightly higher.

In the next step ageing simulations at 28 °C in darkness was done over 10 weeks to determine the influences of carbohydrate additions on the beers at a consumer common storage temperature.

The results of beer analyses after ageing are summarized in table 4.



**Fig. 9** Correlation of EAP- and  $\text{SO}_2$ -consumption to calculate the approximately "Beverage Antioxidative index (BAX)" [37]

As expected after storage a slightly colouration was detectable in all brews but the increase in colour was on highest level in the brews with isomaltulose and fructose addition at the beginning of wort boiling.

Furthermore, the brews with isomaltulose and fructose addition consumed the origin available  $\text{SO}_2$ -content faster during storage than all other brews (Tab. 3 and 4). In tendency the same effect is observable in the decrease of the endogenous antioxidative potential (EAP-value); the fructose brew showed the strongest decrease during storage with 257 min. The decrease in the isomaltulose brew stays in the range of control, maltose or sucrose addition because of its lowest start EAP-value in fresh beer (Tab. 3 and 4).

As demonstrated in figure 8 after ten weeks of storage the ageing components showed great differences between the brews. Even though glucose beer was missing in these analyses a clear tendency could be determined. In this context the described oxidation indicators 2-/3-MB were on the highest levels in isomaltulose beer, followed by fructose. Whereas the sucrose and maltose addition leads to a more moderate increase in the ageing components during storage and show comparable contents to the control brew. 2-F as an indicator of thermal treatment was in the range of 300  $\mu\text{g/L}$  for all brews; this demonstrates comparable storage conditions.

To get a better insight into the pro- and antioxidative properties of the different beer matrices, the consumption rate of the important antioxidant  $\text{SO}_2$  was calculated in correlation to the EAP-decrease during storage (10 weeks/28 °C) and additional forcing tests (15 cycle days 0 °C/60 °C) (Tab. 5) according to the so called Beverage Antioxidative Index (BAX) [37]. Figure 9 shows the correlation between EAP- and  $\text{SO}_2$ -consumption result-

ing in the calculated approximately BAX as slope of regression lines [37].

The calculated slopes or BAX-values are in a range between 26 and 40  $\text{min} \times \text{L/mg}$ , whereby the isomaltulose and fructose brew with 25.5  $\text{min} \times \text{L/mg}$  or 29.8  $\text{min} \times \text{L/mg}$  led to fastest consumption rate of  $\text{SO}_2$  (lowest BAX) during storage. With a clear distance and significant lower consumption rate follow the three brews with glucose, maltose and sucrose addition in a range between 35.2–35.9  $\text{min} \times \text{L/mg}$ . The lowest  $\text{SO}_2$ -consumption (highest BAX) during storage could be detected in the control brew with 39.5  $\text{min} \times \text{L/mg}$ .

In this connection, it is noticeable that the  $\text{SO}_2$ -consumption rates additionally correlate linear ( $r = 0.87$ ) with the detected radical generation ( $T_{\text{max}}$ -value) and oxidative wort stability at the end of wort boiling. Furthermore figure 10 presents the regression analyses between "BAX" – as slope between EAP- and  $\text{SO}_2$ -consumption (Fig. 9) – and the 2-/3-MB formation during storage, respectively.

The development of the ageing components during beer storage followed in the same tendency as the Beverage Antioxidative Index and the linear correlation coefficients were calculated to  $r = 0.882$  and  $r = 0.895$  between BAX-value and 2-/3-MB formation, respectively. On the one hand the calculated correlations demonstrate the dependence between the "BAX" and beer ageing. On the other hand it makes the prior described influences of the different carbohydrates on oxidative process (Tab. 4; EAP- and  $T$ -value) and the formation of aging components (Fig. 8) during storage obvious.

Owing to the demonstrated prooxidative influences of the carbohydrate isomaltulose during wort boiling (Tab. 2 and 4,  $T_{\text{max}}$ -value), the influences of isomaltulose addition at different process steps during brewing were investigated in further trials to get more information about the right handling of non-fermentable carbohydrates to increase the palate fullness with as small as possible influences on oxidative stability (methods 2.2).

