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QUANTITATIVE CHROMATOGRAPHY OF THE OLIGOSACCHARIDES IN WORT, BEER AND BREWING SYRUPS

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SUMMARY

A method for the separation and quantitative analysis of the oligosaccharides from four to fifteen glucose units in brewing materials is described. The procedure is to separate the oligosaccharides on a heated cellulose column by elution with a water–*n*-butanol–ethanol gradient mixture. Quantitative analysis is then achieved by an automatic analyser which mixes and reacts the eluent stream with anthrone/sulphuric acid and then measures the absorbance on an integrating recorder.

INTRODUCTION

The "dextrin" fraction in wort and beer has been investigated by many workers using such techniques as acetone precipitation[1], ethanol extraction[2], paper[3–7] and charcoal column[8,9] chromatography. More recently ENEVOLDSEN[10] has used paper chromatography to separate wort oligosaccharides both by molecular weight and into α-1,4-linked and α-1,6-linked fractions.

DELLWEG *et al.*[11–13] have successfully separated up to fifteen homologous glucose oligomers on polyacrylamide gel columns using an Auto-Analyzer. The lower sugars can be readily separated by gas chromatography[14–18] and this technique has been extended for the analysis of starch hydrolysates of up to seven glucose units[19].

THOMA *et al.*[20] have used partition chromatography on cellulose columns for the preparative separation of the oligo (1–10 glucose units) and megalosaccharides (10–20 glucose units) from starch hydrolysates. This technique has been developed into a manual analytical method for the quantitative analysis of oligosaccharides in brewing materials and was described in our previous report[21].

This present paper describes the further development of a faster automated method for the separation and determination of the oligosaccharides derived from malt and other cereals encountered in the brewing industry.

EXPERIMENTAL

The chromatographic and analyser system is illustrated in Fig. 1. All tubing unless otherwise stated is of 1-mm-bore Teflon. Between tube connections were made from 1/16 in. I.D. Viton tubing (Watson-Marlow) made air-tight by twisting Nichrome wire tightly around the tubing.

Gradient elution

The column eluent was pumped from a gradient elution device consisting of a 250-ml conical flask filled to 200 ml with a mixture of water–ethanol–*n*-butanol (24.5:23:52.5) connected to an empty 50-ml flask, which is connected in turn to a 1-l flask containing 800 ml of water-ethanol–*n*-butanol (42:25:33). All solvents were de-aerated and kept air-free by immersing the gradient elution apparatus in a water bath maintained at 60° by circulating water through the bath by means of a Churchill thermo-circulator. The eluent was pumped to the top of the chromatographic column, by means of a micro-pump at a fixed rate of 28 ml/h.

