Peculiarities of seven refermented Belgian strong ales and their corresponding industrial yeasts

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ABSTRACT

Seven commercial Belgian pale top-fermented beers with high alcohol content and refermentation in the bottle (one trappist, three abbey and three strong blond beers) were characterised for classic beer features and their content of total nitrogen, proteins, FAN, polyphenols, gluconic acid, δ-gluconolactone and viable yeast cells were determined. The saccharide profile of the beers was investigated by determining the fermentable saccharides (HPLC), the higher saccharides (HPAEC-PAD) and the saccharide spectrum, as a distribution of fractions of different molecular mass obtained by ultracentrifugation using filters with cut-offs of 1, 5, 10 and 50 kDa. The results reveal that similar top-fermented beers can have quite different saccharide spectra.
Refermentations were performed combining each of the seven beers with glucose or maltose as extract and with each of the seven corresponding industrial yeast strains. After three weeks of refermentation, bottles were uncorked and refermentation continued for another two weeks. Total counts of viable yeast cells were determined by plating to control if lack of refermentation was due to low yeast viability.
Beer refermentation performance is strongly dependent on the type of beer, the yeast strain used and the type of extract added. Especially, beers with a high alcohol percentage or a high CO_2 level were poorly or not refermented at all. All yeasts could easier referment glucose than maltose and maintained longer their viability if glucose was used as extract instead of maltose. Also the yeast strain and the type of beer influenced the yeast viability. Yeast which had low viability counts after five weeks showed limited refermentation of the extract.

INTRODUCTION

Top-fermented beers with a secondary fermentation in the bottle belong to the Belgian well-known specialties. Trappist and many abbey and strong blond beers are refermented in the bottle. These beers are economically important since their consumption and export still increase (Buelens and Vansevenant, 2005). Specific problems associated with refermentation are extensively discussed (Derdelinckx *et al*., 1992; Vanbeneden *et al*., 2005; van den Berg and Van Landschoot, 2003; Vanderhaegen *et al*., 2002; Vanderhaegen *et al*., 2003; Van Landschoot *et al*., 2003; Van Landschoot, *et al*., 2004). One of the qualities attributed to bottle-fermented beers is that the yeast in the bottle protects against oxidation giving these beers a longer shelf life. This holds as long as the yeast cells are at least intact, but preferably the yeast should remain viable as long as possible. It was shown that to maintain a high viability during refermentation and conservation of the beers, preference should be given to freshly propagated yeast over harvested yeast from the main fermentation (Van Landschoot *et al*., 2004). The sugar composition of the added extract for refermentation of beer in bottles also influences yeast viability. The use of sugar syrups with high maltose content may lead to irreproducible or stuck fermentations, variable batch-to-batch quality and rapid loss of yeast viability during conservation. This probably is related to residual extract promoting inconsistent yeast depot stability (Vanbeneden *et al*., 2005).
In this study, seven commercial Belgian pale top-fermented beers with high alcohol content (one trappist, three abbey and three strong blond beers) are refermented with glucose or maltose as added extract and with the seven corresponding industrial brewing yeasts. The objectives of this study are to 1) examine the influence of the composition of the added fermentable extract (glucose

and maltose) on the fermentation rate and final attenuation of bottle refermentation, 2) determine the influence of the type of beer and the yeast strain on the fermentability of the added extract, 3) study the influence of carbon dioxide pressure on the refermentation performance and 4) determine the influence of the added extract, type of beer and yeast strain on yeast viability.

MATERIALS AND METHODS

Characterisation of the used beers

Seven commercial Belgian pale top-fermented beers (one trappist, three abbey and three strong blond beers) with high alcohol content and refermentation in the bottle (33 cl) were used in the study. The beers are indicated with the letters a, b, c, d, e, f and h (The letter g is not used because it is used as abbreviation for the glucose extract). Beers of two production batches were used of beer f: f1 and f2.

The CO_2 content was determined with the Inpack CO_2-meter ICM2000 and was corrected for the total inpack-oxygen measured with the Inpack airmeter IAM (Haffmans, Venlo, NL).

The DA-310 Density/Specific Gravity Meter DA-310 from Kyoto Electronics was used for density measurements to determine apparent extract, real extract, original extract, alcohol content, apparent and real degree of fermentation.

