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Improvement of the Oxidative Wort and Beer Stability by Increased Unmalted Barley Proportion

The influence of unmalted barley on the brewing process and the quality of the resulting beer-like beverages was investigated with the main focus on the oxidative stability by using traditional beer analyses and EPR-Spectroscopy (EAP-, T450-value). Although all analytical values of the final beverages were within the normal range according to MEBAK, a slight decrease in total polyphenol and FAN content caused by an increased barley proportion in the grist was measured. In direct correlation an increase of higher molecular proteins and ß-glucan were detectable. Based on these results, it can be said that beers with a barley proportion up to 75 % will achieve comparable or higher final attenuation of the "beer" due to a combined effectiveness of malt and technical enzymes. The missing heat exposure and oxidative stress by the malting process resulted in lower values of TBI and wort respectively beer colour with increasing barley proportions in the grist. Furthermore, it was observable that an increase of barley content leads to higher oxidative stability (EAP-value) and a lower EPR signal intensity (T450-value) as an indicator for the radical generation in the wort and final beverage. In comparison to beer produced with 100 % of malt, the beers brewed with up to 50 % barley proportion were slightly preferred and up to 75 % comparable in sensory analyses. Only the brew with a barley proportion of 90 % showed a more astringent bitter taste.

Descriptors: unmalted barley, flavour stability, barley proportion, oxidative beer stability, brewing, Electron Spin Resonance Spectroscopy (EPR)

1 Introduction

The first commercial beer with 100 % barley was brewed and sold in 1963. Due to the improving knowledge about brewing with barley using technical enzymes during the recent years, it is possible to brew beverages with a barley proportion up to 100 % without technological problems in the brewery.

The use of barley instead of malt in the brewing process is interesting in an economic point of view since the material, energy and human resources for malting cause remarkable costs. However, in the cost calculation it is important to consider that unmalted barley has a considerable enzyme deficit against malt due to the lack of a germination process. Therefore, the addition of technically produced enzymes is required to compensate the enzyme deficit during the brewing process. Accordingly, the extract yields as well as the price difference between barley and malt is significant despite of the cost of enzymes. There are different calculations, which assume about 115 kg barley is required to get the same extract yield as from 100 kg malt [27]. Concerning the difference in water content between barley and malt of about 11 %, approximately 104 kg barley is needed. This means that the yield based on the dry matter is only a few percent lower than from malt.

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For beers which are not brewed in accordance to the German purity law, the use of technical enzymes expands the possibilities to affect the mashing process. In this case it is particularly advantageous that various enzymes can be selected and added at different times during the mashing brewing process. In this way, the mashing conditions can be varied and it is possible to have more influence on the wort composition [21].

However, the influences of unmalted barley on the beer quality are discussed controversially by many authors [2, 4, 6, 8, 10, 16, 19, 21, 22, 23, 29]. Previous investigations showed a decrease in colour, bitterness and free amino nitrogen (FAN) content and poor results in sensory analyses. In opposite an increase of β-glucane content could be observed [10, 16, 22].

The aim of this study was to get a deeper insight into the influences of unmalted barley on the brewing process and the quality of resulting beer-like beverages with the main focus on the oxidative wort and "beer" stability. According to this, five brews with grists containing 0, 25, 50, 75 and 90 % barley were produced in a pilot plant brewery. The mashing parameters were set based on laboratory pre-trials which showed a sufficient lautering performance. Considering the results of the preliminary tests, a specific combination of technical enzymes (α-amylase, protease, pullulanase, glucanase, xylanase) calculated to the barley proportion was used to compensate lower or missing enzyme activities in barley.

For this study traditional beer analyses according to MEBAK [17] and some new EPR methods [13, 18] were used to investigate the influence of barley on the wort and beer quality with a special focus on the oxidative stability.

2 Experimental procedures

Barley beers with barley proportions of 0, 25, 50, 75 and 90 % (spring barley, Marthe, protein content 11%) were produced in the pilot plant brewery of the TU Berlin consisting of a two-roller mill (gap 1.7 mm for malt dry milled and 1.0 mm for barley conditioned milled), Künzel, Mainleus, Germany; a 1 hL-mash kettle, a lauter tun, a 2 hL-wort / whirlpool kettle with external boiler, Steinecker, Freising, Germany.

The trials were done twice up to 75 % but only the results of the second trial including a barley proportion of 90 % are completely shown and discussed in this paper because of the extensiveness of the data. An exception is the presentation of the EPR measurements of both trials which are useful to explain the influence of barley on the oxidative stability. Due to the different used raw materials (malt, hops) and different yeast conditions the results are approximately similar and showed the same trends.

Based on laboratory scale pre-trials (mashing of pilot scale milled malt or barley in a congress mashing apparatus, Bender & Hobein, Bruchsal, Germany and simulated lautering in folded filters) the optimal mashing program was used and a specific combination of several technical enzymes depending on the barley proportion was added to the mash (ppm = g per kg barley): α-amylase 2500 ppm; pullulanase 3100 ppm; protease 1200 ppm; glucanase and xylanase 300 ppm).

Mashing program: mashing in 45 °C; rest 30 min; heating within 15 min to 60 °C; rest 30 min; heating within 4 min to 64 °C; rest 30 min; heating within 14 min to 78 °C; rest 10 min

The "kettle-full"-wort was adjusted to the same extract values before boiling and boiled under atmospheric pressure for 60 min and bottom fermented for 6-7 days at 10 °C until rest extract < 3.5 %.

