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Enhancing Flavour Stability in Beer Using Biological Scavengers Part 2: Screening of Yeasts

The diffusion of oxygen into the bottles and the subsequent oxidation of beer components is the main cause for deteriorating flavour during storage, limiting the shelf life of the beer. Unfiltered beers coming into vogue in recent years still contain active yeasts. Active yeasts are known to be capable to metabolize oxygen and subsequently to act as biological oxygen scavengers, which offers the opportunity of enhancing the shelf life of beer in a natural way. Yeasts from a number of traditional, unfiltered beers were isolated. A total number of nine yeasts from these beers and additionally three standard yeasts from VLB (Versuchs- und Lehranstalt für Brauerei in Berlin (Research and Teaching Institute for Brewing in Berlin, Germany)) yeast strain collection were examined for their ability of scavenging the oxygen in beer, taking into account the impact on sensorics as well as on basic analytics in beer. The yeasts were rated for their ability to be used as biological scavengers following a test protocol recently described in a previous paper. Especially one selected yeast (yeast I (PA-2)) showed very encouraging results.

Descriptors: biological scavengers, flavour stability, beer, bottle fermentation

1 Introduction

Brewer's yeast as a facultative anaerobic microorganism is capable to grow aerobically. In this case, oxygen plays a major role as final electron acceptor of the respiratory chain in the catabolism. Aerobic catabolism of the yeast is suppressed when sugar is present in a concentration > 0.1 g/l (Crabtree-effect). Nevertheless oxygen is still taken up by the yeast as aerobic catabolism is not completely inhibited and furthermore, oxygen in comparable small amounts is still required for the anabolism for the formation of cell membrane components like sterols as well as saturated and unsaturated fatty acids [1, 2, 3, 4]. In finally attenuated beer conditions are not favourable for yeast growth as almost no sugars are left and the ethanol acts as a cell toxin even at comparably low concentrations starting at 0.7 %-v/v [1]. But even without sugars yeast is capable to grow if oxygen is present; in this case, ethanol acts as the main energetic source. Another mechanism to render oxygen in beer innocuous is the formation of SO_2 by the yeast. SO_2 acts as an antioxidant and hence as an oxygen scavenger and furthermore is capable of masking carbonyls which are formed during the ageing of the beer [5, 6].

During sugar-based catabolism, the coenzyme Nicotinamide adenine dinucleotide (NAD^+) is reduced to NADH during glycolysis, which is then, under aerobic conditions, oxidized again and hence recovered by electron transfer to oxygen. In case of the absence of oxygen, which is the case during fermentation and maturation, NADH has to be recycled in an alternative way, which is achieved by reducing acetaldehyde to ethanol. This type of reaction is not limited to acetaldehyde, as can be seen easily by the degradation of diacetyl to butanediol by active yeast during maturation [7, 8, 9], and which provides an interesting approach of rendering stale aldehydes harmless. Stale aldehydes amongst others are responsible for the deterioration of the smell and taste of the beer caused by oxidation. They are mainly formed by Strecker degradation and Maillard-reaction [10, 11]. The ability of yeasts to reduce stale aldehydes into their corresponding alcohols was already demonstrated by [12, 13]. The authors first used an inoculum of $1 * 10^6$ cells/ml and added additionally sucrose at a concentration of 6 g/l to stale beer and later on lowered the inoculum to $1 * 10^4$ cells/ml without sucrose addition. In all cases, the yeasts were capable to reduce the stale aldehydes clearly [13]. As a consequence bottle conditioned or refermented beers are back in the focus of interest [14, 15, 16, 17].

The present project targets the isolation and characterization of yeasts from commercially available bottle conditioned beers for their use as biological scavengers. The methodology presented in part 1 of this paper is applied [18].

https://doi.org/10.23763/BrSc18-04schildbach

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Table 1 Oxygen concentration in beer after inoculation (n=3); n.s. = not significant, * = significant (probability of error < 5 %), ** = highly significant (probability of error < 1 %), *** = most significant (probability of error < 0.1 %) compared to the corresponding reference value (n=3)