**Tab. 5** Forcing test at 60 °C/0 °C; data of EPR-measurements and  $\text{SO}_2$ -contents

Beer analyses	Unit	Warm day	Control	Glucose	Fructose	Maltose	Sucrose	Iso-maltulose
$T_{\text{max}}$ -value ( $\times 10^6$ )	EPR s.i.	1	1.68	0.95	1.16	1.07	1.04	1.77
EAP-value	min	1	0	317	274	408	281	0
$\text{SO}_2$	mg/L	1	<0.1	8.3	5.2	10.1	5.0	<0.1
$T_{\text{max}}$ -value ( $\times 10^6$ )	EPR s.i.	2	1.78	1.06	1.15	0.92	–	1.99
EAP-value	min	2	32	320	273	353	–	0
$\text{SO}_2$	mg/L	2	<0.1	8.3	6.4	6.1	–	1.2
$T_{\text{max}}$ -value ( $\times 10^6$ )	EPR s.i.	5	1.18	1.48	1.55	1.24	1.46	2.35
EAP-value	min	5	0	79	151	0	106	0
$\text{SO}_2$	mg/L	5	0.2	1.6	3.8	0.0	2.2	<0.1
$T_{\text{max}}$ -value ( $\times 10^6$ )	EPR s.i.	15	2.43	2.17	2.43	1.92	1.93	3.10
EAP-value	min	15	0	0	0	0	0	0
$\text{SO}_2$	mg/L	15	0.0	0.0	0.0	0.0	0.0	0.0

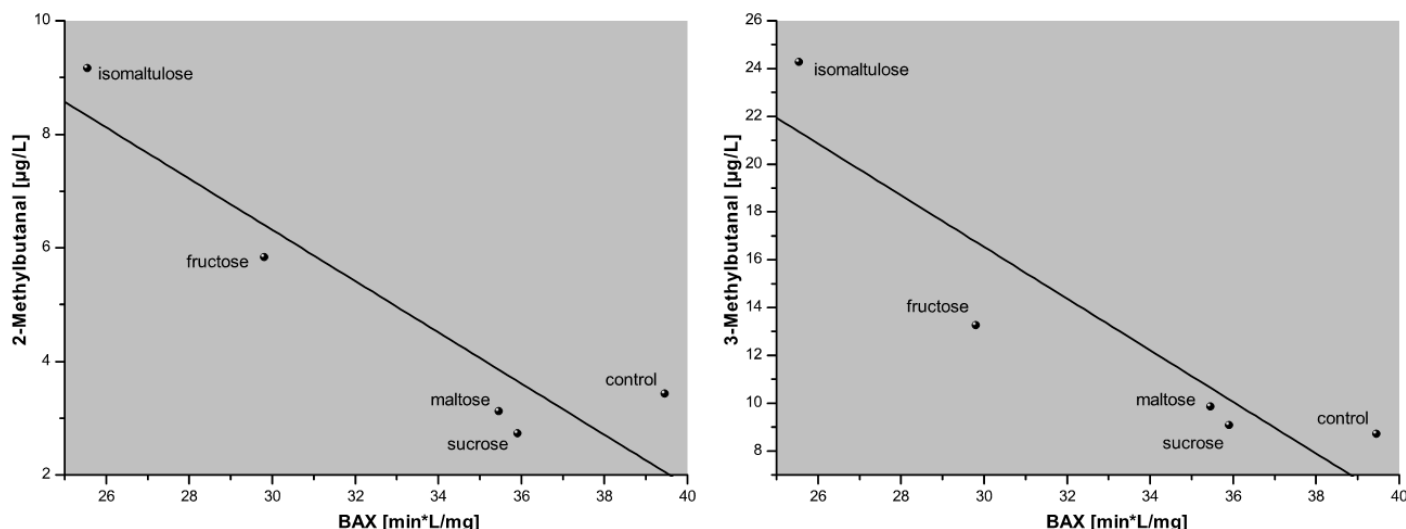


Fig. 10 A: Correlation between BAX and 2-MB amounts in aged beers (28 °C) ( $r = 0.895$ ;  $y = 19.84 - 0.45 x$ ). B: Correlation between BAX and 3-MB amounts in aged beers (28 °C) ( $r = 0.882$ ;  $y = 48.95 - 1.08 x$ )