The beer colour was calculated from the absorbance at 430 nm multiplied by a factor 25 according to the Analytica EBC method 9.6.

Analytica EBC methods were used for the determination of the total nitrogen and proteins (Kjeldahl method), FAN or Free Amino Nitrogen (ninhydrin reagent) and polyphenols (Fe-ammonia reagents).

Gluconic acid and δ-gluconolactone were determined with the D-Gluconic acid Kit (for 25 tests) of Stag NV.

Yeast viability was determined by citrate-buffered methylene blue coloration as described by Smart *et al.* (1999).

The fermentable sugars, glucose, fructose, sucrose, maltose and maltotriose, were determined by HPLC. A HP 1100 Series HPLC with an automated sampler and a HP 1047 A refractive index detector were used. Elution of a carbohydrate amino column (Alltech Associates) was with acetonitrile/MilliQ water (75:25 v/v %). Prior to analysis, beer samples were degassed by filtration. Proteins were precipitated by adding to 10 ml degassed beer 200 µl Carrez-I-solution (10.6 g potassiumhexacyanoferate/100 ml distilled water) and 200 µl Carrez-II-solution (21.9 g zincacetate/100 ml distilled water).

For saccharide profile determination, beer samples were degassed, filtered and submitted to an alcohol distillation according to the EBC Analytica method 9.4. Prior to ultracentrifugation, the residue was diluted to a final saccharide concentration of 1.41 to 1.56% (w/w). The filters of the centrifugation tubes had cut-offs of 5, 10 and 50 kDa (Sartorius Vivaspin 20) or 1 kDa (PALL Macrosep). The saccharide concentration (expressed in °P) in the four filtrates was determined by density measurements with an Anton Paar DMA 4500 density meter. The saccharide concentration (expressed as a percentage) in the fractions 0-1 kDa, 1-5 kDa, 5-10 kDa, 10-50 kDa and > 50 kDa was calculated from these values.

Higher saccharides up to DP10 (Degree of Polymerisation 10) were analysed at the Laboratory for Glycobiology, Universiteit Gent, with High Pressure Anion Exchange Chromatography (HPAEC) coupled to Pulsed Amperometric Detection (PAD). The CarboPac PA100 of Dionex was used and elution was according to Application Note 46 of Dionex (Anonymous, 1997).

Industrial ale yeasts

The industrial brewing yeasts A-H for the study were isolated from a bottle of the corresponding beers a-h by plating some drops of the yeast sediment of the bottle. The yeast received the same letter code as the corresponding beer in capital letters. The yeast growth on plates didn't match the viability as determined by methylene blue viability staining (Table 1). On the contrarily to what was expected from the methylene blue viability stain, yeast E showed good growth while two other yeasts with high viability according to the staining, didn't grow on the agar plates even after different trials. These two yeasts were kindly provided by the corresponding breweries.

The brewing yeasts for the refermentation experiments were freshly propagated on wort, at 22°C, in shaking flasks. Yeast cell counting was performed with a Thoma cell counting chamber. The glycogen and trehalose contents of the pitching yeast were calculated from the released glucose after the treatment with trehalase and amyloglucosidase respectively. The glucose was determined spectrophotometrically at 505 nm after staining with Glucose, *GOD-PAP* kit (Dialab).

Refermentations

The beers a-h were pasteurised in the bottles prior to refermentation to destroy the viable yeast cells and to denature the enzymes. The necessary pasteurisation units were determined to be 27.5. This equals about one minute at 70°C; but for practical reasons 15 hours in an oven at 50°C were chosen.

All bottles were decapsulated just before addition of yeast and sugar to minimize carbon dioxide loss. Each beer was combined with each yeast (1 million viable yeast cells/ml beer) (49 combinations) and each combination was used for two bottle refermentation set-ups: one supplemented with 0.4 °P glucose and one supplemented with 0.4 °P maltose (a total of 98 combinations). For each combination, one blank sample was prepared, which received water instead of yeast. The blanks were used to assess residual microbial or enzymatic activity. The bottles were recapsulated immediately after addition of the yeast (or water) and the sugar. Fermentation took place in a "warm chamber" of 24°C. The apparent extract content was measured every week. After three weeks of refermentation the remaining bottles were decapsulated and the beers were covered with oil, making free diffusion of carbon dioxide possible, but keeping the metabolism fermentative. Fermentation was continued for another two weeks and the apparent extract was determined every week. After about five weeks of refermentation in the bottle, the yeast viability was determined by plating on wort agar.