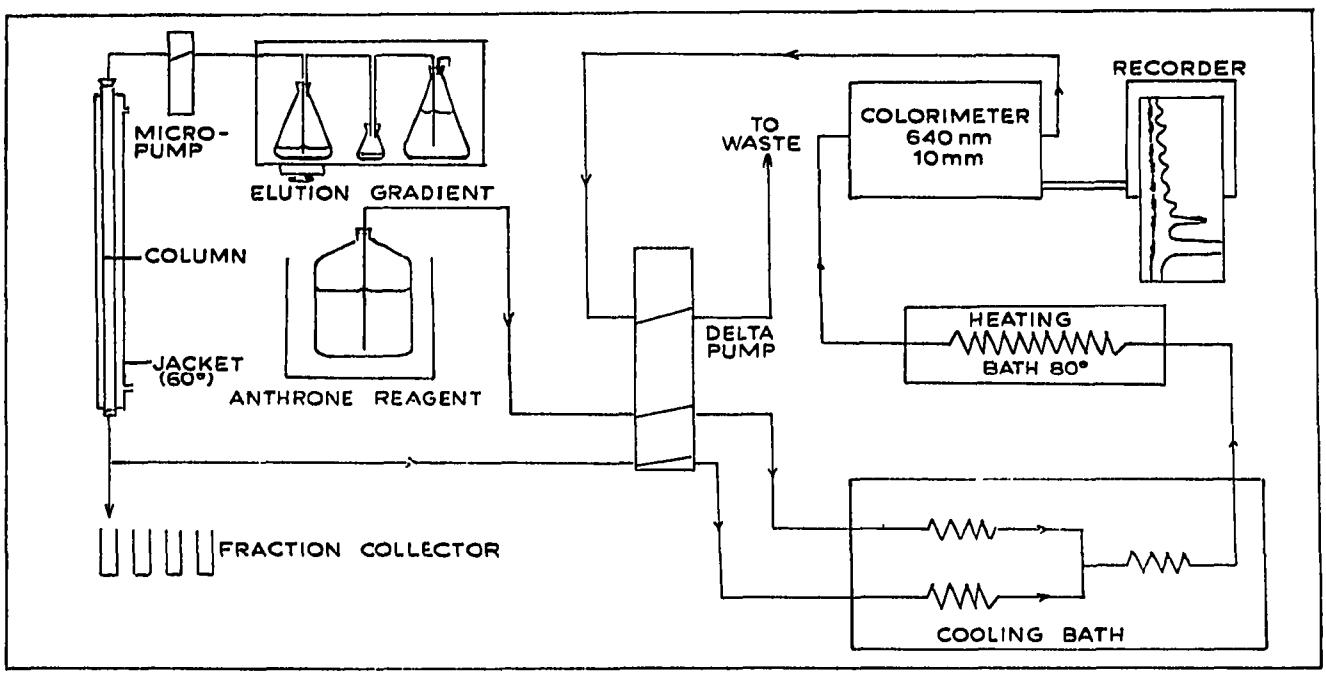

Fig. 1. Chromatography and analyser flow diagram.

Chromatographic column

The column used was a Whatman precision column 45 cm long × 1.5 cm diam. fitted with a water jacket. The Teflon end fittings were removed and replaced by silicone rubber bungs bored to take 1-mm-bore Teflon tubing (Fig. 2). The temperature of the column was maintained at 60° by connecting the water jacket in series with the bath heating the gradient elution device. The column was packed to a depth of 43 cm with Whatman No. CF12 cellulose powder. Prior to use, the fines were removed from the cellulose by stirring 50 g of the powder with 1 l of distilled water

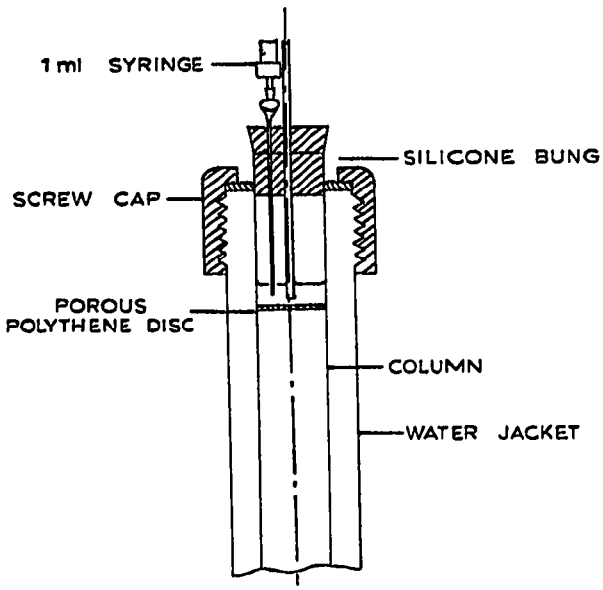

Fig. 2. Chromatography column upper end assembly.

and allowing to settle. The supernatant liquid along with the fines was then poured off and this procedure repeated five times.

