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The influence of wort aeration and yeast preoxygenation on beer staling processes

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Abstract

The influence of oxygen on the beer staling process is considered to be of major importance. Therefore, the impact of wort aeration, which is thought to cause wort oxidation processes, on beer ageing, has been examined. Pilsner and ale beers were produced with the classical wort aeration technique or by the use of the yeast preoxygenation process, in which yeast cells are exposed to oxygen before fermentation. The staling of these beers was studied using P&T GC–MS and sensory analysis. GC–MS analyses of the natural and forced aged beers showed no significant differences between the two treatments. Sensory evaluation of natural and forced beers confirmed these results. Thus, normal wort aeration (8 mg/l) does not appear to determine flavour stability in a direct manner. This is probably due to the short contact of the wort with oxygen at low temperatures before the onset of fermentation.

Keywords: Wort aeration; Yeast preoxygenation; Beer ageing; Oxygen; Flavour stability

1. Introduction

Oxidation processes, due to oxygen uptake during beer production, are considered to be a major cause of stale flavour development in beer. It is often reported that absorption of oxygen in the mash, during filtration, during boiling, in wort and beer, leads to oxidation, which can damage the flavour. The general belief is that wort aeration has a negative impact on wort quality, which results in more rapid beer staling, though literature describing aeration related wort oxidation processes is very scarce. Narziss (1986) and Narziss et al. (1999a) found that mashing in with low air content and a controlled aeration at pitching was favourable for flavour stability, as oxygen diminishes the amount of reducing substances during wort production. Also, Wilson (1978) reported that wort oxygenation could cause oxidation of wort constituents, resulting in unpleasant colour and flavour changes. Minimal oxygen pick-up during filtration and bottling, on the other hand, can diminish all efforts made during the earlier stages of brewing to avoid oxygen inlet (Narziss, Back, Miedaner, & Lustig, 1999b). Minimizing the absorption of oxygen and the formation and activity of reactive oxygen species (ROS) in wort and beer would thus be a first goal for improving beer flavour stability.

Unfortunately, in the classical beer production process, before fermentation, a wort aeration step is still necessary to ensure sufficient yeast growth and a good fermentation performance. Because yeast cells contain insufficient sterols and unsaturated fatty acids (UFA) at the beginning of fermentation, yeast growth only starts when appropriate levels of these essential membrane compounds are

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synthesized (David & Kirsop, 1973). Oxygen is essential for the biosynthesis of UFA and ergosterol (O'Connor-Cox, Lodolo, & Axcell, 1993) and therefore, wort is inevitably exposed to relatively high oxygen concentrations (8 mg/l) at the start of fermentation.

Beer staling processes comprise oxidative as well as non-oxidative reactions. Ageing processes, unaffected by the oxygen concentration, are involved in the formation of certain ethyl esters, heterocyclic compounds and carbonyl components and the degradation of esters produced by yeast during fermentation. For example, many heterocyclic components in aged beers are the result of oxygen-independent Maillard reactions. Oxygen-dependent processes, on the other hand, mainly involve reactions with ROS. Bamforth and Parsons (1985) established the importance of reactive oxygen species in beer staling. These species (O_2^-, $HOO^{\bullet}$, H_2O_2 and $HO^{\bullet}$) react, among other things, with polyphenols, *iso*-α-acids and alcohols in beer. The reaction of ROS with alcohols (ethanol and higher alcohols) gives rise to the formation of aldehydes. Furthermore, amino acids can also be degraded to aldehydes through Strecker degradation reactions, which are enhanced when oxygen is present. In addition, *iso*-α-acids degrade in the presence of ROS (Kaneda, Kano, Osawa, Kawakishi, & Kamada, 1989), but the *trans*-isomer seems to be more affected by this degradation than does the *cis*-isomer (De Cooman, Aerts, Overmeire, & De Keukeleire, 2000). This oxidation process gives rise to several organic acids in the beer. Furthermore, isohumulones can also be subject to oxidative-type degradation in the absence of oxygen (Huvaere et al., 2003). Finally, oxidation of fatty acids was also reported to occur (Dale & Pollock, 1977), but on the other hand, Lermusieau, Noel, Liegeois, and Collin (1999) found that the release of *trans*-2-nonenal, a major fatty acid oxidation product, in beer, was not influenced by oxygen concentration in the bottled beer.