Contamination was checked on MRS medium.

The set-up of the experiments in the study is schematically presented in figure 1.

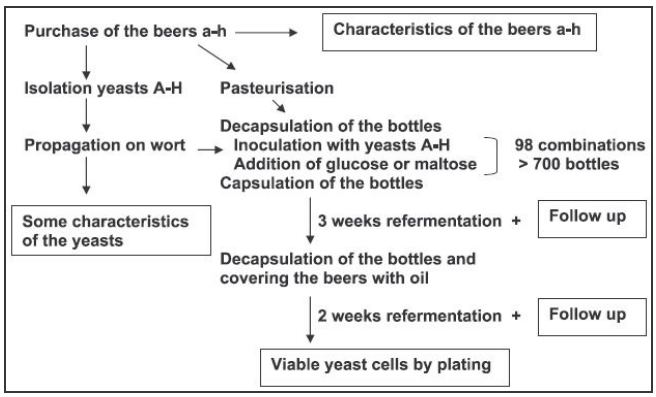

Figure 1: Scheme of the experimental set-up. Results are obtained from the parts marked with a frame.

RESULTS AND DISCUSSION

Characteristics of the beers (Table 1)

The seven beers used are of the same type: pale top-fermented with high alcohol content and refermentation in the bottle. The colour varies between 7 and 19 EBC and the alcohol content between 7.69 and 9.97 v/v %. This corresponds to an original extract between 16.70 and 20.37 °P. As mentioned previously, the % viable yeast cells in the bottle (determined by methylene blue viability staining) doesn't correspond to the real viability of the yeasts.

All beers contain residual fermentable sugars either from the added refermentation extract or from the main fermentation. Beer e has only a small amount of residual fructose from the refermentation extract, while the beers a and f2 contain a considerable amount of residual maltose and/or maltotriose from the main fermentation. Beer f1 contains an appreciable amount of residual maltotriose from the main fermentation and a large amount of glucose and fructose. Probably, sucrose, used as refermentation extract, was only converted to glucose and fructose by yeast invertase activity. Many of the other parameters are beer or brewery related and are not typical for the type of beer itself. The volume of air in the bottle, the carbon dioxide content and the gluconic acid and δ-gluconolactone content depend mainly on the processing. The gluconic acid/δ-gluconolactone content can account for the contact of the wort and/or the beer with oxygen, because these compounds can be formed by chemical oxidation of glucose.

The degree of fermentation and the content of total nitrogen, proteins, FAN, polyphenols and saccharides are related to the raw materials used in the brewery. The seven beers show big differences in saccharide content. The concentration of non-fermentable saccharides with a polymerisation degree of four or higher varies between 4.2 and 5.9 °P, matching a difference of more than 30%. Differences (up to 20%) in the content of higher saccharides are also found for lager beers with alcohol content between 4.7 and 5.2 v/v % (unpublished results).

The spectra of the saccharides of the seven beers, as determined by ultrafiltration, are not uniform at all. HPAEC-PAD analysis of the beers reveals that some beers contain high contents of α(1-4)-linked malto-oligosaccharides, while others mainly have oligosaccharides with other types of linkages such as α(1-6). The HPAEC-PAD chromatograms are shown in figure 2. The saccharide pattern of beer c clearly differs from that of beer d and h. Beer c contains almost no linear α(1-4) malto-oligosaccharides and has high amounts of non-linear α(1-4) malto-oligosaccharides. Beers d and h exhibit the opposite saccharide pattern. The big difference in saccharide composition in the beers mainly originates from the use of different sugar sources (syrup) in the brewing process. Starch hydrolysates added to the wort, will result in high contents of linear malto-oligosaccharides with typical α(1-4)-linkages in the beer. Beers exclusively brewed with starch sources undergoing the full mashing process, mainly contain oligosaccharides with one or more α(1-6)-linkages between the glucose residues as the majority of the α(1-6)-linkages in amylopectin appear to survive the brewing process (Briggs *et al*., 2004). The non-fermentable saccharides, the maltodextrins, create the body of beer. The higher the original gravity of the beer, the more full-bodied the mouthfeel of the beer is. Not only the total content of higher saccharides has an influence on the body of beer, but also the type of the higher saccharides (for example molecular mass) is important (unpublished results).