2.1 Beer Analyses according to MEBAK [17]

Bitterness (2.22.1); Colour (2.13.2); Extract (2.10.3); Foam Stability (2.19); Viscosity (2.28); FAN (2.8.4.1); pH value (2.14); Total Nitrogen (2.8.1); β-glucane (2.5); Coagulable Nitrogen (2.8.2); Magnesium Sulphate precipitable Nitrogen (2.8.3.1); Alcohol (2.10.7); TBI (2.4); Total Polyphenols (2.17.1); Anthocyanogenes (2.17.2)

2.2 EPR measurements: Endogenous antioxidative potential (EAP) and determination of the T_{450}-value [12, 13, 18, 26]

The determination of the "Endogenous Antioxidative Potential" (EAP-value) [13, 18] is based on the application of a specific spin trap reagent (POBN α-(4-Pyridyl-1-oxide)-N-tert-butylnitrone) which decreases the falsifications of the so-called lag-time caused by the spin trap reagent PBN (α-pyridyl- N-tert-butylnitrone) due to a pH-effect on the radical generation [9, 13, 18, 25]. The principle of the "EAP Determination" is based on the indirect detection of the radical generation during accelerated beer aging (60 °C). For a certain time, the radical generation can be delayed or prevented by the endogenous anti-oxidative substances in beer. After consumption of antioxidants, the EPR signal increases when stable spin-trap adducts, mainly hydroxyethyl radicals, are generated. The intersection of the two linear slopes gives a relative measure of the stability and the time at the intersection is defined as the EAP value (see Fig. 1).

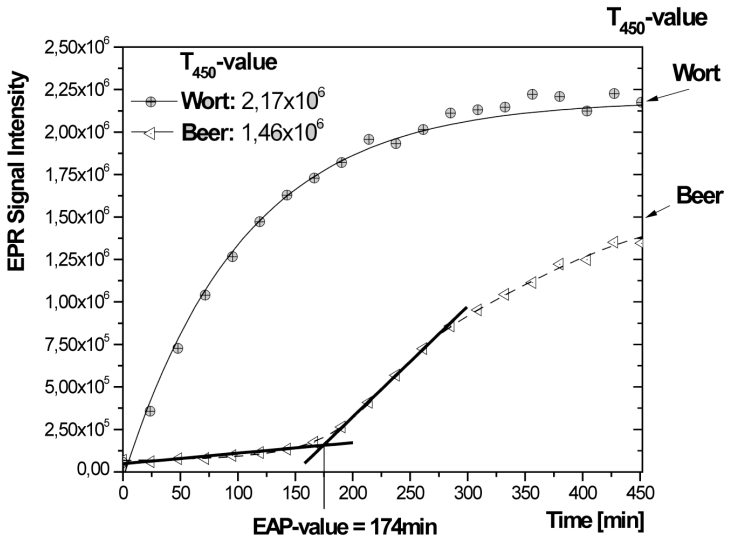


Fig. 1 Evaluation of the EPR measurement

An additional parameter of the EAP-determination for evaluation of the antioxidative behaviour of a beer or wort sample is the T-value which is defined as the EPR signal intensity measured after a certain reaction time. The T-values qualitatively indicate the content of radicals that are generated under forced aging conditions at a temperature of 60 °C depending on the pro- and antioxidative substances of the beer matrix (e.g. T_{450}-value after 450 min in Fig. 1).

Beer samples are degassed in an ultrasonic bath for 15 min (max. 20 °C). For each EPR sample 8.4 mg POBN were diluted in 50 µL of distilled water (3.5–3.6 mMol POBN). For the sample preparation 150 µL of ethanol were added to 12 mL of degassed beer and transferred into vials. 50 µL of the POBN-solution were added at the start of the measurement and the samples are directly placed in an auto sampler.

Instruments: EPR spectra were obtained with an X-band Spectrometer, ESP-300, cavity-type Bruker 4108 TMH, Nr. 8603 and e-scan, Bruker, Rheinstetten, Germany. Settings were as follows: center field 3484 G; attenuation 0 dB; sweep width 14 G; receiver gain 2.0 10^3; resolution 512; mod. amplitude 1,49 G; modulation frequency 86 kHz; conversion time 10 ms; time constant 40 ms; scans: 30 (wort) and 20 (beer).

2.3 SO_2 determination using Continuous Flow Analysis (CFA) [14].

The determination of SO_2 was carried out by CFA under an optimized procedure using a Teflon membrane. Evaporated SO_2 is released from beer at high temperatures and dialyzed by a Teflon membrane into a formaldehyde solution. P-rosaniline is added and the molecule binds with the formed sulfur dioxide-formaldehyde complex at a temperature of 45 °C forming a red coloured complex measured by spectrometry at a wave length of 560 nm.

2.4 Sensory beer analyses according to DLG [17]

The beer was rated in accordance to the testing method of the German agriculture organization (DLG – Deutsche Landwirtschafts-Gesellschaft e.V.). The taste panel consisted of at least ten expert assessors who evaluate the odour, taste, palate fullness, the freshness (rezenz) and the bitterness depending on the beer type. The marks range between 0 and 5 points for each attribute whereas a 5 is the best and 0 the worst mark. After the tasting the average of the single evaluations are averaged (MEBAK 2.10.3).

3 Results and discussion

3.1 Wort analyses

The results of the wort analyses (Table 1) show only slightly different extracts in the pitching worts ranging between 11.8–12.0 % due to an extract adjustment before wort boiling. Independent of the extract adjustment a slightly higher or approximately comparable extract yield, indicated by a higher "kettle-full" volume after lautering could be observed due to a combined effectiveness of malt enzymes and technical enzymes with a barley proportion of 25 %. The 50 %-barley wort had a slightly lower yield and the 75–90 %- barley wort showed a significant decrease due to the higher water content in barley. The barley brews also showed higher final attenuation limits with higher barley proportion up to 75 %.