A new technique, the preoxygenation process, offers the possibility to avoid the aeration (and oxidation) of wort, by exposing the yeast cells to oxygen in a controlled process before fermentation. It was previously shown (Depraetere, Winderickx, & Delvaux, 2003) that the yeast fermentation performance and physiological condition, which might influence flavour stability (Guido, Rodrigues, Rodrigues, Gonçalves, & Barros, 2004), is not altered by yeast preoxygenation. This technique thus provides the opportunity to examine the precise influence of wort aeration on beer ageing, without altering the fermentation and beer production characteristics.

In this study, the effect of wort aeration on beer ageing is evaluated according to a staling test, in which ten indicators, which are representative of the main staling processes in beer, are analyzed (Table 1). Pilsner beers as well as ale beers, produced with the classical aeration and the preoxygenation technique, were the subject of study and their characteristics, before and after natural or forced ageing, were determined.

2. Materials and methods

2.1. Yeast strains

The experiments were carried out with an industrial bottom (CMBS SD01) and a top fermenting (CMBS SD11) yeast strain (*Saccharomyces cerevisiae*).

2.2. Preoxygenation

Preoxygenation was performed at 20 °C in a membrane loop reactor according to Depraetere et al. (2003). Yeast slurries (3 l) (75–80% moisture) were circulated at 750 ml/min through the system. Oxygen was delivered to the slurry during 5 h via a membrane sparger to obtain an oxygen concentration of 8 mg/l in the slurry. Maltose (3%) was added to the yeast slurry prior to preoxygenation.

2.3. Brewing

Wort was prepared in a 5 hl pilot brewery. The wort was fermented in two separate vessels (2.5 hl), using two different conditions of aeration. The control wort was aerated to an oxygen level of 8 mg/l and pitched with untreated yeast cells. For the preoxygenation (Preox) beer, de-aerated wort, obtained by nitrogen sparging, was pitched with preoxygenated yeast cells.

Four pilsner brews (12 °P) and two ale brews (18 °P) were produced, resulting in eight pilsner beers and four ale beers.

2.4. Pilot-scale production of pilsner beers

Wort (12.5 °P) was prepared with 80% Esterel barley malt (Dingemans, Stabroek, Belgium) and 20% maize flakes (Brouwland, Beverlo, Belgium). The pH of the mash was adjusted with lactic acid to 5.5 and the following specific temperature–time profile was used: 55 °C (5 min), 63 °C (45 min), 72 °C (20 min) and increase to 78 °C. After separation of the wort and the spent grains in a traditional lauter tun, the wort was adjusted to pH 5.2 and boiled for 90 min in the copper. Hop pellets (Styrian Golding (9.9% α-acids), Saaz (1.8% α-acids)) were added to obtain a final beer bitterness of 22 EBU. The resulting wort was transferred to the whirlpool for clarification (20 min). The wort was further cooled to 12 °C and pitched with *S. cerevisiae* (CMBS SD01, 15×10^6 cells/ml) and fermented at 12 °C. At the end of fermentation, yeast cells were cropped and reused (with or without preoxygenation) in a subsequent fermentation process. This procedure was thrice repeated, resulting in four brews.