Refermentation profiles

The refermentation profiles for the beers are expressed as apparent extract decrease (°P). Each yeast has 14 refermentation profiles: 7 with glucose as extract and 7 with maltose as extract. The profiles of yeast B are shown in figure 3. Beer f1 always reached a higher attenuation than the expected one from the added extract (0.4 °P). This is due to fermentation of residual extract in the beer. All yeasts ferment glucose to a higher extent than maltose in all seven beers. None of the seven yeasts can completely ferment the added maltose during the three weeks of refermentation in the capsulated bottles. This corresponds with the findings of Vanbeneden *et al*., 2005. The maltose uptake is an active process that requires energy (Maskell *et al*., 2001) and for maltose fermentation the products of at least one of five independent *MAL*-loci are needed. Each *MAL*-locus is made out of three genes encoding for a maltase, a transport protein for maltose and a positive regulatory protein respectively (Kodama *et al*., 1993; Novak *et al*., 2004). Glucose is transported into the yeast by facilitated diffusion through multiple hexose transporters (Debs-Louka *et al*., 1999; Diderich *et al*., 1999), requiring no cellular energy. This explains partly the low maltose utilization during refermentation compared to the higher glucose utilization in similar refermentation experiments. Vanbeneden *et al*. (2005) established that carbon dioxide pressure is the major factor inhibiting the uptake of maltose. Compared to the monosaccharides, the uptake of maltose is very sensitive to elevated carbon dioxide contents. The membrane bound maltose permease is possibly structurally affected at high carbon dioxide pressures, while hexose transporters are less susceptible to environmental effects (Stewart *et al*., 1995). Carbon dioxide can also cause internal acidification disturbing the proton gradient (Debs-Louka *et al*., 1999; Shimoda *et al*., 2001). Since the maltose permease depends on this pH gradient, it can be sensitive to the carbon dioxide content in the beer.

Table 1: Characteristics of the seven Belgian pale top-fermented beers with high alcohol content and refermentation in the bottle.

Ferm. is used as abbreviation for "fermentation" or for "fermentable". ND is noted when the value is not determined. Nd stands for "not detectable concentrations" of the saccharides in the HPAEC-PAD chromatograms. M4, M5, M6, M7, M8 and M9 are maltotetraose, maltopentaose, maltohexaose, maltoheptaose, malto-octaose and maltononaose.

The concentrations of the fermentable saccharides (g/l) are determined by HPLC. The content of higher saccharides (polymerisation degree of four or higher) in the beers is calculated from the real extract content minus the total concentration of lower saccharides (DP ≤ 3). The percentage of higher saccharides in the beers equals the percentage of higher saccharides in the original extract and is calculated according to: (total higher saccharides/original extract) x 100.

The saccharides in the fractions (%) are calculated from the density values of the filtrates after ultrafiltration of samples of the seven top-fermented beers with centrifugation tubes with cut-offs of 1, 5, 10 and 50 kDa.

The concentrations of individual saccharides (in µM) of the beers are determined by HPAEC-PAD. The total content of saccharides in the beer samples used for HPAEC-PAD, determined by density measurements, is 0.38 % w/w.