In agreement with previous investigations [8, 16, 22], higher barley proportions led to a continuous increase of β-glucans without a significant influence on the wort viscosity. In comparison to the barley brews, the viscosity was higher without using barley. Considering the barley worts, also a slight increase in viscosity was observable with higher barley proportions.

The brews produced with up to 50 % barley showed comparable results in the polyphenol- and anthocyanogene content compared to the all-malt brew. With higher barley proportion the content decreased by about 10 %. Additionally, the usage of barley led to a slight increase in bitterness of the pitching wort as described in recent investigations [8, 10, 16, 22].

The influence of barley on the total nitrogen and the nitrogen fractions, calculated to 12 % extract, are graphically demonstrated in figure 2 and 3. These important parameters of the wort analyses showed a continuous decrease in total nitrogen with increasing barley proportion. The same effect could be detected in the content of free amino nitrogen (FAN) with the exception that the decrease started with 50 %-barley wort. Additionally, a change in the composition of the nitrogen fractions was observable. In negative correlation to the total nitrogen and FAN content, the higher molecular fractions, indicated by the magnesium precipitable and coagulable nitrogen showed a continuous increase with increasing barley proportion. This gives an explanation for the fact that the reduction of the total nitrogen content in comparison to the reduction of the FAN content is proportionally smaller and shows the decrease of protease activity with higher barley proportion.

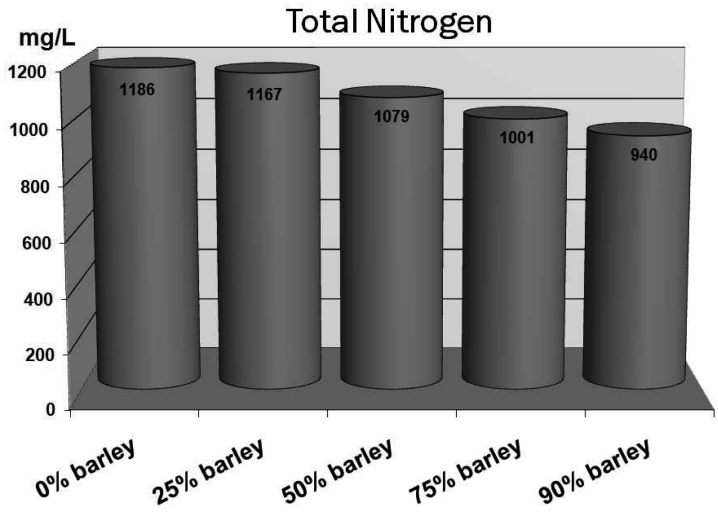


Fig. 2 Wort analyses – total nitrogen and nitrogen fractions

The results of the photometric determination of the iodine number (Fig. 4) demonstrate a continuous reduction of the iodine number with increasing barley proportion. Only the results between 75 and 90 % barley proportioned wort did not show significant differences. The results give an advice for a sufficient technical enzyme addition of α-amylase, pullulanase and reflect an unsatisfactory amylolytic activity for the all-malt brew by applying the mashing process which was optimized for the brewing with barley.

Comparing the fermentable and unfermentable extracts (Fig. 5), only a slight increase of the unfermentable extract, varying between

Table 1 Wort analyses

Wort Analyses	Unit	0 % barley	25 % barley	50 % barley	75 % barley	90 % barley
Original extract	%	11.82	12.01	12.03	11.78	11.90
Final Attenuation	%	85.9	87.6	86.6	87.9	84.3
pH-value		5.60	5.70	5.80	5.70	5.80
Viscosity	mPa·s	1.654	1.568	1.571	1.584	1.597
ß-Glucane	mg/L	162	238	269	341	362
Bitter Units	BU	39	42	43	43	44
Total Polyphenols	mg/L	171	167	169	151	151
Anthocyanogenes	mg/L	40	40	40	36	37