2.5. Pilot-scale production of ale beers

Wort (14.5 °P) was prepared with 100% Esterel barley malt (Dingemans, Stabroek, Belgium). The pH of the mash was adjusted with lactic acid to 5.5 and the following

Table 1
Indicators used in the staling tests

Staling process	Indicator	Flavour threshold (μg/l)	Reference
Maillard reaction	2-Furfural	150,000	Moll (1994)
	5-Methyl-2-furfural	17,000	Moll (1994)
Furanic ether formation	2-Furfuryl ethyl ether	6	Vanderhaegen et al. (2003)
Oxidation of alcohols, Strecker degradation	3-Methyl-butanal	600	Moll (1994)
	Benzaldehyde	2000	Moll (1994)
Release of aldehydes produced in lipid oxidation	Hexanal	350	Moll (1994)
Degradation of hop bitter acids	4-Methyl-pentan-2-one	60,000	Moll (1994)
	Ethyl-3-methyl-butyrate	18–20	Williams and Wagner (1979)
Ethyl ester formation	Ethyl hexanoate	210	Moll (1994)
	Ethyl-3-methyl-butyrate	18–20	Williams and Wagner (1979)
Degradation of esters produced by yeast during fermentation	Isoamyl acetate	1200	Moll (1994)

specific temperature–time profile was used: 63 °C (30 min), 72 °C (15 min) and increase to 78 °C. After separation of the wort and the spent grains in a traditional lauter tun, the wort was adjusted to pH 5.2 and boiled for 90 min in the copper. Hop pellets (10.5% α-acids, Northern Brewer) were added at the start of the boiling process to obtain a final beer bitterness of 30 EBU. At the end of boiling, sucrose was added to obtain a final wort density of 18 °P. The resulting wort was transferred to the whirlpool for clarification (20 min). The wort was further cooled to 22 °C and pitched with *S. cerevisiae* (CMBS SD11, 5×10^6 cells/ml) and fermented at 22 °C. At the end of fermentation, yeast cells were cropped and reused (with or without preoxygenation) in a subsequent fermentation process.

2.6. Beer ageing conditions

The pilsner and ale beers were bottled (250 ml) with total oxygen levels below 0.2 mg/l. The headspace contained less than 0.5 ml air/bottle. The beers were subjected to different ageing conditions in the dark at 20 °C (natural ageing) and 40 °C (forced ageing).

For the pilsner beers, samples were taken after 0 and 7 days of ageing at 40 °C. After 0, 6 and 12 months of storage at 20 °C, samples for each storage condition were also analyzed in duplicate. For the ale beers, samples were taken after 0 and 28 days of ageing at 40 °C. After 0, 6 and 12 months of storage at 20 °C, samples for each storage condition were also analyzed in duplicate.

2.7. Chemicals

The following substances, with corresponding purities were supplied by Sigma Aldrich Chemie GmbH (Munich, Germany): isoamyl acetate (99.7%), ethyl hexanoate (99+%), ethyl 3-methyl-butyrate (99.7%), *n*-hexanal (98%), 3-methyl-butanal (98%), 4-methyl-pentan-2-one (99%), benzaldehyde (99.5%), 5-methyl-2-furfural (99%) and 2-furfural (99%). 2-Furfuryl ethyl ether, with a purity of 95%, was purchased from Narchem Corporation (Chicago, IL, USA).

2.8. Analysis of volatile compounds with P&T GC–MS

Volatile compounds were analyzed and quantified, in duplicate, according to Vanderhaegen, Delvaux, Daenen, Verachtert, and Delvaux (2006).

Mean values of the different compounds for the different beers are depicted. Statistical analysis for the pilsner beers (4 beers) was performed based on a Student's *t*-distribution. Confidence intervals around the mean values are created with a 95% confidence level ($\alpha = 0.05$).

2.9. Sensory analysis of beer

2.9.1. Centres

Trained panels of the Centre for Malting and Brewing Science and of Danbrew (Denmark) performed sensory analysis.

2.9.1.1. Centre for Malting and Brewing Science. Sensory tests on aged beers were carried out using a trained panel of 10 members. Four beers were randomly presented in one session to the panellists. Results were analyzed according to the European Brewery Convention (1998).

Fresh aroma and taste were evaluated for seven aspects, while the naturally aged flavour profile was evaluated for eleven aspects, by giving a score from 0 to 8. The selected fresh aspects were sweet, bitter, after-bitter, mouthfullness, fruity, hops and sulfurs (pilsner beers) or alcoholic flavours (ale beers). The aged beer flavour aspects were sweet, bitter, after-bitter, pungent, fruity, solvent, papery/cardboard, port/Madeira, ribes (red fruit), caramel and sulfurs. A score of 0 meant that the particular flavour aspect was not present, whereas a score of 8 meant that the particular flavour aspect was extremely strong.