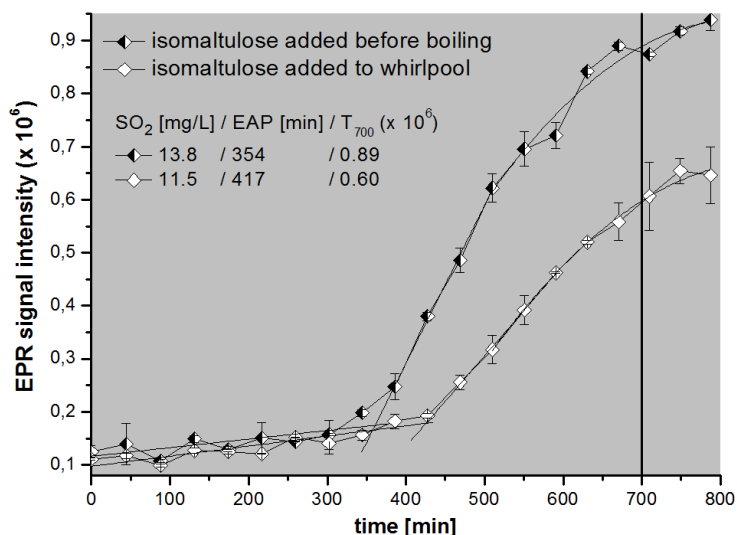


Fig. 11 EPR-analyses (EAP- and T<sub>700</sub>-value) and SO<sub>2</sub>-contents of green beers with isomaltulose added at different process steps during brewing - isomaltulose added before boiling or whirlpool rest. Error bars represent ±1 standard deviation, n = 2

The diagram in figure 11 compares the results of SO<sub>2</sub>-contents and conducted EPR-analyses (EAP-/ T<sub>700</sub>-value) of green beers firstly with an isomaltulose addition before wort boiling and secondly with an addition before whirlpool rest.

The standard analytics (not figured) of alcohol, real extract and pH showed nearly no differences between the brews. However it was noticeable that, although the produced green beer with boiled isomaltulose resulted in a slightly higher final SO<sub>2</sub>-content (13.8 to 12.9 mg/L) but a significant lower EAP-value (354 to 417 min) in comparison to the brew with later added isomaltulose to whirlpool rest. Also the prooxidative acting radical generation as indicated by the EPR signal intensities (T<sub>700</sub>-value) was significantly accelerated in the green beer with isomaltulose addition at beginning of wort boiling. The long thermal treatment of isomaltulose during boiling and additional whirlpool rest led obviously to a higher prooxidative influence on the wort and beer matrix than the short heating during whirlpool which was done to achieve a good carbohydrate solution.

#### 4 Conclusion

In summary the results suggest a carbohydrate dependent influence on oxidative processes in correlation to their reduction potential in different pH-ranges caused by the Fenton/Haber-Weiss reaction system and the activation of oxygen per electron transfer resulting in an increasing formation of reactive oxygen species. For the explanation prior published reaction mechanisms of substances with a strong reduction potential and typical reductone or enediol structures on oxidative processes with the involvement of metal ions like iron have to be in consideration as described in the introduction.

In solutions the most carbohydrates can be found in cyclic forms, but in case of mutarotation hemiacetal rings open in dependence to pH and temperature temporary and close again. Mutarotation is relatively slow at low beer pH and low temperatures but during wort boiling the reactions of the carbohydrates are accelerated, owing to higher pH-values and temperatures [19, 26].

Wort with Isomaltulose and fructose addition showed the lowest oxidative stability in all trials; the Isomaltulose α-1,6 glycosidic bond opens relatively fast [35, 39] in comparison to maltose with α-1,4 glycosidic bond. A view into the chemical structure exhibits that isomaltulose has two primary hydroxyl groups and one reactive carbonyl function; both make isomaltulose very reactive. By oxidation of hydroxyl groups produced aldehydes are also reactive and could further react to dicarboxylic acids [33]. In open-chain aldehyde structure the isomaltulose has a prooxidative effect, based on its relative strong reduction potential against iron with oxidation step 3+ which leads to an acceleration of radical generation caused by the catalytic effect on the Fenton/Haber-Weiss reaction system and the activation of oxygen per electron transfer, resulting in an acceleration of reactive oxygen species generation (ROS),

The high reduction potential of fructose occurs from a high ability to open its ring structure at lower pH in comparison to glucose, sucrose or maltose. In this connection the results of the model trials with buffer solutions (Fig. 3) and the later brewing trials confirm the work of Evans et al [17, 35], who described that glucose loses the reduction potential against fructose with pH < 7 and demonstrated

clearly the low reduction potential of the so-called “reducing sugar” glucose in beverages with low pH. The results indicate that the formation of the open-chain aldehyde structure of glucose is continuously inhibited with decreasing pH-value which makes glucose less reactive against  $\text{Fe}^{3+}$  in wort or beer in comparison to isomaltulose and fructose.