	Reference		Rh (BF)		68 obg. (TF)		S. ludwigii		Yeast A (BW)		Yeast B (PA-1)		Yeast C (TR-1)		Yeast D (GU-1)		Yeast E (GU-2)		Yeast F (LA-1)		Yeast G (LA-2)		Yeast H (TR-2)		Yeast I (PA-2)	
	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD	AVE	STD
Day	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
0	2.723	0.084	2.393	0.206	2.600	0.488	2.087	0.227	2.517	0.110	2.460	0.452	2.663	0.071	2.387	0.087	2.497	0.203	2.647	0.207	2.677	0.115	2.040	0.347	2.463	0.433
1	2.237	0.064	2.017	0.206	2.183	0.416	1.697	0.205	2.170	0.085	2.050	0.429	2.257	0.050	2.040	0.100	2.140	0.201	2.133	0.212	2.267	0.121	1.653	0.333	2.140	0.382
2	2.200	0.087	1.910	0.235	2.057	0.484	1.513	0.200	1.993	0.061	1.790	0.400	1.953	0.051	1.980	0.101	2.067	0.215	1.632	0.161	1.803	0.161	1.297	0.355	1.970	0.343
3	2.137	0.093	1.768	0.250	1.793	0.563	1.295	0.182	1.787	0.038	1.430	0.288	1.443	0.021	1.887	0.104	1.978	0.222	0.631	0.080	0.819	0.138	0.584	0.399	1.577	0.369
4	2.050	0.104	1.570	0.312	1.397	0.599	0.910	0.132	1.513	0.055	0.826	0.188	0.821	0.055	1.800	0.114	1.893	0.217	0.198	0.050	0.336	0.082	0.257	0.200	0.750	0.189
5	1.960	0.098	1.157	0.386	0.810	0.501	0.430	0.078	1.183	0.070	0.428	0.053	0.436	0.098	1.687	0.099	1.787	0.206	0.000	0.000	0.113	0.051	0.128	0.115	0.397	0.090
6	1.860	0.089	0.833	0.429	0.470	0.394	0.180	0.062	0.640	0.158	0.215	0.015	0.209	0.080	1.533	0.101	1.710	0.176			0.031	0.027	0.056	0.069	0.159	0.075
7	1.780	0.095	0.611	0.364	0.223	0.223	0.087	0.033	0.270	0.043	0.086	0.009	0.074	0.037	1.447	0.090	1.643	0.174					0.030	0.040	0.109	0.050
8	1.690	0.118	0.437	0.267	0.121	0.199	0.051	0.015	0.131	0.045	0.026	0.015	0.030	0.024	1.300	0.082	1.573	0.180					0.022	0.033	0.079	0.031
9	1.640	0.131	0.293	0.192	0.078	0.132	0.035	0.008	0.057	0.014	0.015	0.008	0.010	0.008	0.961	0.062	1.450	0.192							0.030	0.021
10	1.577	0.117	0.200	0.151	0.042	0.072	0.008	0.008	0.025	0.007	0.006	0.003	0.003	0.005	0.548	0.096	1.297	0.136							0.016	0.012
11	1.493	0.119	0.097	0.090			0.004	0.001	0.009	0.007	0.002	0.002			0.337	0.096	0.789	0.092							0.002	0.001
12	1.417	0.125	0.028	0.037			0.001	0.001	0.001	0.002					0.173	0.106	0.388	0.022							0.000	0.000

Day		Rh (BF)	68 obg. (TF)	S. ludwigii	Yeast A (BW)	Yeast B (PA-1)	Yeast C (TR-1)	Yeast D (GU-1)	Yeast E (GU-2)	Yeast F (LA-1)	Yeast G (LA-2)	Yeast H (TR-2)	Yeast I (PA-2)
0		n.s.	n.s.	*	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
1		n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2		n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
3		n.s.	n.s.	*	*	n.s.	**	n.s.	n.s.	**	**	*	n.s.
4		n.s.	n.s.	**	*	*	**	n.s.	n.s.	**	**	**	**
5		n.s.	n.s.	**	**	**	**	n.s.	n.s.	***	**	**	**
6		n.s.	*	**	**	***	**	n.s.	n.s.		***	**	**
7		*	**	**	**	**	**	*	n.s.			**	**
8		*	**	**	**	**	**	*	n.s.			**	**
9		**	**	**	**	**	**	*	n.s.				**
10		**	**	**	**	**	**	**	n.s.				**
11		**		**	**	**		**	*				**
12		**		**	**			**	**				**