The overall staling of the beers was assessed for the forced aged beers, by giving a score from 0 to 5. A score of 0 meant that the staling flavour was not present, whereas a score of 5 meant that the staling aspect was extremely strong.

2.9.1.2. Danbrew. Sensory tests on aged beers were carried out using a trained panel of 7 members. Beers were randomly presented to the panellists.

The overall staling of the beers was assessed for the forced aged pilsner beers, by giving a score from 0 to 5. A score of 0 meant that the staling flavour was absent, whereas a score of 5 meant that the staling aspect was very strong.

3. Results and discussion

3.1. Staling

The staling of pilsner and ale beers, produced with the classical wort aeration and the yeast preoxygenation technique, was studied. The natural and forced ageing characteristics of the different beers were examined according to a staling test, based on the analysis of ten indicators, which are representative of the main staling processes in beer (Table 1).

3.2. Influence of wort aeration on beer ageing in pilsner beers

3.2.1. Evolution of beer staling indicators

Analyses of four different pilsner beers, made with a yeast preoxygenation step or with a wort aeration step, were performed. Therefore, the beers were aged during 1 week at 40 °C (forced ageing) and during 1 year at 20 °C. The mean concentrations of the different staling indicators are shown in Table 2.

No significant differences between the fresh beers, made with or without wort aeration, could be observed, based on statistical analysis. After the forced ageing treatment, differences in the concentrations of the different indicators could be detected, in comparison with the fresh beers. This resulted in an average twofold concentration increase of the different staling indicators, except for the isoamyl acetate concentration that decreased during staling, because the beers were not pasteurized and esterases were thus still active. Moreover, between the preoxygenated and the wort-aerated beers, the concentrations of the compounds involved in Maillard reactions, lipid oxidation, degradation of hop bitter acids, oxidation of alcohols, Strecker degradation and ester formation/degradation did not vary considerably. This means that the concentrations of 3-methyl-butanal, benzaldehyde, hexanal, 4-methyl-pentan-2-one and ethyl 3-methyl-butyrate, which are all oxygen-dependent staling compounds (Kaneda et al., 1989; Narziss, Miedaner, & Lustig, 1999a; Vanderhaegen et al., 2003), did not differ much between the diverse beers. Only the concentration of furfuryl ethyl ether (FEE) was always slightly but significantly lower (with a confidence level of 95%) in the force-aged preoxygenated beers.

To confirm the results obtained by forced ageing, the GC–MS profile of naturally aged pilsner beers was also examined (Table 2). The mean staling increased three and sevenfold after 6 and 12 months of storage at 20 °C, respectively. Additionally, no straightforward variations between the control and the preoxygenated beers could be observed thereafter, resulting in similar ageing profiles. In particular, the concentrations of 3-methyl-butanal and benzaldehyde, two well-known oxygen-induced staling indicators (Narziss et al., 1999a), did not vary significantly between the preoxygenated and the control beers. In contrast to the force-aged beers, no differences in 2-furfuryl ethyl ether concentrations could be observed in the naturally aged beers. According to Vanderhaegen et al. (2004), this component is a good marker of the thermal load to which the beer is subjected and is thus not dependent on oxygen. For these beers, the "thermal load" is identical as the beers are produced in exactly the same manner. Indeed, it was only, just before the fermentation process, after the cooling of the wort, that one batch of wort was divided into two fermentation tanks. Furthermore, it is striking that the differences in ageing were more pronounced between the different beers, than between the preoxygenated and control beers, made from the same wort (results not shown).