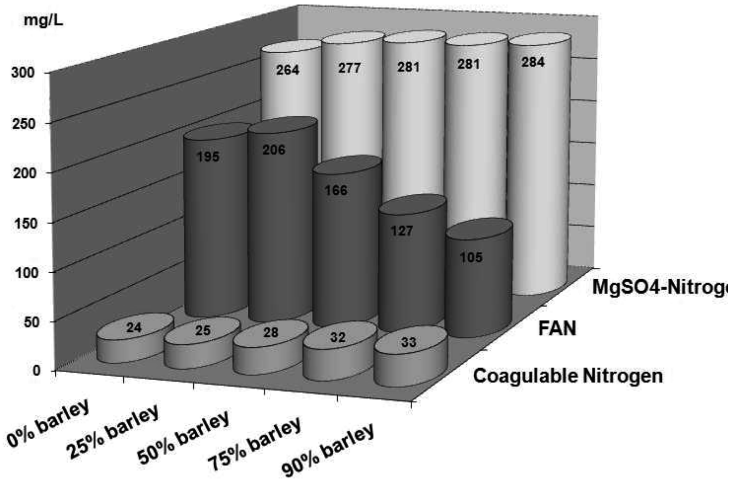


Fig. 3 Wort analyses – Nitrogen fractions

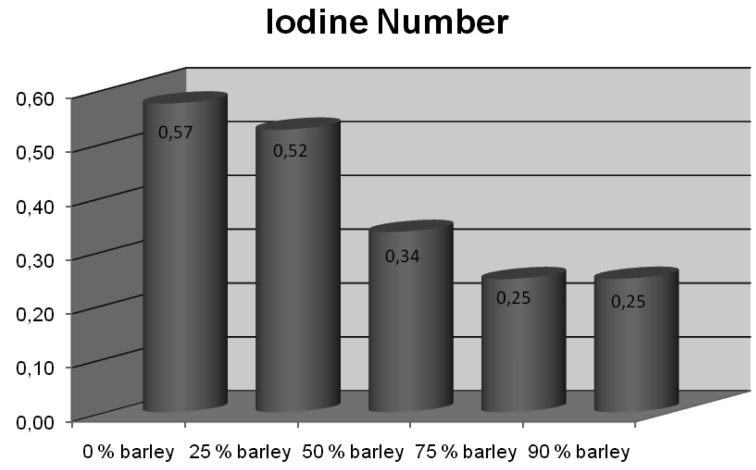


Fig. 4 Wort analyses – Iodine number

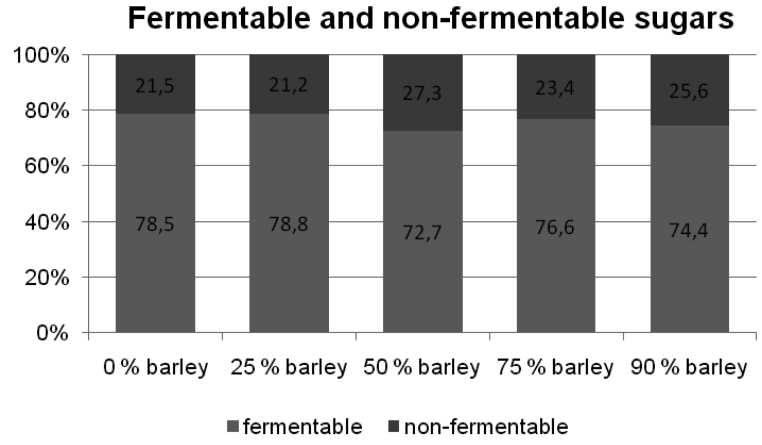
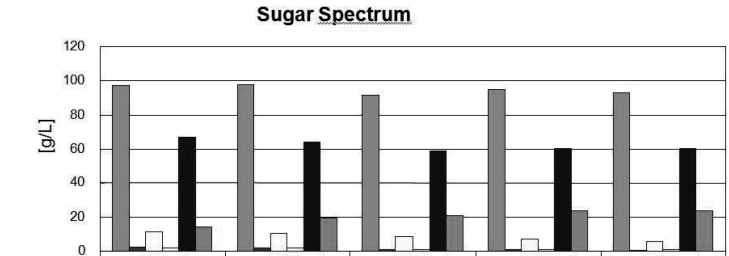


Fig. 5 Wort analyses – fermentable und unfermentable extract

21.2 and 27.3 % with higher barley proportions was observable. All the results in figure 4 and 5 demonstrate that the assigned technical enzyme addition of α-amylase and pullulanase could adjust the enzyme deficit respectively lower amylolytic activity of barley. The results also implies that the used mashing program led to approximately optimal conditions for the brews with barley but reflect an unsatisfactory amylolytic activity in the all-malt brew.

The corresponding sugar spectra of the different worts determined by HPLC are represented in figure 6.

Considering the small deviations as errors within the measuring tolerance, clear tendencies can be seen. Based on the used technical enzyme combination and concentration the barley proportion has no influence on the maltose content of the wort. The maltose content correlates with the total sugar content and shows similar deviations. On the other hand, it was observable that the concentration of the monosaccharides glucose and fructose and the proportion of the disaccharide sucrose decreased with higher barley proportions. In negative correlation, a clear increase of maltotriose was detectable. This implies that the proportion of short oligosaccharides increases with higher barley proportions but the amylolytic enzyme addition was sufficient for an improved starch degradation compared to the all-malt brew indicated by the decreasing iodine number with higher barley proportions.



	0% barley	25% barley	50% barley	75% barley	90% barley
Sugar (total)	97.03	97.83	91.55	94.42	92.63
Fructose	2.49	2.06	1.59	1.10	0.87
Glucose	11.38	10.32	8.71	7.14	5.87
Saccharose	2.21	1.92	1.49	1.47	1.56
Maltose	66.67	64.17	59.02	60.47	60.18
Maltotriose	14.28	19.36	20.75	24.24	24.15

Fig. 6 Wort analyses – sugar spectrum

Further important parameters were the thiobarbituric acid index (TBI), which should be below 45 for pale worts according to the MEBAK specifications, and the colour (norm 7–11 EBC for pale worts) which are graphically demonstrated in figure 7. The results of the colour as an indicator of Maillard reaction products in wort show a clear decrease with increasing barley proportion. Compared to the all-malt wort, a continuous brightening approximately up to 25 % was detectable. At the same time, the TBI as an indicator for thermal treatment also significantly decreased from 35 to 18 with higher barley proportions in the grist up to 90 %. This fact can be attributed to the missing heat exposure and oxidative stress which is exposed to the malt during withering and kilning in the malting process.