The method of packing the column is important for good separation characteristics. The column was filled with a slurry of 50 g (less fines) of cellulose in 800 ml of water at 90° and allowed to settle under gravity with no flow. The packing was consolidated by sucking the surplus water through the outlet with a 10-ml hypodermic syringe. This procedure was repeated until the cellulose was packed to the required depth. When correctly packed, the column should be capable of maintaining a flow rate of 30–40 ml/h and 0.2 ml of universal pH indicator should separate into bands, and run evenly down the column, using hot water as eluent.

The water was displaced by percolating at least 500 ml of water–ethanol–*n*-butanol (24.5:23:52.5) through the column.

Microgranular cellulose did not show any improvement of separation, tended to pack too tightly and to have a reduced flow rate.

Regeneration

After each run it was necessary to wash the column by running hot de-aerated water through it overnight and then to displace the water by 500 ml of the eluting solvent (water–ethanol–*n*-butanol, 24.5:23:52.5).

Analyser

The effluent from the column was split in the ratio 3:1, 7 ml/h being fed to the analyser system and 21 ml/h flowing to a fraction collector.

The flow to the analyser was controlled at 7 ml/h by a Watson-Marlow delta pump fitted with 2 mm I.D. M.S. (high-grade PVC, Esco Rubber Ltd.) pumping tubes.

The eluent was pumped firstly through a chilling coil made from 2 m of Teflon tubing maintained at less than 4° using a Churchill chiller circulator (or ice) to a glass

'T' piece where it met the chilled anthrone reagent flowing at 14 ml/h (effluent to reagent ratio, 1:2.0). The reaction mixture was then fed through a chilled mixing coil made from 2 m of Teflon tubing and then into a Teflon reaction coil of 7-ml capacity (≏ 14 m Teflon tubing) immersed in a water bath at 80°. This coil volume gives a reaction time of 20 min. The absorbance of the effluent stream was measured in a Vitatron colorimeter (Fisons Ltd.) using a flow through cell of 10 mm light path and 0.08 ml capacity and filter No. 641 (640 nm) and then recorded with a Vitatron lin/log integrating recorder. The reaction mixture was then pumped through the delta pump to waste, using a ¼ in. I.D. Viton tube.

Detection reagent

The reagent used for colour development was 0.1% anthrone in 85% sulphuric acid freshly prepared at the beginning of each run. The anthrone reagent stock bottle was kept below 4° and the reagent stream was pumped at 14 ml/h through the delta pump using 1/8 in. I.D. Viton tubing (Watson-Marlow), then through a cooling coil of 2 m length to the 'T' piece, where it was mixed with the column effluent.

Preparation of sample

The beer, wort or syrup was dried under vacuum in a rotary evaporator or alternatively by freeze drying and 0.1–0.2 g of the residue was dissolved in the minimum amount of degassed water. 90% dimethyl sulphoxide was found to be a better solvent for some syrups.

The viscous solution obtained was layered onto the top of the column by injecting through the silicone rubber bung using a 1-ml hypodermic syringe (Fig. 2).

Quantitation of results

n-Butanol which is contained in the column eluent interferes with the reaction between anthrone and carbohydrate. It has been shown[21] that this interference can be reduced by reaction at 60° instead of the more usual 100° (refs. 22–24).

YADAV *et al.*[25] showed that if the acid concentration of the anthrone reagent is reduced from 95% to 85% a constant molar extinction, dependent only on the glucose equivalent of the sugar, is obtained. This modification was adopted and it was found, using 0.1% anthrone in 85% sulphuric acid, that the most reproducible results were obtained when the mixture was heated at 80° for 20 min.

The ratio of reagent to column eluent is important and should be kept to 2.0:1 (Fig. 3).

Some batches of commercial *n*-butanol gave different absorbance values when used in the column eluent; for this reason it is necessary to check each batch of *n*-butanol and redistill when required.

