# Characterization of Beer Polysaccharides by Enzymes and Gel Permeation Chromatography

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## ABSTRACT

Analyses were made on beer polysaccharides eluting in the excluded volumes of different polyacrylamide-gel bead columns. The distribution of polysaccharides in certain molecular-weight ranges was obtained. An example is a comparison of the excluded volume contents of Bio-Gel® P-2 and P-4 columns, with minimum molecular weights of 1800 and 4000, respectively. The range between 1800 and 4000 is calculated by difference. Analyses of six American and one German beer showed similar distributions of molecular-weight ranges. The carbohydrate of the molecular-weight range of 1800 and greater could be partially decreased not only by amyloglucosidase but also by β-glucanase or pronase. Thus, there is residual β-glucan and protein-bound carbohydrate, in addition to dextrin. There was no significant difference in the amount of carbohydrate in the excluded volumes of P-10 and P-30 columns, showing that the contents of the former contain polysaccharide with a minimum molecular weight of 40,000. The action of amylases during malting and mashing should leave little dextrin of this molecular size; nevertheless, a considerable portion of this fraction is degradable by amyloglucosidase.


Key words: *Beer, Gel permeation, Polysaccharides.*

The polysaccharides of beer are known to be principally dextrin. There does, however, appear to be significant polysaccharide remaining in beer after incubation with amyloglucosidase. Some beer carbohydrate is peptide-bound (10,11,12). Enevoldsen and Schmidt (3) suggested that β-glucan may be present in beer, although the levels would be very low.

The higher dextrins are generally believed to contribute to the desirable properties of beer, such as foam stabilization, mouth-feel, and flavor. However, Otter *et al.* (8) concluded that beer dextrins have no flavor.

In order to obtain more information about the size and composition of beer polysaccharides, it was necessary to devise techniques for measuring polysaccharides beyond the sizes reported by Dellweg *et al.* (1), Enevoldsen (5), and Enevoldsen and Schmidt (3,4).

This study presents results obtained by using a combination of the polyacrylamide-gel column technique of Dellweg *et al.* (1) and Enevoldsen and Schmidt (4) and the multicolumn method for protein reported by Guenther and Stutler (6).

It was anticipated that this new procedure would give a polysaccharide molecular-weight separation according to the properties of the various Bio-Gel® columns used. The carbohydrates found in the excluded volumes should therefore separate according to the scheme shown in Table I.

Beer was also treated with protease, amylase, and β-glucanase to determine if the various molecular-weight polysaccharides would be further degraded by any of these enzymes.

## EXPERIMENTAL

### Analytical Methods

Total carbohydrate was determined by the phenol-sulfuric acid method (2). A tannin-turbidimetric method (7) was used for protein determination. Reducing groups were measured by the Nelson method (from ref. 9).

### Enzymatic Treatment of Beer

The susceptibility of the beer polysaccharides to enzymatic attack was tested using pronase, amyloglucosidase, pullulanase, pseudomonas isoamylase, β-glucanase, *Bacillus subtilus* α-amylase, and barley β-amylase. These enzymes were all available commercially. Sigma Chemical Co. was the source of α-amylase (#A-6380), pronase (#P-5130), and amyloglucosidase (#A-7255). The latter was also obtained from Bio-Dynamics/bmc (#7384405). Isoamylase was obtained from Hyashibara of Japan. β-amylase was a product of Calbiochem (#17157). Pullulanase was obtained from the Enzyme Development Corporation. The β-glucanase had to be purified, as described below.

Each beer sample was adjusted to a pH optimal for the particular enzyme used except for amyloglucosidase and isoamylase, which were incubated at beer pH. The pH conditions are listed in Table II.

In each case, an excess of enzyme was used to ensure complete degradation of any possible substrate. All incubations were at room temperature, and proceeded for 16 hr. At the end of the incubation period, portions of the sample were injected into each of the Bio-Gel P columns used.