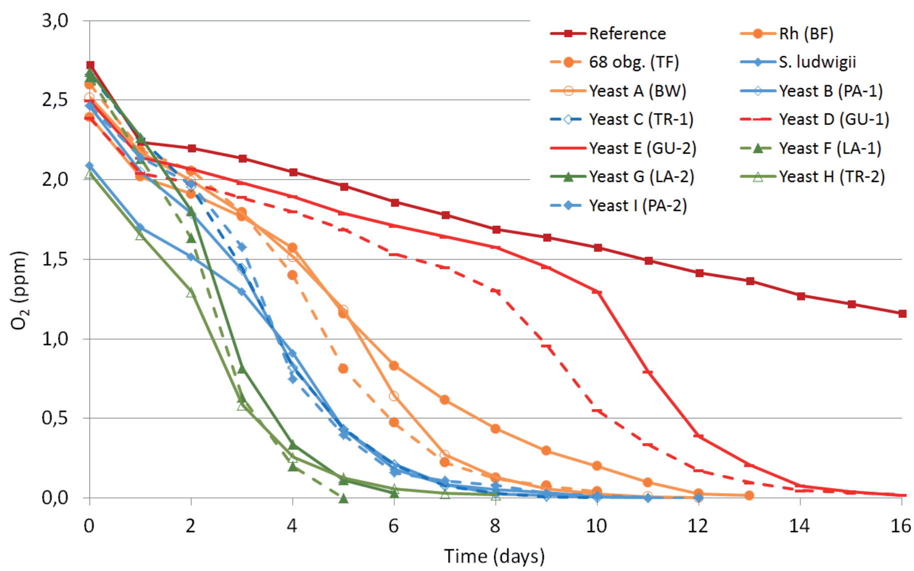

Fig. 1 **Oxygen concentration in beer after inoculation with different yeasts compared to the reference sample without addition of yeasts (mean values, n = 3, standard deviations given in table 1 only)**

These three standard yeasts were already used in preliminary trials as described in part 1 [18].

In total 15 commercially available beers were used for yeast selection. The turbid beers were chosen as it was expected that living yeast cells were present in view of the referring beer type and origin. The bottle closures were disinfected using a paper cloth soaked with ethanol 70 % for 10 min. After removing the closures the beers were carefully decanted transferring the final approx. 30 ml into sterile wort bouillon (Carl Roth Germany), which was incubated for five days at 28 °C. The incubated samples were checked for living yeast cells after methylene blue (Löfflers Methylene blue, Merck Germany) stain with a microscope. In eleven out of 15 different beers including Berliner Weiße (BW), Pale Ales (PA), Belgian Triple (TR), Gueuze (GU) and Lambic (LA) yeasts were found, with nine of them containing culturable yeasts (partly more than one). From these yeasts, nine were selected for the screening programme.

Table 2 **Sensory evaluation concerning stale flavours acc. to Eichhorn, weighted overall score ranging from 1 = fresh to 4 = extremely oxidised; acceptance (in %) only related to staling (n = 1)**

	Weighted Overall Score Weeks Storage			Acceptance (%) Weeks Storage		
	4	8	12	4	8	12
Original	1.0	1.1	1.6	99	96	79
Reference	2.0	2.6	2.5	41	38	42
Rh (BF)	1.2	1.1	1.3	93	93	93
68 obg. (TF)	1.4	1.2	1.0	63	97	97
S. ludwigii	1.2	1.0	1.4	93	100	90
Yeast A (BW)	1.5	1.3	1.1	87	84	93
Yeast B (PA-1)	1.2	1.0	1.0	87	96	100
Yeast C (TR-1)	1.7	1.1	1.2	70	96	97
Yeast D (GU-1)	1.3	1.2	1.1	83	97	90
Yeast E (GU-2)	1.3	1.1	1.1	83	93	90
Yeast F (LA-1)	1.1	1.0	1.2	97	100	90
Yeast G (LA-2)	1.1	1.1	1.0	97	93	97
Yeast H (TR-2)	1.1	1.1	1.1	97	90	100
Yeast I (PA-2)	1.0	1.0	1.0	100	97	100

2 Materials and methods

Altogether twelve different yeasts were used for the experiments, whereas three yeasts served as standards (reference stocks from Versuchs- und Lehranstalt für Brauerei (VLB) in Berlin):

- Rh bottom fermenting (BF) strain of *Saccharomyces carlsbergensis* (abbr. Rh (BF));
- 68 obg. top fermenting (TF) strain of *Saccharomyces cerevisiae* (abbr. 68 obg. (TF));
- *Saccharomycodes ludwigii* 3448 (abbr. *S. ludwigii*).