	Beer a	Beer b	Beer c	Beer d	Beer e	Beer f1	Beer f2	Beer h
Air in the bottle (ml)	0.60	0.85	6.50	2.80	3.30	0.75	2.10	2.05
Carbon dioxide (g/l)	9.5	7.7	6.2	7.8	8.0	4.2	8.1	7.3
Apparent extract (°P)	1.76	2.31	1.32	2.40	2.06	3.06	2.46	3.43
Real extract (°P)	5.09	5.04	4.52	5.48	5.54	5.97	5.57	6.55
Ethanol content (v/v %)	8.00	7.69	9.24	8.71	9.97	8.22	8.99	8.60
Original extract (°P)	17.17	16.70	18.41	18.55	20.37	18.32	18.77	19.40
Apparent degree of ferm. (%)	90	86	93	87	90	83	87	82
Real degree of ferm. (%)	70	70	76	71	73	67	70	66
Colour (EBC)	7	15	16	14	14	10	10	19
Total nitrogen (mg/l)	303	417	452	371	301	ND	259	424
Proteins (mg/l)	1892	2603	2822	2319	1881	ND	1619	2647
FAN (mg/l)	64	97	99	88	41	ND	41	85
Polyphenols (mg/l)	214	178	189	153	221	ND	102	240
Gluconic acid (mg/l)	34.1	51.5	33.0	ND	63.5	ND	31.2	50.0
δ-Gluconolactone (mg/l)	30.9	46.7	30.0	ND	57.7	ND	28.3	45.4
Viable yeast cells (%)	40	> 90	> 75	40	0	> 90	> 90	50
Saccharide concentrations from HPLC analysis								
Fructose (g/l)	0.34	0.46	0.61	0.46	0.52	3.07	< 0.06	1.49
Glucose (g/l)	< 0.06	0.25	0.33	< 0.13	< 0.13	2.65	< 0.09	0.22
Sucrose (g/l)	< 0.09	< 0.09	< 0.09	< 0.09	< 0.09	< 0.09	< 0.09	< 0.09
Maltose (g/l)	3.30	1.23	0.58	< 0.12	< 0.12	< 0.12	< 0.09	< 0.12
Maltotriose (g/l)	3.40	< 0.19	1.21	1.22	< 0.16	6.15	8.70	4.01
Total ferm. saccharides (g/l)	7.0	1.9	2.7	1.7	0.6	11.9	8.7	5.7
Higher saccharides (°P)	4.4	4.8	4.2	5.3	5.4	4.8	4.7	5.9
Higher saccharides (%)	26	29	23	29	27	26	25	30
Saccharides in the fractions (%) from ultrafiltration with centrifugation tubes with cut-off filters								
Fraction 0-1 kDa (%)	34	38	46	38	36	ND	32	35
Fraction 1-5 kDa (%)	19	13	14	6	15	ND	12	19
Fraction 5-10 kDa (%)	23	19	7	17	6	ND	19	11
Fraction 10-50 kDa (%)	13	10	9	19	15	ND	17	19
Fraction > 50 kDa (%)	11	20	24	20	28	ND	20	16
Linear α(1-4) malto-oligosaccharides (µM in samples of 0.38 °P) from HPAEC-PAD analysis								
Retention time 4.7 min (M4)	372	367	65	391	273	ND	269	521
Retention time 6.9 min (M5)	129	136	Nd	168	131	ND	60	235
Retention time 10.4 min (M6)	46	95	Nd	161	74	ND	24	187
Retention time 13.9 min (M7)	17	31	Nd	57	17	ND	Nd	34
Retention time 16.8 min (M8)	Nd	12.6	Nd	21	Nd	ND	Nd	Nd
Retention time 20.5 min (M9)	21	20	Nd	36	23	ND	18	47
Non-linear α(1-4) malto-oligosaccharides (µM in samples of 0.38 °P) from HPAEC-PAD analysis								
Retention time 5.6 min	83	15	236	13	46	ND	12	18
Retention time 8.7 min	116	116	208	105	168	ND	137	85
Retention time 12.5 min	107	111	121	110	157	ND	128	75
Retention time 15.5 min	86	106	71	115	162	ND	131	60
Retention time 18.2 min	57	62	26	84	104	ND	89	56
Retention time 21.7 min	35	44	46	45	51	ND	53	12
Retention time 23.5 min	37	52.4	33	69	72	ND	73	13
Retention time 25.0 min	43	51	28	53	78	ND	75	21