The same correlation could be found considering the radical generation of the wort samples in both trials. The radical generation is quantified by the T_{450}-values of the EPR measurements shown in figure 8 a, b and describes the influence of barley on the oxidative wort stability. Generally, the radical generation in the pitching wort decreases significantly with an increasing barley proportion

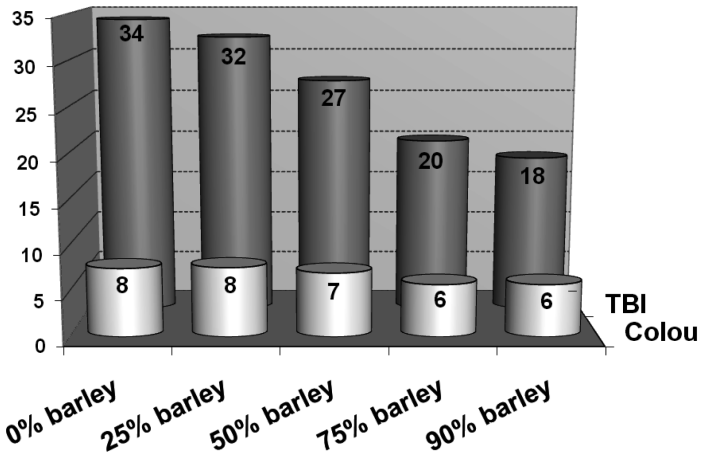


Fig. 7 Wort analyses - TBI and colour

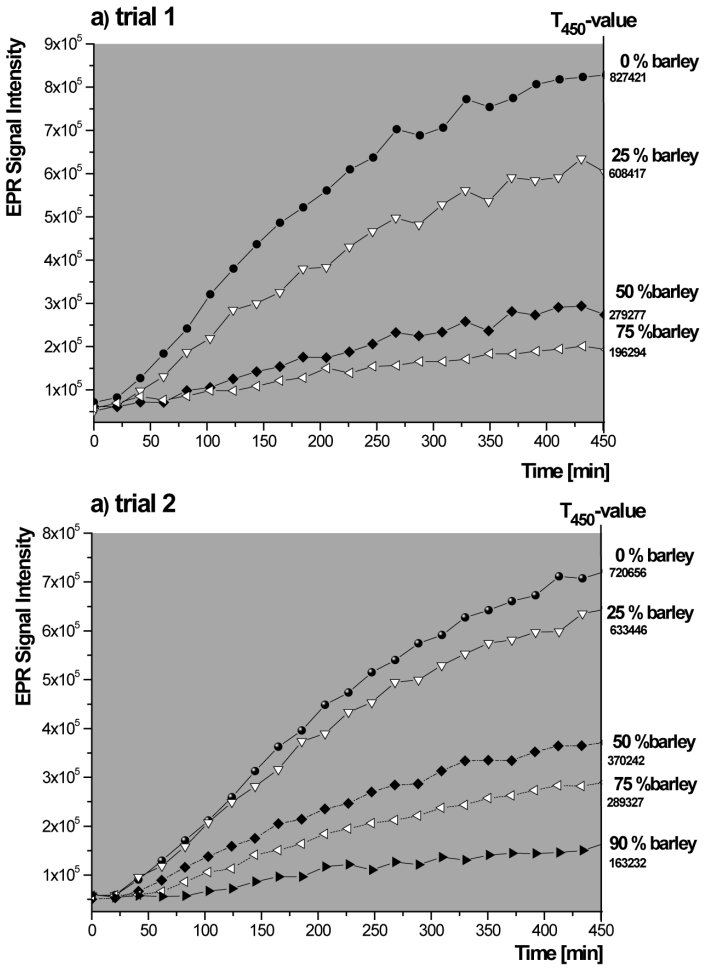


Fig. 8 EPR measurement of pitching worts a) trial 1; b) trial 2

in the grist. In this context, it can be pointed out that the advantage on the radical generation and oxidative wort stability is direct correlated to the barley proportion and the radical generation can be extremely inhibited respectively reduced by increasing barley proportion. Finally, in the first trial the 75 %-barley wort and in the second trial the 90 %-barley wort showed just approximately 25 % of the radical generation in comparison to the all-malt wort.

3.2 Beer analyses

Necessarily, the results of the beer analyses (Table 2), show similar trends like the wort analyses. The colour of the beer was reduced approximately up to 2 EBC by the replacement of malt up to 90 %. The detected slight increase in the bitterness of the wort samples could not be confirmed in the results of the beer analyses. The values of bitterness and foam stability were approximately in the same range in all beer samples.

In direct correlation to the wort analyses the brews up to 50 % barley showed comparable results in the polyphenol and anthocyanogene content and a slight decrease with higher barley proportion. The obtained results of all nitrogenous components in the beers are directly comparable with the results of the wort analysis. Generally, higher barley proportions in the grist led to lower total nitrogen and FAN contents. Negatively correlating to this, a constant increase of the high molecular nitrogen fraction indicated by coagulable nitrogen and magnesium sulphate precipitable nitrogen could be observed. The described negative correlation follows the wort

Table 2 Beer analyses

Beer Analyses	Unit	0 % barley	25 % barley	50 % barley	75 % barley	90 % barley
Extract	**%**	12.16	12.28	12.24	12.06	12.02
Attenuation	%	86.4	87.9	87.0	87.7	84.4
Alcohol	Vol. %	5.32	5.35	5.41	5.43	5.02
pH-value		4.58	4.58	4.64	4.60	4.60
Colour	**EBC**	6.3	5.8	5.4	4.6	4.3
Bitter Units	BU	21	19	20	20	19
Foam Stability (NIBEM)	sec	301	319	301	302	306
Viscosity (12 %)	mPa×s	1.495	1.432	1.364	1.397	1.406
ß-Glucane	mg/L	164	157	170	189	136
Total Nitrogen	mg/L	894	870	828	736	687
TotalPolyphenols	**mg/L**	154	157	147	139	132
Anthocyanogenes	mg/L	32	29	33	30	29
FAN	mg/L	141	129	112	73	54

analyses and can be explained by decreasing protease activities with increasing barley proportion.