All yeasts (standards and isolates) were then cultivated 48 h prior to inoculation in freshly prepared wort bouillon and incubated at 28 °C. For adjusting the inoculum to 2000 cells/ml, living yeast cells in the incubated wort bouillon were counted after methylene blue stain using a Thoma chamber.

If not otherwise stated the methodology applied for measuring the oxygen concentration in the samples was the same as already described in part 1 [18]. Therefore PET bottles (PET with a passive barrier, screw caps without chemical scavenger, provided by VLB Berlin) were equipped with optical-chemical sensor spots (SP-Pst3-NAU-DS-YOP, PreSens Germany) allowing measuring the oxygen concentration in the bottle without opening them. PET bottles were chosen for safety reasons in case of excessive CO_2-formation by the yeasts.

Like in part 1 bottled beer was sourced commercially from a big-sized brewery in glass bottles. Disinfection of the PET bottles was done by ethanol 70 % (in- and outside) and then dried under the clean bench before filling with media and inoculation. After transferring the beer under clean bench conditions into PET bottles the oxygen concentration increased to approx. 3 ppm. Additionally, un-inoculated PET bottles were treated under the same conditions in order to measure the oxygen consumption caused only by the beer matrix as well as to serve as the comparison for the inoculated samples (same oxygen stress like the inoculated samples, but no biological scavengers). These samples refer to as "reference". Furthermore, samples in the original glass bottles stayed unopened (referred to as "original") and were stored under the same conditions (dark storage in a box at room temperature in an upright position) like the PET bottles.

Statistical evaluation was done by comparing the oxygen con-

Table 3 **Sensory evaluation concerning overall beer quality acc. to DLG, weighted overall score ranging from 5 = very good to 1 = very bad; crosses in bold indicate a strong characteristic (n = 1)**

	Weighted Overall Score Weeks Storage			Description											
	4	8	12	Oxidation	sulfury	estery	fruity	solvent-like	alcoholic	fatty acids	adstringent	earthy	soapy	leathery	currant
Original	4.7	4.3	3.8	x											
Reference	3.2	2.9	2.6	**x**											
Rh (BF)	4.0	4.1	3.9												
68 obg. (TF)	2.4	3.5	3.9		x	x	x	x				x			
S. ludwigii	4.0	4.3	3.3		x							x			x
Yeast A (BW)	3.1	2.9	3.4		x	x	x				x				
Yeast B (PA-1)	3.7	4.1	4.0			x	x	x	x					x	
Yeast C (TR-1)	3.5	4.0	4.1			x	x		x		x				
Yeast D (GU-1)	2.8	2.7	2.9		**x**	**x**		x							
Yeast E (GU-2)	2.7	3.0	2.8		**x**	**x**	x	**x**		x					
Yeast F (LA-1)	3.6	4.1	3.3		**x**	x	x	**x**			**x**	x		x	
Yeast G (LA-2)	4.1	3.4	4.1		x	x	**x**				**x**				
Yeast H (TR-2)	3.6	3.8	3.6		x	x				x			x		
Yeast I (PA-2)	4.3	3.8	4.6		x	x	x								

centration in the inoculated samples with the one in the reference sample using the t-Test.

Sensory evaluation was carried out by a panel of six people. Training before the trials focussed on the stale flavour of the beer. Different evaluating schemes were used, starting assessing beer ageing by the scheme from *Eichhorn* [19]. Marks are given for smell, taste and bitterness all in respect to beer ageing, ranging from 1 = fresh, 2 = slightly aged, 3 = strongly aged to 4 = extremely aged. A weighted average is calculated with the marks for smell and taste being factored twice, for bitterness once. Additionally and in line with Eichhorn [19] percentage values in 20-%-steps are given for acceptance in respect to oxidation, with 100 % acceptance indicating a fresh and 0 % acceptance indicating a severely aged beer. Secondly, the procedure acc. to Deutsche Landwirtschafts-gesellschaft (DLG) [20] was applied in order to gain an overview of any off-flavours in general apart from oxidation/beer ageing. Marks for the smell, purity of taste, mouthfeel and quality of bitterness range from 5 = very good to 1 = very bad. Divergent to the DLG-scheme carbonation taste was not rated due to losses of carbon dioxide caused by the handling during filling of the PET-bottles. Again weighted averages were calculated, factoring smell, purity of smell and quality of bitterness twice and mouthfeel only once. All sensory analyses were conducted after four, eight and twelve weeks without replicates.