Comparison of the forced and naturally aged samples confirmed the temperature dependency of the different

Table 2
Mean beer characteristics and concentrations of staling indicators in fresh, force-aged (1 week at 40 °C) and naturally-aged (20 °C) pilsner beer samples produced without (C) or with (Preox) preoxygenated yeast

	CV(%)[a]	Pilsner beers							
		Fresh		40 °C		6 Months 20 °C		12 Months 20 °C	
		C	Preox	*C*	Preox	*C*	Preox	*C*	Preox
Alcohol % (v/v)		5.3	5.3	5.4	5.4	5.4	5.4	5.3	5.4
pH		4.5	4.5	4.5	4.6	4.5	4.5	4.5	4.5
Indicators (μg/l)									
2-Furfural	6.4	240	241	447	403	1973	1769	7341	6920
5-Methyl-2-furfural	6.5	255	246	296	270	465	514	894	934
2-Furfuryl ethyl ether	10.5	0.3	0.3	2.3	1.9	3.8	3.6	7.6	7.3
3-Methyl-butanal	7.5	244	246	304	305	317	341	283	315
Benzaldehyde	10.7	1.3	1.4	1.7	1.5	1.6	1.9	2.5	2.6
Hexanal	9.4	74	103	113	110	139	146	170	155
4-Methyl-pentan-2-one	3.3	69	73	79	78	92	102	117	123
Ethyl-3-methyl-butyrate	7.3	3.8	3.9	4.5	4.3	6.8	7.8	10.8	11.3
Ethyl hexanoate	4.2	365	368	331	323	365	374	384	384
Isoamyl acetate	5.1	528	496	455	428	281	275	222	251

[a] The coefficient of variance (CV) is given for each staling indicator.

ageing reactions (Bright, Patino, Schroedel, & Nyarady, 1993). Indeed, for a certain temperature increase, the reaction rate increase depends on the reaction activation energy, which differs with the reaction type. Therefore, a disproportionate effect on the development of staling flavours in beers could be observed. This has an impact on the use of forcing tests to analyze staling, as compounds could develop during warm storage, which do not usually develop within realistic timescales of "normal" storage (Bamforth, 2000; Bright et al., 1993; Walters, Heasman, & Hughes, 1997). For example, isoamyl acetate reduction at 40 °C was only 10%, while its concentration decreases were approximately 50% and 60% after 6 months of storage at 20 °C for pilsner and ale beers, respectively. FEE formation also changed as a function of temperature: at 40 °C its formation was markedly higher than that at 20 °C. These findings are in accordance with the results of Vanderhaegen et al. (2004). However, forced ageing procedures remain in use since the examination of natural ageing processes is a time-consuming phenomenon.

3.2.2. Sensory analysis

Figs. 1 and 2 show the mean scores for each flavour aspect for the pilsner and ale beers in a spider web diagram. For each aspect, the mean score of all the beers and all the panellists is given. As can be seen in Fig. 1a, the fresh aroma profiles of the control and preoxygenated pilsner beers are almost identical. Sweet, bitter and after-bitter taste, as well as the mouthfullness and the fruity, hoppy and sulfury aroma of the beers were appreciated on the same scale. Ageing of the beers during 1 year at 20 °C resulted in a whole range of staling flavours (Fig. 2a). Beers became sweeter, less bitter, less fruity and hoppy. In addition, pungent taste increased, together with a complex aroma, consisting of solvent, papery/cardboard, ribes, Madeira, caramel and sulfuric ageing notes. However, no obvious differences in aged aroma profile between control and preoxygenated beers could be detected by sensory analysis. Our sensory observations regarding beer ageing are mainly in accordance with the results of Dalgliesh (1977) and Zufall et al. (2005), who