Calibration

The fractions corresponding to each peak were collected together and evaporated to dryness under vacuum in a rotary evaporator. The solids obtained were dissolved in hot water treated with charcoal, precipitated with excess ethanol, filtered and dried at 95° under vacuum. Aliquots (1 mg) of each oligosaccharide were then accurately weighed on a micro balance and dissolved in a range of volumes (4–10 ml) of a mixture of ethanol, water and *n*-butanol corresponding to the column

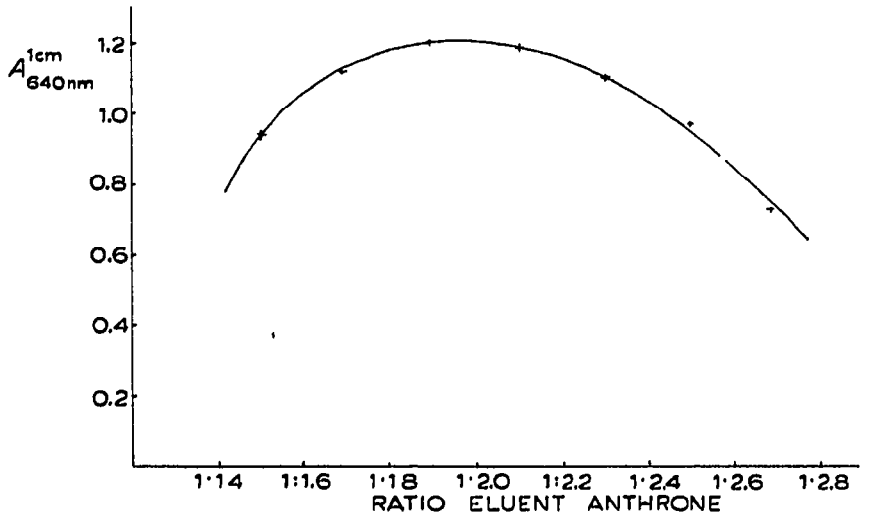

Fig. 3 Variation of absorbance with the ratio of eluent to anthrone reagent

eluent which would elute that oligosaccharide. These solutions were then pumped through the analyser and the area corresponding to 1 mg was found both in counts and by planimeter (Table I).

TABLE I

ANALYSER CALIBRATION

Peak area corresponding to 1 mg of each oligosaccharide. Results are the average of three determinations. Recorder 6 cm/h and minimum count rate.

	Peak area (counts/mg)	*Peak area (sq. cm/mg)*
Glucose	359 ± 26	41.6 ± 0.9
Fructose	420 ± 24	48.0 ± 1.6
Sucrose	406 ± 24	46.1 ± 0.9
Maltose	333 ± 18	37.5 ± 0 9
Maltotriose	320 ± 28	36.4 ± 1.5
Maltotetraose	275 ± 30	31.1 ± 1.4
Five glucose units	280 ± 15	31 4 ± 0.7
Six glucose units	267 ± 23	29.0 ± 1.1
Seven glucose units	274 ± 11	31.4 ± 0.1
Eight glucose units	293 ± 17	34.7 ± 0.9
Nine glucose units	304 ± 11	34.7 ± 0.3
Ten glucose units	251 ± 23	28.7 ± 1.3
Eleven glucose units	303 ± 17	34.5 ± 1.4
Twelve glucose units	278 ± 8	31 9 ± 0.9
Thirteen glucose units	293 ± 10	32.3 ± 0.9
Fourteen glucose units	209 ± 8	24.7 ± 0.4
Fifteen glucose units	223 ± 29	24.2 ± 1.3

Calculation of results

$$\frac{\text{Flow rate off column}}{\text{Flow rate to analyser}} \times \frac{\text{Area (counts)}}{\text{Area (counts) per mg}} \times \frac{100}{\text{wt. of sample (mg) put on column}} = \%\ \text{oligosaccharide in sample.}$$