Contrary to the results of the wort analyses, the increasing barley proportion led to slight differences in the content of the β-glucans without a direct correlation to the beer viscosity and showed the influence of the beer filtration.

Furthermore, the EAP- and T_{450}-value determination using the EPR spectroscopy, as shown in figure 9 a, b, was applied in both trials to get more information about the influence of barley on the endogenous antioxidative potential and antiradical behaviour of the beer matrix. The results of the EAP-determinations pointed out a direct correlation between the barley proportion and the radical generation after the consumption of the EAP-value. A higher radical generation normally describes a lower oxidative stability of the beer matrix but just in the first trial it was possible to detect a direct correlation between the barley proportion and the EAP-value (coefficient of determination 0.98). This can be explained by the fact that the endogenous antioxidative potential of the beer is strongly dependent on the sulphur dioxide content as one of the most important antioxidant in [1, 11, 12, 13, 20]. The SO_2 in beer is formed as a secondary metabolite of the yeast during fermentation. The formation of the SO_2 in turn is strongly dependent on the used yeast strain, wort aeration and fermentation conditions. Therefore also the FAN content influences the EAP-value. The different conditions in the second trial resulted in too strong varying SO_2-contents thus the EAP-values could not optimally be used to evaluate the oxidative stability but the T_{450}-values were useful to get information about the consumption of the existing antioxidative potential during beer aging and the influence of the beer matrix on the oxidative stability. The decreasing T_{450}-values with increasing barley proportion as found in the worts of both trials were transferred to the final beers. Finally, in the both trials the 75 %- and 90 %-barley beers showed approximately 50–60 % of the radical generation (T_{450}-values) in comparison to the all-malt beer.

All EAP-values were within the range of the MEBAK-specifications (2.14.3) for average oxidative beer stability (60–200 min). The SO_2-content of the beers in the first trial were approximately comparable and thus the highest EAP-value could be detected in the 75 %-barley brew with 157 min followed by the 50 %-, 25 %-barley- and all-malt brew with 138, 108 and 91 min.

The results of the sensory analyses in table 3 show that the brews with barley proportions up to 50 % were comparable or better in

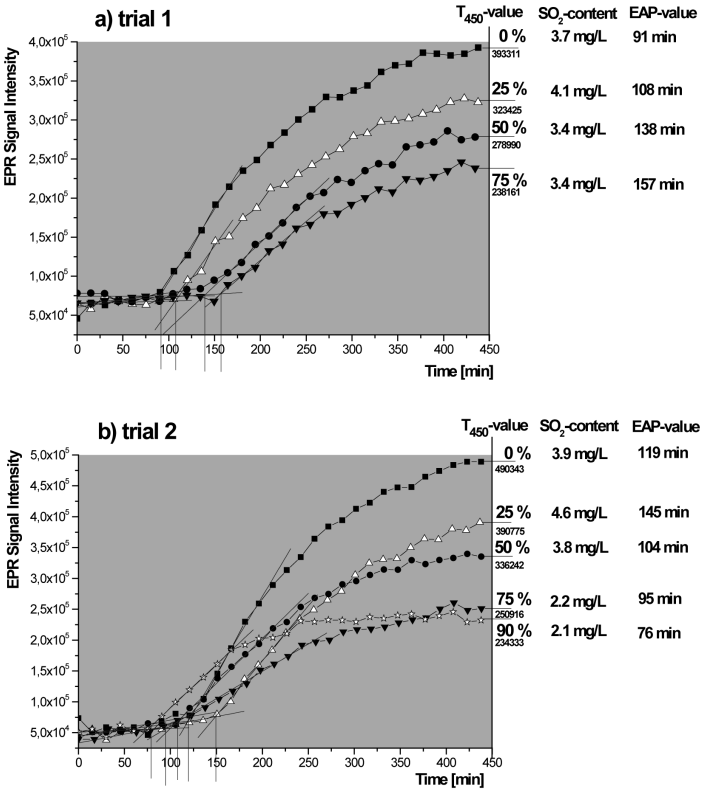


Fig. 9 EPR measurement of final beers a) trial 1; b) trial 2

terms of the quality parameters aroma and taste than the control beer (100 % malt). The brews with a barley proportion of 75 % or more were rated lower whereas the brew with a barley proportion of 90 % scored with the lowest mark in odour and taste. In the assessment criteria, palate fullness is unbelievable similar to "malt beer", fizziness and quality of bitterness, all beers were rated similarly.

4 General discussion

The brewing with a conditioned dry milling and a barley proportion up to 90 % using the described technical enzymes combination was done without any technological problems in the brewery. The results of the beer analyses follow in most cases the results of the wort analyses but the slightly improved bitterness yield in wort could not be confirmed in the final beers. Previous investigation [16, 22] reported on higher bitterness and foam stability in the finished beer with increasing barley proportion in the grist but

Table 3 Sensory analyses

Sensory features	0 % barley	25 % barley	50 % barley	75 % barley	90 % barley
Odour	3.8	4.1	4.1	3.6	3.6
Taste	3.6	3.9	3.9	3.4	3.3
Palate fullness	4.1	4.4	4.1	4.1	4.0
Freshness	4.3	4.4	4.3	4.4	4.3
Bitterness	3.8	3.9	3.8	4.0	3.9

under the used conditions all beers are on an almost identical level of bitterness and foam stability. The detected slightly increasing bitter values of the barley wort are probably eliminated during the fermentation and filtration process. The same effect could be observed in the analysis of the β-glucans. The increasing barley proportion in wort led to a continuous increase in the β-glucan content without a significant influence on the viscosity. Perhaps, this can be explained by the analysis of β-glucans which gives no information about the molecular size and long-chained β-glucans have a higher influence on the viscosity. On the other hand, the increasing β-glucan content of the barley wort was eliminated during the fermentation and filtration process. It seems that the higher molecular respectively long-chained β-glucans were removed from the barley beer by the used membrane filtration system (sterile filtration 0.45 µm).