Chemical-technical analyses were done by beer measuring station DMA 4500M and Alcolyzer Beer ME (Anton Paar Austria), pH by PH539 (WTW Germany) and turbidity as light scattering by LTP5 (Dr Lange Germany).

In the second set of trials, the inoculum was decreased in an attempt to limit side-effects like the formation of off-flavours. For this approach the standard yeasts Rh (TF), 68 obg. (TF) and *S. ludwigii*, as well as yeast I (PA-2) were taken and the inoculum was set to 2000 cells/ml, 200 cells/ml and 20 cells/ml respectively.

Table 4 **Chemical-technical analyses of samples after twelve weeks storage time (over-fermenting capability indicated in bold) (n=1)**

	Alcohol (%-v/v)	Turbidity (EBC)	pH ( )
Original	4.77	1.2	4.25
Reference	4.71	1.3	4.23
Rh (BF)	4.90	3.9	4.23
68 obg. (TF)	4.88	6.0	4.27
S. ludwigii	4.78	1.8	4.25
Yeast A (BW)	4.99	18.0	4.20
Yeast B (PA-1)	4.91	9.3	4.24
Yeast C (TR-1)	**5.60**	1.3	4.17
Yeast D (GU-1)	4.82	8.3	4.23
Yeast E (GU-2)	**5.01**	17.8	4.21
Yeast F (LA-1)	**5.52**	27.5	4.22
Yeast G (LA-2)	**5.34**	17.8	4.21
Yeast H (TR-2)	4.94	4.5	4.27
Yeast I (PA-2)	4.90	6.3	4.25

3 Results and discussion

Figure 1 and table 1 show the course of the oxygen concentration in the beer samples inoculated with the standard yeasts Rh (BF), 68 obg. (TF) and *S. ludwigii* as well as the yeasts isolated from

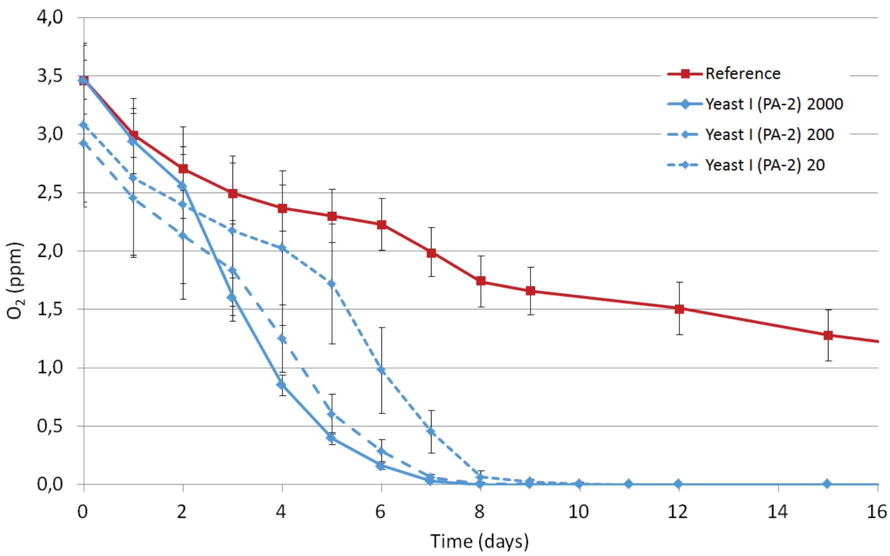

Fig. 2 Oxygen concentration in beer after inoculation with yeast I (PA-2) with different inoculum (2000 cells/ml, 200 cells/ml and 20 cells/ml) compared to the reference sample without addition of yeast (reference n = 5, rest n = 3)), error bars indicating standard deviation

different commercially available beers compared to the reference (beer with oxygen uptake but no yeast, squares), revealing different speeds in oxygen uptake by the yeasts, but with all of them being clearly faster compared to the oxygen consumption caused by the beer matrix.

