Spectrum of polysaccharides degradation products of ales and lager beers

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SUMMARY

The saccharide spectrum, as a distribution of fractions of different molecular mass, of sixteen beers was determined by ultracentrifugation using filters with cut-offs of 1, 5, 10 and 50 kDa. The saccharide concentrations in the filtrates were determined by density measurements. The saccharide composition was examined through High Pressure Anion Exchange Chromatography (HPAEC) coupled to Pulsed Amperometric Detection (PAD). The results were compared with the values of classic features of beers.
The newly developed method provides additional information of the beers and is a simple and fast tool for exploring the effect of the saccharide spectrum on the industrial characteristics. The results revealed that similar top fermentation beers and similar lager beers have different saccharide spectra.

INTRODUCTION

Body is one of the flavour characteristics in the spider web plot of beer [1]. It is a basic concept expressing how heavy or how light a beer feels in the mouth. As a rule, full-bodied beers have more residual sugars, while lighter beers have less. This is a result of how starch has been hydrolysed into fermentable and non-fermentable sugars during the brewing process. Carbohydrases (e.g. α- and β-amylase, limit dextrinase, α-glucosidase, β-glucanase) play a very important role in the brewing process. These enzymes originate from the malt or are exogenous enzymes produced by genetically modified bacteria. In mashing, a very large number of enzymes act simultaneously on the components of the grist (malt and mash tun adjuncts) under conditions that are far from optimal for many of them in terms of substrate concentration and accessibility, pH and enzyme stability. At present a range of brewing procedures are in use and they are carried out in many different types of equipment. The mashing varies from traditional infusion mashing, decoction mashing, double mashing, temperature programmed infusion mashing, 'all-' or 'mainly-adjunct' mashing to 'mixed' mashing [2]. Most of the extract formed during mashing comes from the conversion of starch to a mixture of fermentable saccharides (glucose, maltose and maltotriose), and non-fermentable saccharides (maltotetraose and larger linear and branched maltodextrins). The yeast converts the fermentable sugars in alcohol during the fermentation process, whereas the non-fermentable saccharides, the maltodextrins, create the body of beer. The higher the

original gravity of the beer the more full-bodied it tastes. Not only the total content of higher saccharides has an influence on the body of beer but also the nature of the higher saccharides (for example molecular mass) is of importance (unpublished results).

EXPERIMENTAL

Ultracentrifugation
Seven Belgian pale top fermentation beers with high alcohol content and refermentation in the bottle (a trappist beer, three abbey beers and three strong blond beers) and nine international brands of lager beer (two German, Dutch and French beers and one Belgian, Swiss and South-African beer) were investigated. The samples for the ultracentrifugation were dilutions of the residue of the alcohol distillation of degassed and filtered beer. The starting solutions had an initial saccharide concentration of 1.41 to 1.58% (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 concentrations in the filtrates were determined by density measurements with an Anton Paar DMA 4500 density meter.

Classic features of beers
The following characteristics of the beers were determined with EBC (European Brewery Convention) analytical methods or calculated: the apparent (E_a % w/w), real (E_r % w/w) and original (E_o % w/w) extract, the alcohol content (A % v/v), the real fermentation degree (V_r %), volume air in the bottle (V_{air}), g CO_2/l and colour. The concentrations of the fermentable carbohydrates (glucose, fructose, sucrose, maltose and maltotriose) in the beers were obtained by HPLC analysis [3]. The concentrations of the saccharides with a polymerisation degree of four or higher were calculated from the real extract and the concentrations of the fermentable carbohydrates.

HPAEC-PAD
High Pressure Anion Exchange Chromatography (HPAEC) coupled to Pulsed Amperometric Detection (PAD) was performed to separate the oligosaccharides of the beers. The CarboPac PA-100 of Dionex was used and elution was according to Application Note 46 of Dionex (www.dionex.com).

RESULTS AND DISCUSSION

The results of the ultracentrifugation with tubes with cut-offs of 1, 5, 10 and 50 kDa were expressed as % saccharides in the fractions 0-1 kDa, 1-5 kDa, 5-10 kDa, 10-50 kDa and more than 50 kDa. The results for the seven Belgian pale top fermentation beers with high alcohol content and refermentation in the bottle are gathered in tables 1, 2 and 3. Although these seven beers are of the same type, they do show big differences for the saccharides. The concentrations of the saccharides with a polymerisation degree of four or higher vary between 4.2 and 5.9 % w/w. The spectra of the saccharides, as determined by ultracentrifugation, are not at all uniform for the seven beers. HPAEC-PAD reveals that some beers contain high contents of α1-4 linked malto-oligosaccharides while others merely have oligosaccharides with other types of linkages as probably α1-6. Figure 1 gives the HPAEC-PAD chromatograms for two beers which clearly show different saccharides patterns. The concentrations of individual saccharides

in these beers illustrate the big difference in nature of the saccharides in the two beers (Tabel 4).