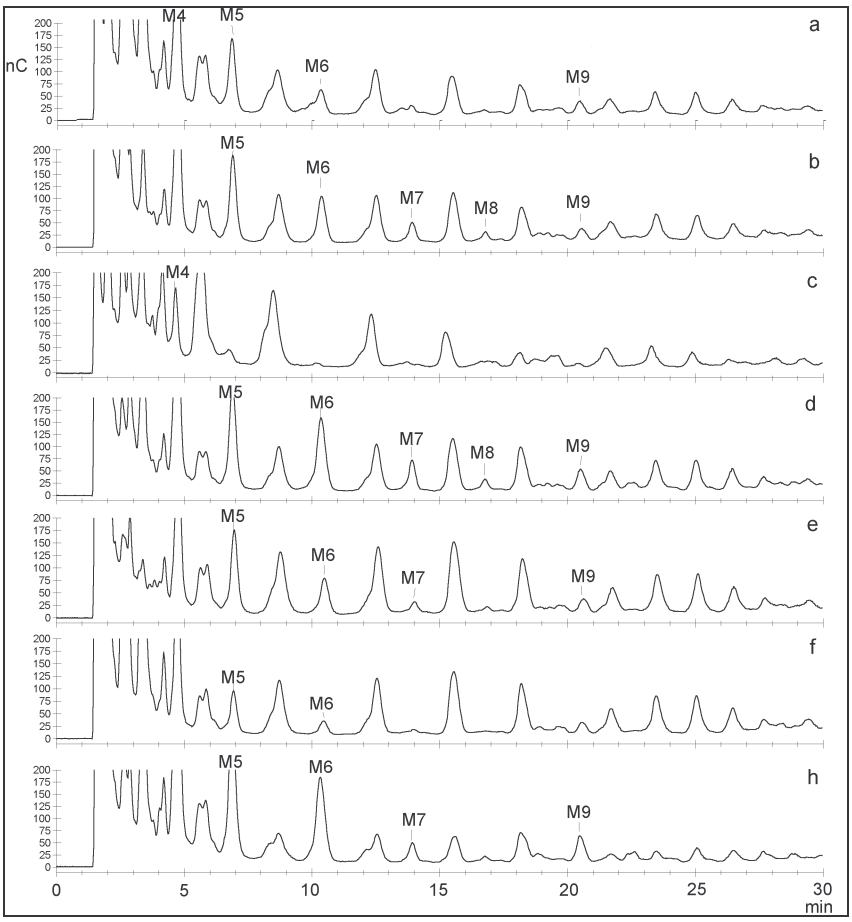

Figure 2: Separation of malto-oligosaccharides in seven Belgian pale top-fermented beers a-h by anion-exchange chromatography with pulsed amperometric detection. f stands for beer f2.
M4, M5, M6, M7, M8 and M9 are maltotetraose, maltopentaose, maltohexaose, maltoheptaose, malto-octaose and maltononaose.

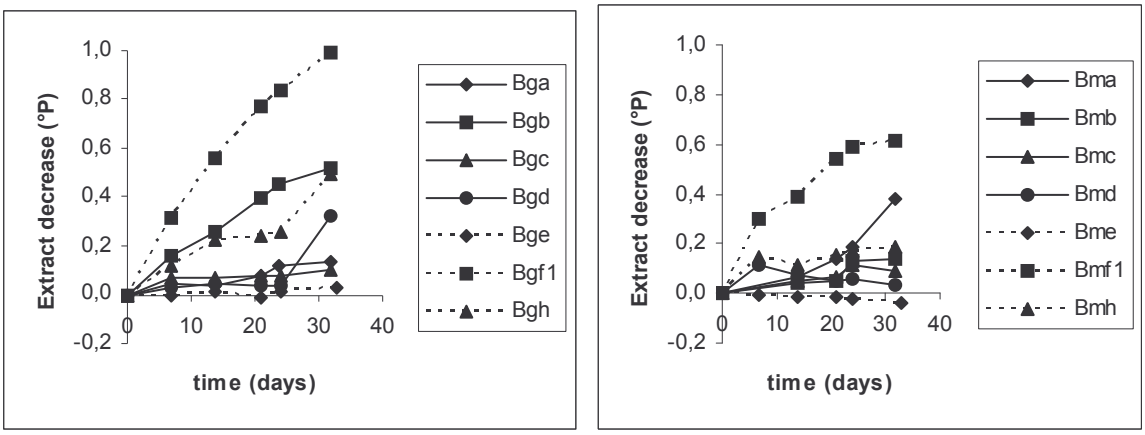

Figure 3: Evolution of the apparent extract decrease (°P) during bottle refermentation of seven top-fermented beers (a-h) with the brewing ale yeast B and glucose (left figure) or maltose (right figure) as extract. The first letter of the code stands for the yeast (B). The second letter of the code is g (for glucose) or m (for maltose). The last letter in the code stands for the beer (a-h).

Table 2: Estimates of the alcohol tolerance, the carbon dioxide tolerance and the viability during conservation of the seven industrial brewing yeasts A-H in the seven Belgian pale top-fermented beers a-h.