Comparably slow are the yeasts from the Gueuze beer (yeasts E and D, minus symbols with continuous and dotted line respectively), whereas Rh (BF), 68 obg. (TF) and Yeast A (BW) cause a faster oxygen decrease (circles). Even better are the other tested yeasts, with Yeast F (LA-1), Yeast G (LA-2) and Yeast H (TR-2) (triangles) being exceptionally fast in oxygen reduction.

Following the storage of the beer samples for twelve weeks, the sensory evaluation according to Eichhorn (Table 2) reveals that all yeasts prevent the beer from staling, whereas the reference sample is strongly affected by the oxygen. Nevertheless it has to be acknowledged that some yeasts (68 obg. (TF), yeast A (BW) and yeast C (TR-1)) show some signs of oxidation at the beginning, but later improve in this respect, which might indicate a reduction in stale aldehydes as already mentioned by [12, 13].

Table 3 provides an overview of further off-flavours. Sensorics was done in line with the DLG-procedure. When giving marks of 3 and below for smell and quality of taste the panellists were asked to provide a description about the off-flavour detected.

As expected the reference beer is strongly oxidised right from the start. Most of the added yeasts cause some sort of off-flavours in the Pilsener beer but corresponding to the sensory evaluation acc. to Eichhorn no oxidation is detected. Some yeasts like yeast D (GU-1) and E (GU-2) yield very low overall ratings throughout, whereas others like Rh (BF) or *S. ludwigii* show good weighted overall DLG-scores at the start or like yeast B (PA-1), yeast C (TR-1), yeast G (LA-2) and especially yeast I (PA-2) at the end of the 12-weeks-period. Most of the off-flavours like sulfury and fruity / estery resemble green beer, which might indicate a restart of some fermentation activity.

Table 5 Sensory evaluation acc. to Eichhorn, "2000" = 2000 yeasts/ml, "200" = 200 yeasts/ml and "20" = 20 yeasts/ml inoculated, weighted overall score ranging from 1 = fresh to 4 = extremely oxidised (n = 1)

	Weighted Overall Score Weeks Storage		
	4	8	12
Original	1.0	1.1	1.1
Reference	2.4	3.0	3.3
Rh (BF) 2000	1.2	1.4	1.5
Rh (BF) 200	1.2	1.4	1.7
Rh (BF) 20	1.1	1.4	1.2
68 obg. (TF) 2000	1.4	1.3	1.2
68 obg. (TF) 200	1.0	1.2	1.3
68 obg. (TF) 20	1.0	1.2	1.2
S. ludwigii 2000	1.2	1.2	1.7
S. ludwigii 200	1.2	1.6	1.5
S. ludwigii 20	1.6	1.2	–
Yeast I (PA-2) 2000	1.0	1.4	1.3
Yeast I (PA-2) 200	1.2	1.5	1.2
Yeast I (PA-2) 20	1.2	1.5	1.3

Table 6 Sensory evaluation acc. to DLG, "2000" = 2000 yeasts/ml, "200" = 200 yeasts/ml and "20" = 20 yeasts/ml inoculated, weighted overall score ranging from 5 = very good to 1 = very bad (original and reference n = 4, rest n = 1)

	Weighted Overall Score Weeks Storage		
	4	8	12
Original	4.6	4.7	4.6
Reference	3.1	2.7	2.4
Rh (BF) 2000	4.1	4.0	3.7
Rh (BF) 200	4.4	3.7	3.6
Rh (BF) 20	4.3	4.1	4.2
68 obg. (TF) 2000	3.3	3.2	3.3
68 obg. (TF) 200	3.5	3.5	3.7
68 obg. (TF) 20	3.4	3.5	3.9
S. ludwigii 2000	3.9	4.0	3.7
S. ludwigii 200	4.0	3.7	3.4
S. ludwigii 20	3.7	3.9	–
Yeast I (PA-2) 2000	4.3	4.0	3.7
Yeast I (PA-2) 200	4.2	3.5	4.0
Yeast I (PA-2) 20	4.1	3.5	3.8

Table 7 Summary of results, + = positive effect, o = neutral, – = negative effect