### β-Glucanase Purification

The starting material was obtained from the Grinsted Corp., Denmark. It contained amylase and protease activity. There was also material present which reduced cupric ions, making the Nelson method for reducing groups inoperable. One liter of this starting material was gradually adjusted to pH 8.1 with 5*N* NaOH. A precipitate was removed by centrifugation (23,000 × *g* for 20 min). The pH was adjusted with glacial acetic acid to 5.5, the optimum for activity, and then the solution was dialyzed exhaustively against running tap water for 10 hr. A change to fresh dialysis tubing was made after 4 hr to prevent digestion of the membrane. Lyophilization gave about 40 g of crusty material.

A DEAE Sephadex A-50 column, 2 × 24 cm, was equilibrated with 0.1*M* tris buffer, pH 7.5. Five hundred milligrams of lyophilized material was dissolved in a minimum of the buffer and applied to the column. Twenty-milliliter fractions were collected. The first eluant was 80 ml of the buffer. The second and third eluants of 80 ml each contained 0.15*M* NaCl and 0.3*M* NaCl, respectively, in the buffer. The β-glucanase activity eluted with the 0.15*M* NaCl. These four fractions were active on both laminarin and p-nitrophenyl-β-D-glucoside.

The pooled fractions still retained some activity on soluble starch (Merck; prepared according to Lintner). It was not clear whether this was residual amylase activity or if the β-glucanase was hydrolyzing minor contaminants in the starch, such as cellulose, or possibly β-glucan.

**TABLE I**
**Molecular Weights of Bio-Gel Exclusion Limits and Polymer Standards**

| Gel No. | Minimum Exclusion | Standards Tested | | Elution Position |
|---|---|---|---|---|
| | | Polymer | Molecular wt | |
| P-2 | 1800 | FTIC Dextran® | 3000 | Excluded |
| P-4 | 4000 | FTIC Dextran® | 3000 | Retained |
| P-4 | 4000 | Inulin | 4000 | Excluded |
| P-6 | 6000 | Inulin | 4000 | Retained |
| P-6 | 6000 | Dextran T-10® | 10,000 | Excluded |
| P-10 | 20,000 | Dextran T-10® | 10,000 | Retained |
| P-10 | 20,000 | Dextran T-40® | 40,000 | Excluded |
| P-30 | 40,000 | Dextran T-40® | 40,000 | Retained |

**TABLE II**
**pH Conditions for Enzyme Treatment of Beer**

| Enzyme Incubated | pH |
|---|---|
| Pronase | 4.0 |
| Pullulanase | 5.7 |
| β-Glucanase | 5.5 |
| α-Amylase | 7.0 |
| β-Amylase | 5.7 |

[1]Presented at the 42nd Annual Meeting, Milwaukee, May 1976.

### Multicolumn Gel Filtration

The flow schemes for the chromatography and the autoanalyzer are shown in Figs. 1 and 2. The diagram in Fig. 1 is essentially that of Dellweg *et al.* (1). Five polyacrylamide columns containing Bio-Gel P-2, P-4, P-6, P-10, or P-30 were used (Fig. 2). Each column was 1.2 × 88 cm in length. The gels were swollen with deionized wat4r. Degassed deionized water was the eluant, with a flow of 0.32 ml/min.

Blue Dextran® was applied to each column to determine the times at which the excluded volume constituents would appear. It was also necessary to determine if the assumptions about the minimum exclusion limits for each column were correct. For example, solutes ≥ 1800 Daltons elute in the excluded volume of Bio-Gel P-2; anything smaller is retained. Solutes ≥ 4000 Daltons elute in the excluded volume of P-4; smaller molecules are retained. Thus, FTIC® Dextran (3000 $\overline{MW}$) should elute in the excluded volume of P-2, but be retained by P-4. Table I shows the molecular weights of the polysaccharide standards, the columns tested with each standard, and whether the standard should elute in the excluded volume or be retained. In our tests, the position of elution for each standard was confirmed.