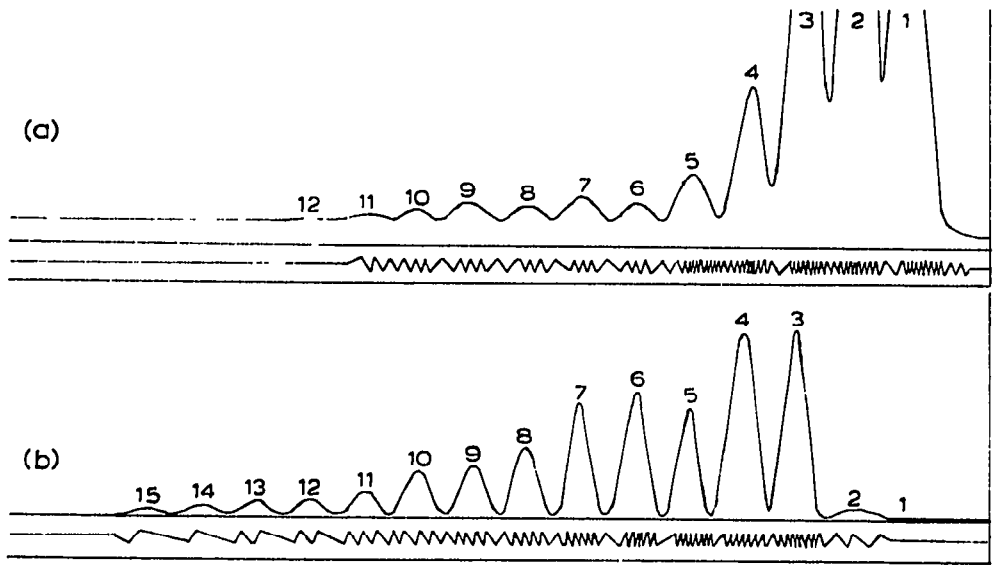

Fig. 4 Typical recorder traces obtained for (a) wort and (b) beer oligosaccharides. Peak numbers also represent glucose units

RESULTS AND CONCLUSIONS

Up to fifteen homologous glucose oligomers can be determined in wort, beer and brewing syrups in a 24-h run and some typical chromatograms are shown in Fig. 4. The fermentable sugars glucose, fructose, sucrose and maltose are present in

TABLE II

TYPICAL ANALYSIS OF THE CARBOHYDRATES IN WORT AND BEER

	Wort, gravity 1033		*Beer extract, gravity 1013*	
	% w/w of solids	*% w/v of sample*	*% w/w of solids*	*% w/v of sample*
Fructose	0.73	0.06	—	—
Glucose	6.42	0 53	—	—
Sucrose	4.12	0 34	—	—
Maltose	53.58	4.42	3.08	0.10
Maltotriose	18 91	1 56	6.76	0 22
Maltotetraose	3 03	0.25	7.08	0.23
Five glucose units	2.06	0 17	2.77	0 09
Six glucose units	0.85	0.07	3.88	0.11
Seven glucose units	1 58	0 13	4.00	0 13
Eight glucose units	1.70	0 14	4.31	0 14
Nine glucose units	1.33	0.11	3.69	0.12
Ten glucose units	0.24	0.02	2 77	0.09
Eleven glucose units	0.12	0.01	2.46	0.08
Twelve glucose units	0 12	0 01	1.54	0.05
Thirteen glucose units	0.24	0.02	1 23	0.04
Fourteen glucose units	—	—	0 61	0.02
Fifteen glucose units	—	—	0 31	0 01
Total	95.03	7.84	44.5	1 43

wort in too great an amount to permit determination by the analyser and these sugars are best determined by GLC or an alternative method.

The quantitative analysis of wort and beer is shown in Table II. The lower fermentable sugars were determined using GLC (ref. 17).

The oligosaccharides are separated on the basis of number of glucose units in the molecule, without regard to whether the linkages are α-1,4 or α-1,6. If information is required about the relative proportions of α-1,4 and α-1,6 branching, the collected fractions corresponding to each peak can be combined and treated with pure α and β amylase (α-1,4 bonds) or pullanase (α-1,6 bonds) and the fractions recycled to examine the relative amounts of the resulting carbohydrate residues.

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