	O_2-Scav.	Oxidation	Off-Flavour	Over-Ferm.	Turbidity
Rh (BF)	o	+	o	+	–
68 obg. (TF)	o	++	o	+	–
S. ludwigii	+	+	o	+	o
Yeast A (BW)	o	+	–	o	–
Yeast B (PA-1)	+	++	o	+	–
Yeast C (TR-1)	+	++	o	–	o
Yeast D (GU-1)	–	+	–	+	–
Yeast E (GU-2)	–	+	–	–	–
Yeast F (LA-1)	++	+	–	–	–
Yeast G (LA-2)	++	++	o	–	–
Yeast H (TR-2)	++	++	o	o	–
Yeast I (PA-2)	+	++	+	+	–

After 30 weeks storage time, the yeast cell count was around 10^6 cells/ml for all yeasts and the vitality ranged from appr. 55 % to > 95 % (results not shown).

Looking on the chemical-technical results after twelve weeks (Table 4) it is obvious that some of the selected yeasts (yeast C (TR-1), yeast E (GU-2), yeast F (LA-1) and yeast G (LA-2)) possess over-fermenting capabilities. This does not only result in an increase in alcohol concentration, but also in an equivalent carbon dioxide formation, which already led to a buckling of the referring PET-bottles. These yeasts are not suitable to be used as biological scavengers due to a high risk of bottle burst during storage. An increase in turbidity depending on the yeast added can be monitored, whereas the pH-value of the beers stays more or less unchanged.

In a further set of trials, the inoculum was decreased in order to minimize side-effects like off-flavour formation by the added yeasts. The oxygen decrease is given exemplarily for yeast I (PA-2) in figure 2, with the other yeasts giving similar results.

Lowering yeast inoculum retards slightly the oxygen decrease, but still, even the sample with the smallest inoculum of 20 cells/ml reaches zero after nine days. The results of the sensory analyses acc. to Eichhorn and DLG as presented in tables 5 and 6 are somehow indifferent and do not disclose any major differences between the varying inoculum concentrations.

It has to be realized that by limiting the inoculum the yeast growth and hence the oxygen uptake is only delayed, but the oxidation preventing effect is still existent even at very low inoculum concentrations, which is in-line with the literature [13]. Nevertheless, the off-flavours are still present, so decreasing the inoculation does not look to be a promising way to limit undesired side-effects.

4 Conclusion/Summary

Different yeasts selected from commercially available bottle conditioned beers showed different speeds in oxygen reduction, which interestingly did not have an impact on the prevention of stale flavour, as all yeasts proved to be efficient in this respect. Off-flavours were present in most cases at different degree, making some yeasts less suitable for their use as biological scavengers. The variation of the inoculum did not show a positive effect concerning the avoidance of off-flavours. Some yeasts have to be sorted out due to the strong formation of off-flavours respectively due to their over-fermenting capabilities.

Table 7 reveals the overall performance of the different yeasts. Yeast I (PA-2) does especially well, followed by the standard yeast Rh (BF), 68 obg. (TF) and S. ludwigii as well as yeast B (PA-1) and H (TR-2). The rest of the yeasts have to be ruled out due to unacceptable negative effects (yeasts A, D and E due to strong off-flavours and yeasts C, E, F and G due to over-fermenting). As all yeasts lead to an increase in turbidity, this is not considered in the overall ranking.

The procedure presented in both parts of this paper might serve as a blueprint for assessing the applicability of different yeasts for their use as biological scavengers in naturally turbid beers. Nonetheless, it has to be acknowledged that there are still some questions to be answered. The oxygen uptake normally will not take place at once as simulated in the trials but will be caused by diffusion through the packaging materials like PET-bottles and closures over a longer period of time, which might have an influence on the growth of the yeast. Furthermore, storage conditions were quite gentle (no mechanical stress, stored in dark conditions) in order to focus on the staling effect of dissolved oxygen only. In practice harsher conditions have to be taken into account, so a bigger focus has to be put on the autolysis of the yeast.

Nevertheless, enhancing flavour stability of beer in a natural way by adding suitable yeasts is a promising approach and gives new momentum for unfiltered beers.

Acknowledgement

Special thanks go to Hessian Ministry for Science and Culture for funding within the programme „Forschung für die Praxis“.

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Received 8 March 2018, accepted 12 April 2018