The sucrose pre-trials and brews showed no significant differences to glucose trials in all EPR-analyses; only in pitching wort the reduction potential of sucrose added wort against iron is slightly higher. An explanation could be given by the acidic hydrolysis of sucrose at high temperatures resulting in the formation of glucose and more reactive fructose [35, 39, 49, 51]. The acidic hydrolysis occurs faster at beer pH than in wort pH but sucrose is mostly fermented in beer. Maltose has a reactive aldehyde group on the glycosidic carbon atom of its second glucose molecule that seems to have an inhibited ability to open the chain structure depending on a low pH either [35, 39, 49, 51].

In correlation to the different ascertained reduction properties of the various used carbohydrate additions at wort boiling, a low or huge impact on oxidative processes, colouration and the formation of staling aldehydes could be detected in the pitching worts.

It is known, that at low pH many amino groups of amino acids are largely protonated and cannot take part in the Maillard reaction any more [32]. Consequently, the Maillard reaction can only be responsible for a defined part of the detected carbohydrate dependent colouration processes. Additionally, the results demonstrated a clear connection between the heat treatment during wort boiling and oxidative caused colouration under participation of the Fenton/Haber-Weiss reaction system. Among other oxidative reactions, which lead to a darker colour, an oxidative colouration induced by polymerization of oxidized polyphenols is easily conceivable. The colouration by polyphenol polymerization is to be emphasized because of its function as a radical scavenger resulting in radical intermediates with mesomer-stabilized radicals. These structures lead to a stronger polymerization of polyphenols caused by oxidation.

Carbohydrates are relatively stable in solutions, as long as they are not heated. 2-F as heat indicator occurs from 3-deoxy-2-pentosulose, a Maillard reaction intermediate from carbohydrate degradation during heating, by cyclization and water elimination which leads to furan structures. As thermal indicator [5, 36, 53, 57], 2-F (Tab. 1) was nearly on the same level in all brews that confirmed a comparable heat treatment during wort boiling; just the concentration in the isomaltulose wort was slightly higher. Altogether the 2-F amounts certified a comparable intensity of the Maillard reactions and heat treatments in all brews.

Generally, the Strecker aldehydes like 2-/3-MB are reduced during wort boiling by its volatility and reproduced by Strecker degradation of desoxyosones under participation of reducing carbohydrates, amino acids or different processes under participation of radical reactions e.g. generated by the Fenton/Haber-Weiss reaction system [67]. However the results demonstrates clearly that the carbohydrates with stronger reduction potential like isomaltulose and fructose yielded in a significant higher value of 2-/3-MB at the end of wort

boiling. The brews where maltose or sucrose were added lay in a comparable range as the control brew just the glucose addition led to slightly higher contents.

During the further brewing process the analysed ageing components were significant degraded during fermentation by reduction and/or bond in reversible carbonyl complexes with generated sulphur dioxide.

For the reduction of staling aldehydes during fermentation different capabilities of aldehyde reduction during fermentation have to be in consideration. Firstly, during fermentation the pH-drop affects free radicals to destroy amino acids, alcohols and volatile aldehyde precursors [60]. Secondly, aldehydes are metabolized by yeast, depending on the strain and coupled to glycolysis activity, and are used as precursors for lipid synthesis in the yeast cell. Thereby mainly pentanal, nonanal, decanal and 2-/3-MB are degraded [65]. Additionally, it is known that different carbonyl functions of aldehydes and ketones can also be bound to sulphur dioxide during fermentation and the flavour compounds are not taste-active any more. In this case there should be equilibrium between free  $\text{SO}_2$  and bound  $\text{SO}_2$ . During the consumption of  $\text{SO}_2$  while beer storage the compounds are set free and get taste active again [2, 13, 15, 31, 40, 59]. The velocity of those releases is influenced by the  $\text{SO}_2$ -content and  $\text{SO}_2$ -consumption rate during storage which, in turn, is influenced by wort boiling conditions and reactions occurring during boiling e.g. under participation of carbohydrates as demonstrated in the results of this study.