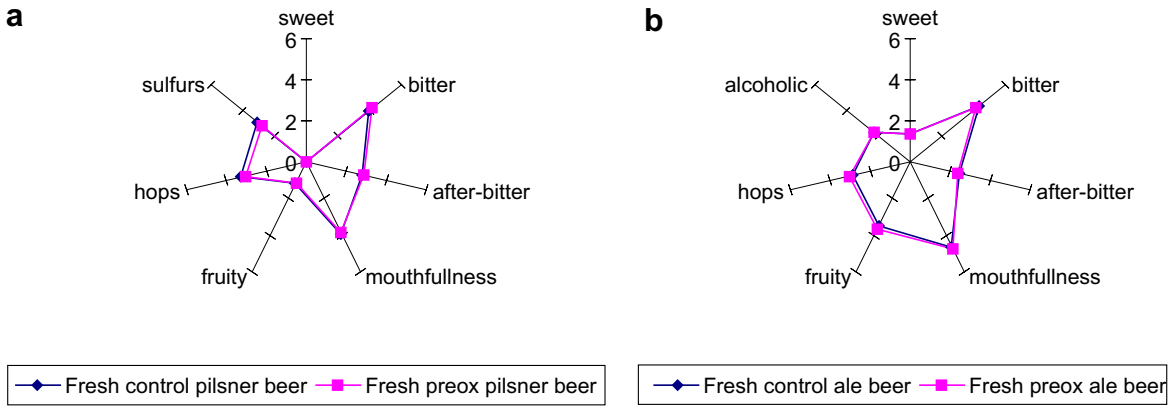

Fig. 1. Spider web diagram of the sensory results of fresh beer tasting. Beers made without (control) or with (preox) preoxygenation step are compared and the mean value of the panel scores for the different beers is given. (a) Pilsner beers and (b) Ale beers.

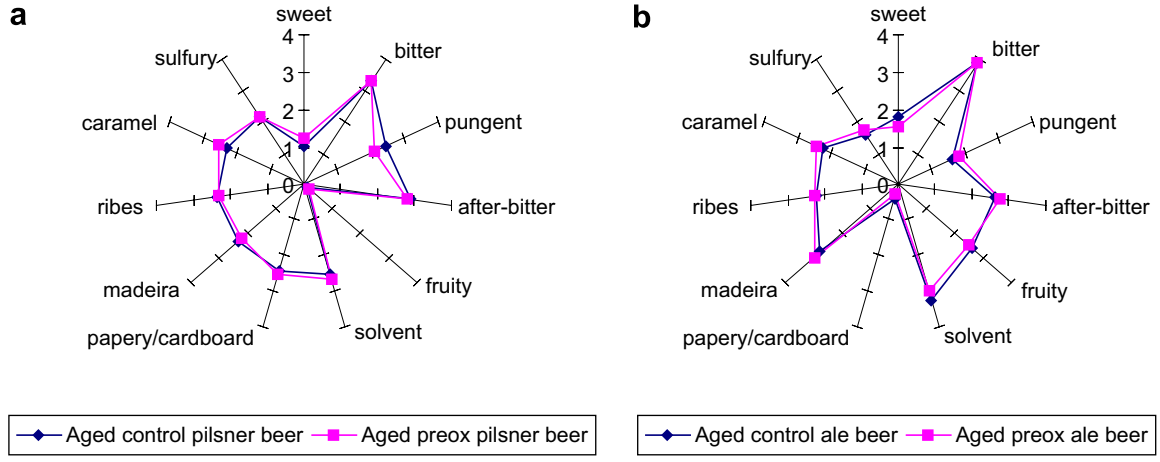

Fig. 2. Spider web diagram of the sensory results of beer tasting after 12 months of storage at 20 °C. Beers made without (control) or with (preox) preoxygenation step are compared and the mean value of the panel scores for the different beers is given. (a) Pilsner beers and (b) Ale beers.

described the sensory evolution during beer storage in general.

The panellists of the Centre for Malting and Brewing Science and of Danbrew were also asked to quantify the overall ageing intensity after forced ageing. Table 3 shows that differences in rating existed between the two panels. However, in general, no overall differences in mean staling score between control and preoxygenated pilsner beer samples could be detected.

Furthermore, a paired comparison test (two sided situation) (European Brewery Convention, 1998) confirmed that no significant difference between preoxygenated and control beers could be found at a significance level of 95%, either before or after (forced) ageing.