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

Beer		V_{air} (ml)	g CO_2/l	E_a % w/w	E_r % w/w	A % v/v	E_o % w/w	V_r (%)	Colour (EBC)
a	Strong blond	0.6	9.5	1.76	5.09	8.00	17.17	70	7
b	Strong blond	0.85	7.7	2.31	5.04	7.69	16.70	70	15
c	Strong blond	6.5	6.2	1.32	4.52	9.24	18.41	76	16
d	Abbey	2.8	7.8	2.40	5.48	8.71	18.55	71	14
e	Trappist	3.3	8.0	2.06	5.54	9.97	20.37	73	14
f	Abbey	2.1	8.1	2.46	5.57	8.99	18.77	70	10
h	Abbey	2.05	7.3	3.43	6.55	8.60	19.40	66	19

Table 2: Saccharide concentrations (g/l) and total of lower saccharides (LS in g/l) in the seven top fermentation beers as determined with HPLC. The content of higher saccharides (polymerisation degree of four or higher; HS in % w/w) in the beers is calculated from E_r and total LS. The percentage of higher saccharides (% HS) in the beers equals the % HS in the original extract and is calculated according to: total HS/E_o . 100.

Beer	Fructose	Glucose	Sucrose	Maltose	Malto-triose	~ Total LS	~ Total HS (% w/w)	% HS
a	0.34	< 0.06	< 0.09	3.30	3.40	7.0	4.4	26
b	0.46	0.25	< 0.09	1.23	< 0.19	1.9	4.8	29
c	0.61	0.33	< 0.09	0.58	1.21	2.7	4.2	23
d	0.46	< 0.13	< 0.09	< 0.12	1.22	1.7	5.3	29
e	0.52	< 0.13	< 0.09	< 0.12	< 0.16	0.6	5.4	27
f	< 0.06	< 0.09	< 0.09	< 0.09	8.7	8.7	4.7	25
h	1.49	0.22	< 0.09	< 0. 2	4.01	5.7	5.9	30

Table 3: Saccharides in the fractions (%) calculated from the density values of the filtrates after ultrafiltration of the seven top fermentation beers with centrifugation tubes with cut-offs 1, 5, 10 and 50 kDa.

Beer	Saccharides in the fractions (%)				
	0-1 kDa	1-5 kDa	5-10 kDa	10-50 kDa	> 50 kDa
a	34	19	23	13	11
b	38	13	19	10	20
c	46	14	7	9	24
d	38	6	17	19	20
e	36	15	6	15	28
f	32	12	19	17	20
h	35	19	11	19	16

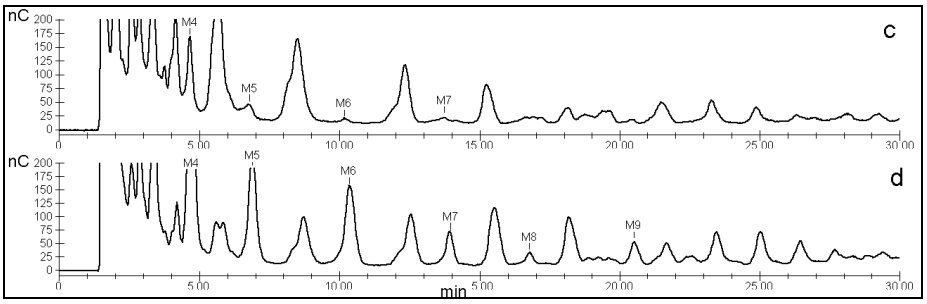

Figure 1: Separation of malto-oligosaccharides in two Belgian pale top fermentation beers (strong blond beer c and abbey beer d) by anion-exchange chromatography with pulsed amperometric detection.

Table 4: Concentrations of individual saccharides (in µM) of the two beers of figure 1. The total content of saccharides in the two beer samples is the same: 0.38 % w/w. Rt is the retention time in minutes. Nd stands for not detectable concentrations of the saccharides. M4, M5, M6, M7, M8 and M9 are maltotetraose, maltopentaose, maltohexaose, maltoheptaose, malto-octaose and maltononaose.

	Linear malto-oligosaccharides (µM)						**Non-linear malto-oligosaccharides (µM)**							
	M4	M5	M6	M7	M8	M9								
Rt (min)	4.7	6.9	10.4	13.9	16.8	20.5	5.6	8.7	12.5	15.5	18.2	21.7	23.5	25
Beer c	65	Nd	Nd	Nd	Nd	Nd	236	208	121	71	26	46	33	28
Beer d	391	168	161	57	21	36	13	105	110	115	84	45	69	53

The nine international brands of lager beer also differ a lot in saccharide total content, saccharide spectrum and nature of the oligosaccharides in terms of monosaccharide binding.

CONCLUSIONS

The developed ultracentrifugation method provides additional information of the beers and is a simple and fast tool for exploring the effect of the saccharide spectrum on the industrial characteristics. The results revealed that similar top fermentation beers, with alcohol contents between 7.7 and 9.9 % (v/v), and similar lager beers, with alcohol contents between 4.3 and 5.2 % (v/v), have different saccharide spectra. This 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 α1-4 linkages in the beer. Beers exclusively brewed with starch sources that undergo the full mashing process mainly contain oligosaccharides with one or more α1-6 linkages between the glucose residues.

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