The described contrarious correlation between the free amino nitrogen (FAN) and higher molecular protein fractions with a barley proportion of more than 25 % is in agreement with previous investigations [16, 22] and can be explained by the low protease activities in barley. It seems that further improvement can be achieved by optimizing the composition and adequate concentration of technical proteolytic enzyme addition to approximate the soluble nitrogen and FAN content to the all-malt brew to get better fermentation conditions. On the other hand, a lower FAN content has advantageous for the flavour stability due to a lower amount of precursors for the generation of Strecker-aldehydes during beer aging.

The results of the wort and beer colour as an indicator for the content of Maillard reaction products showed a reduction of the colour approximately up to 2 EBC with 90 % barley proportion. This fact can be attributed to the missing heat exposure of barley by the malting process and the higher content of colouring Maillard reaction products in malt caused by the withering and kilning process.

The missing heat exposure is also reflected with the results of the TBI content in wort. The TBI as an indicator for the heat treatment was significant reduced from 35 to 18 by increasing barley proportion up to 90 %.

In this study it was possible to show a direct correlation between the barley proportion and the detected EAP-value in the first trial. In the second the EAP-values were not in direct correlation to the barley proportion. This can be explained by the strong correlation between the EAP-value and the SO_2-content as one of the most important antioxidants in beer [1, 11, 12, 13, 20] and the varying SO_2-content in the second trial. Independent of the SO_2-content, the radical generation (T_{450}-value) is an indicator for the velocity of oxidative processes and thus for the consumption rate of the SO_2 during storage. In direct correlation, it could be shown that an increased barley proportion leads to a lower radical generation in the wort- or beer matrix resulting in a higher oxidative stability. The simultaneous increase of radical generation and the wort colour shows a direct correlation to previous investigations of *Cortès et al.* [3] and *Furukawa et al.* [7]. These studies [3, 7] demonstrate that the addition of colour malt generally leads to a decrease of the oxidative wort and beer stability. The authors showed a significant correlation between the radical generation in the wort and the content of Maillard reaction products. Recent investigations [15] have shown that specific Maillard intermediate products with endiol structure like reductones are able to accelerate the radical generation and oxidative processes in the wort or beverage by their strong reducing potential against specific metallic ions resulting in an acceleration of the prooxidativ acting Fenton/ Haber-Weiss reaction system.

Based on the results of this study, it can be concluded that in direct opposite to the influence of colour malt the usage of unmalted barley leads to a lower content of these specific intermediate Maillard reaction products in wort and the final beverage. Consequently, a lower radical generation and higher oxidative stability can be detected with increasing barley proportion.

In comparison to the all-malt beer the sensory evaluation showed up to 50 % barley proportion a slightly preferred or comparable odour or taste and a lower sensory rating of the 75–90 %-barley brews. In the assessment criteria, palate fullness, fizziness (rezenz) and quality of bitterness, all beers were rated with very close marks. Only the brew with a barley proportion of 90 % showed a more astringent bitter taste.

In summary, the results of this investigation illustrate that a barley proportion up to 50 % (max. 75 % by consideration of the water content) in the grist can be used to inhibit the radical generation during the brewing process and to improve the oxidative wort and beer stability without a negative influence on the beer quality or sensory ratings. It seems that a further improvement can be achieved by optimizing the concentration of technical enzyme addition. In consideration of all results it can be said that the production of barley beers up to 90% is not really a technical problem today due to the opportunities of the available technical enzymes on the market.

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5 References

1. Andersen, M. L., Outtrup; H. and Skibsted, L.H. (2000): Potential antioxidants in beer assessed by ESR spin trapping, J. Agric. Food Chem. **48**, pp. 3106-3111.
2. Bley, W.; Hoffmann, M. and Löther H. (1970): Enzyme treatment of raw barley in brewing: Comparative tests, Int. Brew. J. **106** (1261), pp. 67-72.
3. Cortés, N.; Kunz, T.; Furukawa Suárez, A.; Hughes, P. and Methner, F-J. (2010): Development and correlation between the organic radical concentration in different malt types and the oxidative beer stability, J. Am. Soc. Brew. Chem. **68** (2), pp. 107-113.
4. Crisp J. W. M. and East E. (1971): The use of added enzymes in the production of wort, Elsevier Scientific Co., Amsterdam, Netherlands, Proc. Congr. Eur. Brew. Conv. Estoril **13**, pp. 185-190.