Glucose standards were used to prepare standard curves for each column; the range was 30 to 225 μg glucose. This enabled the quantitative measurement of the carbohydrate. Prior knowledge of the elution time for glucose in any column indicated when the column, injected with a beer sample, would be cleared for another injection.

The sample sizes were 200 μl each for the P-30 and P-10 columns, 100 μl for the P-6 column, and 20 μl for the P-2 column. The carbohydrate which was actually measured was that eluted in the excluded volume of each column. The corresponding curves traced on the recording charts were symmetrical. Quantitative measurement could be obtained either by use of a planimeter or by multiplying height times width at half-height.

In order to complete the chromatography of all five columns within a working day, it was necessary to devise a schedule for sample injection, and for connecting and diverting each column eluate. The time intervals for the sequence of operations, along with the corresponding recorded chromatogram, are shown in Fig. 3, a and b. The sequence of injections started with the P-30 column, followed by columns P-10, P-6, P-4, and finally P-2. As the curve for the excluded volume from a column appeared on the chart, that column effluent was diverted to "excess" (Fig. 2) and the effluent of the next column was connected. The timing of the diversion relative to the appearance of the curve on the chart differed for each column, because of differences in each gel.

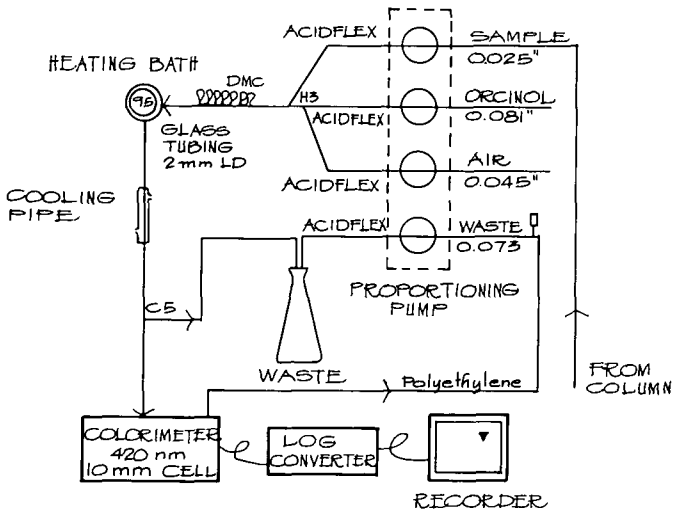


Fig. 1. Flow scheme for chromatography and analysis of carbohydrates using autoanalyzer system.

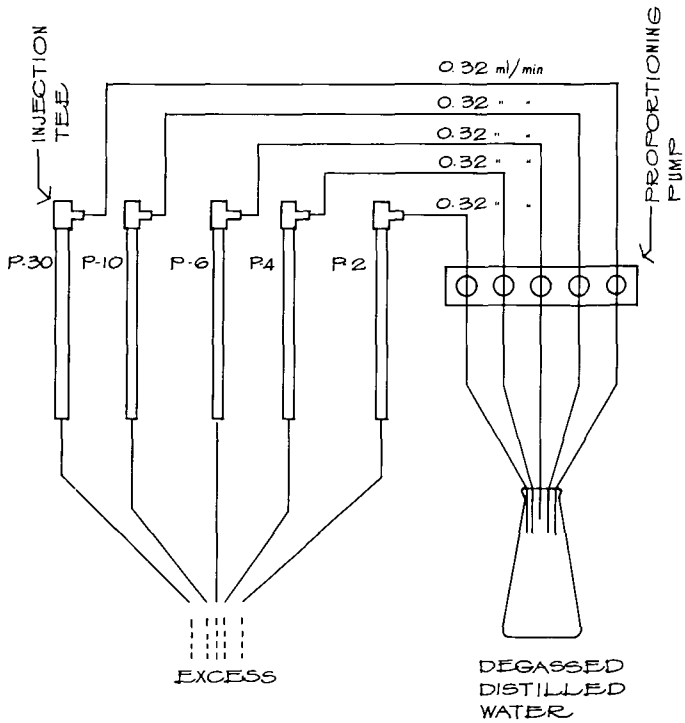


Fig. 2. Multicolumn chromatography flow scheme.