In consequence, an accelerated release of bound aldehydes in sulphite carbonyl complexes during storage would be expectable in the brew with isomaltulose addition resulting in the lowest oxidative stability and fastest consumption rate of  $\text{SO}_2$  (lowest BAX). After complete consumption of  $\text{SO}_2$  during storage and the achievement of EAP-zero-value all staling aldehydes should be released; thereby the earlier detected values from pitching wort should be approximately or minimum reached. Without consideration of new generated aldehydes and small losses based on  $\text{CO}_2$ -formation during fermentation and the volatility of aldehydes, the described correlation can be approximately observed in development of the staling aldehydes 2-/3-MB during beer storage. Accordingly, the isomaltulose brew showed the highest amount of staling aldehydes after 10 weeks at 28 °C. In all brews the amount of aldehydes in pitching wort was not already reached after storage because the  $\text{SO}_2$  was not completely consumed. Anyway, the brews with the fastest  $\text{SO}_2$ -consumption rates or lowest “BAX” showed the strongest increase in the staling aldehyde contents during storage. In consequence, the isomaltulose added beer contained, after consuming most of  $\text{SO}_2$  (12,6 -> 2,6 mg/l), nearly the same quantities of 2-/3-MB that were detected in wort after boiling. Thereby it is noticeable that up to the point of complete  $\text{SO}_2$  consumption no significant new-formation of ageing compounds was detected in case of 2-/3-MB. This hypothesis can be proven with the calculated correlation coefficients between the  $\text{SO}_2$ -consumption rates and the amounts of ageing compounds. The coefficients are 0.87 for 3-MB and 0.86 for 2-MB. Based on this it can be expected that the staling aldehyde content will be just slightly higher direct after completely consumption of  $\text{SO}_2$ . In comparison to isomaltulose, the brew with fructose addition

resulted in an also high SO<sub>2</sub>-consumption rate; there is a gap to isomaltulose based on the comparable higher SO<sub>2</sub> content directly after fermentation. With a clear distance, recognizable by significant lower consumption rates and lower development of staling aldehydes the three brews with glucose, maltose and sucrose addition follow.

Based on the described facts, it can be assumed that the ageing compounds become bound to the SO<sub>2</sub> generated during fermentation in a great extend in form of sulphite carbonyl complexes and those aldehydes are set free again during SO<sub>2</sub>-consumption while beer ageing which was explicit described by Baert et al [4] and other research groups [9, 15, 28, 45–48, 50, 55, 61–63].

This context makes the often used description of the Strecker aldehydes 2-/3-MB as oxidation indicators in literature [5, 36, 53, 57] understandable.

In principal, a decrease in oxidative wort stability caused by boiling conditions (time, temperature) or e.g. carbohydrate addition or e.g. oxygen entry during brewing and beer storage can be responsible for a faster SO<sub>2</sub>-consumption rate during storage and acceleration in release of staling aldehydes like 2-/3-MB from sulphite carbonyl complexes.

Beside the described release also different reactions like Strecker degradation or damaging reactions under participation of specific prooxidative acting radicals can be responsible for a further increase of aldehydes during beer storage as described in the introduction [67].

The verified acceleration of oxidative processes in correlation to the reduction potential of used carbohydrates against oxidized catalytically acting metal ions like Fe<sup>3+</sup> (Fig. 1) [39, 43, 44] leads to an increase in the hydroxyl and hydroxyethyl radicals generation. In consequence this kind of mechanisms will accelerate the formation of staling aldehydes under participation of prooxidative acting radical reactions during brewing, especially during high temperature while wort boiling.

Altogether the results give a clear advice that carbohydrate addition during brewing has far reaching consequences, especially in case of fermentable fructose and non-fermentable isomaltulose. Also the addition of glucose-fructose-syrup (invert sugar), which is often used in beer and beverages, has to be done carefully and at the right process step knowing the taste-damaging impact of fructose. In general the isomaltulose, fructose, glucose-fructose syrup (invert sugar) addition should not be done during heating process steps of beverages.

The experiences of this work lead to the further suggestion that an addition of non-fermentable carbohydrates, like isomaltulose, to increase the palate fullness during brewing, should be done direct prior fermentation. With the carbohydrate addition directly prior fermentation the unwanted prooxidative acting influences during wort boiling can be avoided and the positive influences on the SO<sub>2</sub>-generation during fermentation caused by the increase of osmotic pressure [47] can be used to compensate a part of the accelerated oxidative processes during beer storage.

In consideration of earlier investigations [39] and the demonstrated effects of carbohydrate addition in this study it seems that the carbohydrate profile of wort in the brewing process caused by the used mashing process should be generally characterized by a low content of maltotriose and fructose to produce a beer with better oxidative stability.

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