5. De Schutter D.P.; Saison D.; Delvaux F.; Derderlinckxs G. and Delvaux F.R. (2008): The chemistry of beer aging, Beer in health and disease prevention, pp. 375-388, ed. Victor R. Preedy, Elsevier, London, UK.
6. Enari, T.-M.; Mikola, J. and Linko, M. (1964): Restriction of proteolysis in mashing by using a mixture of barley and malt, J. Inst. Brew. 70, pp. 405-410.
7. Furukawa Suárez, A.; Kunz, T.; Cortés, N.; MacKinlay, J.; Hughes P. and Methner, F.-J. (2011): Impact of color adjustment on flavor stability of pale lager beers with a range of distinct coloring agents, Food Chemistry **125** (3), pp. 850-859.
8. Goode, D.L.; Wijngaard, H.H. and Arendt, E.K. (2005): Mashing with unmalted barley – Impact of malted barley and commercial enzyme (*Bacillus* spp.) additions, Tech. Q. Master Brew. Assoc. Am. **42** (3), pp. 184-198.
9. Kaneda, H.; Kano, Y.; Osawa, T.; Kawasakishi, S. and Kamada, K. (1989): The role of free radicals in beer oxidation, J. Am. Soc. Brew. Chem. **47**, pp. 49-53.
10. Klopper, W. J. (1969): Gerste als Rohstoff zur Bierbereitung, BRAUWELT **40**, p. 753.
11. Kotake, Y. and Janzen, E. (1991): Decay and fate of the hydroxyl radical addukt of α-phenyl-N-tert-butylnitrone in aqueous media, J. Am. Chem. Soc. **113**, pp. 9503-9506.
12. Kunz, T.; Stephan, A.; Methner, F.-J.; Kappl, R. and Hüttermann, J. (2002). Grundlegendes zur Elektronenspinresonanz-Spektroskopie (EPR) und Untersuchungen zum Zusammenhang zwischen oxidativer Bierstabilität und dem SO_2-Gehalt., Monatsschrift für Brauwissenschaft **55** (7/8), pp. 140-153.
13. Kunz, T.; Methner, F.-J.; Kappl, R. and Hüttermann, J. (2005): Method for determining the endogenous antioxidative potential of beverages by means of EPR spectroscopy. Patentanmeldung U30121, TU-Berlin/ Universität des Saarlandes, IPAL. DE 10 2005 043 113 A1, Patent US 20080248580.
14. Kunz, T.; Schiwek, V.; Harms, D. and Methner, F-J. (2009): Optimized analysis methods for the determination of SO_2 in beer and malt, BRAUWELT International **27**, pp. 216-220.
15. Kunz, T.; Strähmel, A.; Cortés, N.; Hense, W.; Kroh, L.W. and Methner, F.-J. (2010): Pro- and antioxidative effects of the Maillard reaction products in malt on the oxidative beer stability, 73rd ASBC Annual Meeting, Rhode Island, Providence, USA.
16. Kunz, T.; Strähmel, A.; Cortés, N.; Hense, W.; Kroh, L.W. and Methner, F.-J. (2011): Influence of intermediate Maillard reaction products with endiol structure on the oxidative stability of beverages, J. Am. Soc. Brew. Chem. Food Chemistry **(in review).**
17. Macey, A.; Stowell, K.C. and White, H. B. (1967): Experimental brewing procedures using unmalted cereals and enzymes, Proc. Congr. Eur. Brew. Conv., Madrid 11, Elsevier Scientific Co., Amsterdam, Netherlands.
18. MEBAK. Brautechnische Analysenmethoden, Band II. 1993, 3rd ed. Freising-Weihenstephan: MEBAK (Methodensammlung der Miteleuropäischen Brautechnischen Analysekommision).
19. Methner, F.-J.; Kunz, T. and Schön, T. (2007): Application of optimized methods to determine the endogenous antioxidant potential of beer and other beverages, Proc. Congr. Eur. Brew. Conv., Venice 31: Fachverlag Hans Carl, Nürnberg, Germany.
20. Mikola, J. and Enari, T.-M. (1970): Changes in the contents of barley proteolytic inhibitors during malting and mashing., J. Inst. Brew. **76**, pp. 182-188.
21. Moll, M. (2001): Determination of antioxidants in brewing, Monatsschrift für Brauwissenschaft **54**, pp. 64-69.
22. Nielsen E. B. (1971): Brewing with barley and enzymes – A review, Proc. Congr. Eur. Brew. Conv., Estoril 13: pp. 149-170. Elsevier Scientific Co., Amsterdam, Netherlands.
23. Pfenninger, H. B.; Schur, F. and Wieg, A. J. (1971): Zur Technologie der Rohfruchtverarbeitung mit industriellen Enzympräparaten. Proc. Congr. Eur. Brew. Conv., Estoril 13: pp. 171-184. Elsevier Scientific Co., Amsterdam, Netherlands.
24. Schoenenberg, S. and Kreisz, S. (2010): The use of 100 percent unmalted barley, BRAUWELT International **28** (1), pp. 30-32.
25. Ting, P.L.; Lusk, L.; Refling, J.; Kay S. and Ryder, D. (2008): Identification of antiradical hop compounds. J. Am. Soc. Brew. Chem., **66** (2), pp. 116-121.
26. Uchida, M. and Ono, M. (1996): Improvement for oxidative flavor stability of beer-role of OH-radical in beer oxidation, J. Am. Soc. Brew. Chem., **54**, pp. 198-204.
27. Uchida, M.; Suga, S. and Ono, M. (1996): Improvement for oxidative flavor stability of beer – Rapid prediction method for beer flavor stability by electron spin resonance spectroscopy, J. Am. Soc. Brew. Chem., **54** (4), pp. 205-211.
28. Wieg, A. J.; Holló J. and Varga, P. (1969): Brewing beer with enzymes, Proc. Biochem. **4**, pp. 33.
29. Wieg, A. J. (1973): Brewing adjuncts and industrial enzymes, Tech. Q. Master Brew. Assoc. Am. **10** (2), pp. 79-86.
30. Wieg, A. J. (1987): Barley brewing, Brewing Science **3**, pp. 533-571.

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