	Yeast A	**Yeast B**	**Yeast C**	**Yeast D**	**Yeast E**	**Yeast F**	**Yeast H**
Alcohol tolerance	moderate	moderate	good	moderate	limited	limited	limited
CO_2 tolerance	good	good	very good	moderate	moderate	moderate	good
Viability	moderate	good	very good	very good	limited	limited	moderate

Glucose and maltose refermentation is strongly yeast and beer dependent. The influence of the medium is due to the differences between the beers: the beers have an alcohol content between 7.69 and 9.97 v/v % and a carbon dioxide content between 4.2 and 9.5 g/l. The influence of the yeast strain originates from differences in ethanol and carbon dioxide tolerance. The refermentation performances are translated in rough estimates of the alcohol and carbon dioxide tolerance of the corresponding yeasts (Table 2).

The effect of the release of the carbon dioxide pressure on the uptake of glucose and maltose was evaluated by decapsulating the bottles after three weeks of refermentation and covering the beer with oil. Refermentation was continued for another two weeks and the apparent extract was determined every week. Many beers showed an additional decrease in apparent extract, proving that the carbon dioxide pressure has an inhibiting effect on the fermentation of glucose and maltose. However, complete attenuation was not reached since the yeast is still inhibited by the high alcohol concentration in the beer and, in some cases, has already lost much of its viability.

The results of plating the yeast sediment (expressed as viable cells/ml beer) on wort agar after about five weeks of refermentation in the bottles are gathered in table 3. Figure 4 shows pictures of the agar plates for the beers a-h pitched with yeasts B and F. The yeast viability on glucose is, for each combination, at least equal, but mostly far greater than the viability on maltose. The rapid decrease of yeast viability upon refermentation with maltose is probably due to the limited availability of fermentable carbon sources, since the conditions during bottle conditioning impair the uptake of maltose. Moreover, since flocculation only starts upon the exhaustion of the medium and can be considered as a defense mechanism, the yeast can less well withstand the aversive environmental conditions (Vanbeneden *et al*., 2005).

The viability was not only dependent on the yeast strain used, but also on the beer used for refermentation. This was expected as the beers differ in alcohol (7.69–9.97 v/v %) and carbon dioxide (6.2–9.5 g/l) content. As discussed above, these two parameters have an effect on the refermentation properties of the yeast. There is a clear correlation between the yeast's refermentation capacity and the preservation of its viability during conservation. Yeasts that referment well, maintain longer their viability. A description of the evolution of the viability of the seven yeasts during conservation of the beers is summarized in table 2. The trehalose and the glycogen content after propagation (Table 4) show no correlation with the yeast viability. For instance, yeast D has the lowest level of glycogen and trehalose, but its viability remains quite high during conservation, while yeast F has higher levels of glycogen and trehalose than yeast D, but survives less well.

Table 3: Yeast viability (as viable cells/ml beer) as determined by the plating technique after five weeks of refermentation of glucose (Glu) or maltose (Mal) in the beers a-h. Refermentations with beer f are either with beer f1 or beer f2.

	Yeast A		**Yeast B**		**Yeast C**		**Yeast D**		**Yeast E**		**Yeast F**		**Yeast H**	
	Glu	**Mal**	**Glu**	**Mal**	**Glu**	**Mal**	**Glu**	**Mal**	**Glu**	**Mal**	**Glu**	**Mal**	**Glu**	**Mal**
Beer a	$>10^5$	$>10^4$	$>10^4$	$>10^4$	$>10^5$	$>10^5$	$>10^5$	$>10^5$	$>10^4$	<1	$>10^4$	600	$>10^3$	450
Beer b	$>10^4$	$>10^3$	$>10^3$	$>10^3$	$>10^5$	$>10^4$	$>10^5$	$>10^5$	$>10^4$	$>10^3$	$>10^3$	600	$>10^5$	$>10^3$
Beer c	700	< 1	$>10^4$	$>10^3$	$>10^4$	$>10^4$	$>10^5$	$>10^5$	<1	<1	140	<1	53	14
Beer d	700	450	$>10^3$	600	$>10^5$	$>10^5$	$>10^5$	$>10^4$	$>10^4$	20	$>10^5$	<1	$>10^5$	$>10^4$
Beer e	60	2	700	20	$>10^5$	$>10^3$	$>10^5$	$>10^4$	100	<1	47	<1	450	<1
Beer f1	-	-	$>10^3$	$>10^3$	-	-	-	-	$>10^3$	300	-	-	-	-
Beer f2	$>10^3$	700	-	-	$>10^5$	$>10^4$	$>10^5$	$>10^4$	-	-	700	6	$>10^3$	240
Beer h	$>10^4$	$>10^3$	$>10^3$	$>10^4$	$>10^5$	$>10^5$	$>10^5$	$>10^4$	$>10^3$	$>10^3$	700	15	$>10^5$	$>10^3$