3.3. Influence of wort aeration on beer ageing in ale beers

3.3.1. Evolution of beer staling indicators

Analyses of two ale beers, made with or without a wort aeration step, were also performed after natural and forced ageing. As for the pilsner beers, in general, no major differences between the staling indicators were observed between the control and preoxygenation ale beers after the different ageing treatments (Table 4). The mean staling, for the control, as well as the preoxygenation beers, increased 3.4-fold at 40 °C and 1.5- and 2-fold after 6 and 12 months of storage at 20 °C, respectively. Only small variations between control and preoxygenated beers could thus be detected for specific compounds by GC–MS analysis. Furthermore, these differences were not consistently noticeable among the different staling treatments. Again, diversities in ageing were more prominent between the two subsequently produced beers, than between the preoxygenated and control beers made from the same wort. Finally, differences in the nature of ageing flavours between pilsner and ale beers were found (Tables 2 and 4). But, these results are in accordance with previous research by Vanderhaegen et al. (2006). Furthermore, ageing of the pilsner beers at 40 °C resulted in lower indicator concentrations than at 20 °C, while the converse applied for the ale beers. This is due to the different storage time at 40 °C: the pilsner beers were only stored for one week, while the ale beers were stored for 28 days because the sensory resistance to ageing of the ale beers is better (see Section 3.3.2).

Table 3
Overall ageing intensity scores after forced ageing of the beer samples at 40 °C

	Mean ageing score (scale 0–5)			
	CMBS		Danbrew	
	C	Preox	*C*	Preox
Ale beer 1	2.3	2.6	–	–
Ale beer 2	2.4	2.2	–	–
Pilsner beer 1	2.1	2.0	1.7	1.7
Pilsner beer 2	1.8	1.9	2.2	2.3
Pilsner beer 3	1.9	1.8	2.5	2.7
Pilsner beer 4	1.9	1.6	1.3	2.2

Two trained panels (CMBS and Danbrew) tasted the different beers, made without (*C*) or with (Preox) preoxygenated yeast.

3.3.2. Sensory analysis

The aroma profiles of the ale beers are depicted in Figs. 1b and 2b. No differences existed between the control and preoxygenated beer samples for as well the fresh as the aged flavour profiles. All aged samples lost initial fruity flavours, while, predominantly, Madeira, ribes and caramel flavours appeared. Apparently, only a small cardboard flavour increase was detected, in comparison with the pilsner type beers. In the beers, pungent taste and solvent aroma also developed strongly. These results largely confirm the observations of Vanderhaegen et al. (2003), who detected decreased estery notes and an increase in Madeira/port,

Table 4
Mean beer characteristics and concentrations of staling indicators in fresh, force-aged (28 days at 40 °C) and naturally-aged (20 °C) ale beer samples produced without (*C*) or with (Preox) preoxygenated yeast

	CV(%)[a]	Ale beers							
		Fresh		40 °C		6 Months 20 °C		12 Months 20 °C	
		C	Preox	*C*	Preox	*C*	Preox	*C*	Preox
Alcohol % (v/v)		8.2	7.9	8.1	7.8	8.2	8.0	8.2	7.9
pH		4.3	4.4	4.4	4.4	4.4	4.4	4.4	4.4
Indicators (μg/l)									
2-Furfural	4.1	272	233	2379	2399	516	450	788	727
5-Methyl-2-furfural	2.9	276	232	417	409	300	274	266	266
2-Furfuryl ethyl ether	2.0	4.9	4.2	62	61	24	23	37	37
3-Methyl-butanal	7.1	81	81	294	332	86	85	85	94
Benzaldehyde	3.6	24	22	31	34	25	22	24	29
Hexanal	8.4	129	115	131	147	142	136	158	141
4-Methyl-pentan-2-one	5.1	17	18	24	24	23	21	28	27
Ethyl-3-methyl-butyrate	6.2	3.9	3.8	6.5	6.5	6.1	7.1	8.3	8.5
Ethyl hexanoate	1.4	587	533	461	442	634	622	631	599
Isoamyl acetate	4.8	3118	2976	2737	2535	1308	1120	528	442

[a] The coefficient of variance (CV) is given for each staling indicator.

ribes and caramel aroma in an ale beer stored for 6 months at 20 °C. At 40 °C, a significant solvent and only minor papery flavour appeared, while pungency increased in these beers.

Furthermore, Table 3 shows that no differences in the overall ageing intensity after forced ageing between control and preoxygenated samples could be perceived. As for the pilsner beers, a paired comparison test again confirmed that no significant variation between preoxygenated and control beers could be established at a significance level of 95%, either before or after (forced) ageing. In addition, it is remarkable that the overall sensory ageing intensities at 40 °C of the ale beers (after 28 days) only slightly differed from those of the pilsner beers (after 1 week), although the ale beers demonstrated a more pronounced difference in ageing indicator concentrations than did the pilsner beers (Tables 2 and 4). However, Vanderhaegen et al. (2006) also found that ale beers showed less sensory ageing than did pilsner beers, due to the sensory masking of stale flavours. This is in part due to the higher alcohol content of ale beers than pilsner beers. Moreover, ale beers also contain higher levels of esters (such as isoamyl acetate), which create pleasant fruity flavours and mask the negative staling flavours.

3.4. General discussion

Analysis of these pilsner and ale beers showed no significant differences in beer staling caused by wort aeration, in contrast to the results of Narziss et al. (1999b). However, the two studies cannot be compared directly. Narziss et al. (1999b) aerated the pilsner wort with air during 6, 18 and 24 h and with pure oxygen during 18 h and found that an increased contact with air resulted in a decreased flavour stability, determined by GC analysis of different flavour compounds. This effect was even more pronounced when pure oxygen was used. However, they realized that altering the aeration procedure also had an impact on yeast growth and thus sulfite production. In addition, oxygenation of the wort during 18 h with pure oxygen is not a common practice in breweries. Therefore, they could not conclude whether the differences observed were caused by an oxidation of wort components or an alteration of the yeast growth rate and sulfite production.

Uchida and Ono (2000) also detected that wort aeration and pitching rate influenced the endogenous antioxidant activity (EA) level of pilsner beers and thus flavour stability. They found that EA level drops, when wort aeration rises and EA increases, when pitching rate augments.

Furthermore, Guido et al. (2004) described the impact of the physiological condition of a commercial pilsner brewing yeast on beer flavour stability. They demonstrated that an increased vitality of the pitching yeast improved flavour stability, when assessed by sensory analysis. However, the analyses of *trans*-2-nonenal and 2-furaldehyde did not reflect these sensory observations.

The major advantage stemming from our research is that the yeast's fermentation performance, growth and physiological state are not altered by the preoxygenation process (Depraetere et al., 2003). Therefore, beers could be made with or without a classical wort aeration step, without making any significant alterations to the beer production process. This was not possible in previously conducted researches from different research groups, where only gradations in wort aeration could be compared.

4. Conclusions

In this study, yeast preoxygenation, which has been suggested to have a positive influence on flavour stability, has been used to study the role of wort aeration on beer staling. GC–MS analyses, as well as sensory analysis, did not show significant differences in beer staling between the wort aeration and the preoxygenation treatment, for either pilsner- or ale-type beers.

So, when wort is only exposed to normal oxygen levels (8 mg/l) for two hours before the fermentation process starts and yeast growth begins (as in our research), this period is apparently too short and at too low temperatures (10–25 °C) to cause significant differences in beer ageing. The length of reaction time of oxygen with the wort has been proven to be of great importance (van Gheluwe & Valyi, 1974). The reaction temperature is probably likewise of major importance, as a 10 °C increase in temperature can lead to an approximate doubling of reaction rate (Bamforth, 1999).

This research thus proves that wort oxygenation does not appear to be an important parameter for determining flavour stability. However, this does not preclude that bad aeration practices, such as oxygenation of hot wort, overoxygenation and extended contact with wort without pitching yeast, can still have a noticeably negative impact on beer ageing. In this context, oxygenation of yeast might still be a good alternative to wort aeration.

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