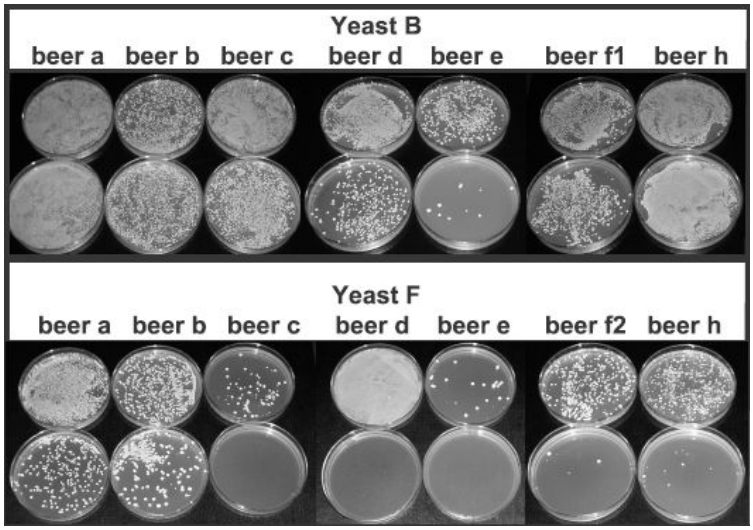

Figure 4: Wort agar plates of yeast B and yeast F after 5 weeks of refermentation in beers a-h with glucose as extract (upper row of plates per yeast) or with maltose as extract (lower row of plates per yeast). The same dilution of each beer was used for plating. The colony counts on the plates equal the number of living yeast cells in 0.66 ml beer.

Table 4: Trehalose and glycogen content, expressed in mg/g wet, freshly propagated yeast.

	Yeast A	Yeast B	Yeast C	Yeast D	Yeast E	Yeast F	Yeast H
Trehalose (mg/g)	8.3	7.1	10.6	5.1	7.5	7.9	5.9
Glycogen (mg/g)	19	20	11	9	17	18	16

CONCLUSIONS

This study supplies extensive information of seven Belgian pale top-fermented beers and their corresponding industrial yeasts. The studied beers were one trappist, three abbey and three strong blond beers. All beers have high alcohol content: between 7.69 and 9.97 v/v %; and an original extract between 16.70 and 20.37 °P. Big differences between the beers were found for total nitrogen, proteins, FAN, polyphenols, gluconic acid, δ-gluconolactone and saccharide content. All beers contained residual fermentable saccharides, ranging between 0.6 g/l up to high levels of 11.9 g/l. The concentrations of the saccharides with a polymerisation degree of four or higher varied between 4.2 and 5.9 °P. The saccharide spectra of the seven beers differed largely in saccharide pattern and in content of individual oligosaccharides. One beer contained almost no linear α(1-4) malto-oligosaccharides and had high amounts of non-linear α(1-4) malto-oligosaccharides, while other beers contained roughly the opposite. The beers were thus brewed with quite different sugar mixture additions.

The refermentation profiles with the seven beers with glucose or maltose as extract and with the seven corresponding industrial yeasts revealed that the refermentation of the beers was strongly dependent on the beer medium, the yeast strain and the added extract used for refermentation. Especially, the extracts in beers with a high alcohol percentage or a high CO_2 level, refermented poorly or not at all. All yeasts could referment glucose better than maltose. Yeast cells maintained longer their viability if glucose was used as extract instead of maltose. The yeast strain and the beer influenced the yeast viability during refermentation and conservation. Yeast which had low viability counts after five weeks, also showed limited refermentation of the extract.

The seven industrial brewing yeasts were evaluated according to their alcohol and carbon dioxide tolerance and the preservation of their viability during refermentation. Yeast C scored the best for the three criteria and yeast E and F had the lowest assessment.

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