### Isolation of Beer Dextrin

A 400-ml sample of beer was dialyzed in a membrane which retained solutes ≥ 3500 Daltons (Spectrapor Co., Los Angeles, Calif.). After exhaustive dialysis, the dialysate was adjusted to pH 3 and passed through a Dowex 50 × 8 ($H^+$) to remove protein. The eluate was lyophilized and yielded slightly over 4 g.

### Isolation of Beer Protein

Beer was treated with an equal volume of 40% trichloroacetic

**TABLE III**
**Size Distribution (Daltons) of Polysaccharides in Beer and Wort**

| | Per Cent of Total Carbohydrate | | | | | |
|---|---|---|---|---|---|---|
| Sample | ≥40,000 | 20,000–40,000 | 6000–20,000 | 4000–6000 | 1800–4000 | ≤1800[a] |
| Beer | 3.6 | 0.4 | 2.2 | 4.0 | 23.1 | 67.7 |
| Wort | Over 20,000 1.3 | | 0.6 | 4.7 | 7.0 | 86.4 |

[a]Difference between the total carbohydrate and P-2 excluded volume carbohydrate.

**TABLE IV**
**Polysaccharide Size Distribution in Commercial Beers**

| Sample | ≥40,000 | 20,000–40,000 | 6000–20,000 | 4000–6000 | 1800–400 | ≤1800[a] |
|---|---|---|---|---|---|---|
| U.S.: | | | | | | |
| Beer A | 2.1 | 2.3 | 1.0 | 2.9 | 32.2 | 67.8 |
| Beer B | 1.3 | 1.6 | 1.8 | 2.1 | 29.6 | 70.4 |
| Beer C | 1.5 | 2.2 | 0.9 | 1.3 | 25.8 | 74.2 |
| Beer D | 2.3 | 2.8 | 1.6 | 1.5 | 30.9 | 69.1 |
| Beer E | 1.0 | 1.5 | 1.8 | 2.2 | 23.7 | 76.3 |
| Beer F | 1.6 | 2.4 | 2.5 | 2.2 | 28.9 | 71.1 |
| Beer G | 3.6 | 0.4 | 2.3 | 4.0 | 23.1 | 76.9 |
| German | 3.1 | 4.7 | 0.5 | 0.9 | 30.2 | 69.8 |

[a]Difference between the total carbohydrate and P-2 excluded volume carbohydrate.

acid and held at 0°C overnight. The pellet obtained upon centrifugation was dialyzed to remove the trichloroacetic acid, and then lyophilized.

**Preliminary Isolation of a Carbohydrate-Bound Protein**

A 50-mg sample of beer protein was applied to a 2.5 × 62-cm Sephadex G-100 column, equilibrated with 0.1 *M* $NH_4OH$ adjusted to pH 10 with HCl. This pH 10 solution was used as eluant. Fractions were monitored for carbohydrate by the phenol-sulfuric acid method and for protein by tannin turbidimetry. Fractions with a high carbohydrate-to-protein ratio, and which eluted shortly after the excluded volume, were collected from duplicate columns and pooled.

## RESULTS

**Size Distribution of Polysaccharides**

Typical size distribution patterns of polysaccharides in beer and wort are shown in Table III. The principal difference between the two sets is that much more of the wort carbohydrate is fermentable sugar which does not appear in the excluded volumes.

A comparison of polysaccharide size patterns for some commercial beers is given in Table IV. There is an overall similarity between brands. The polysaccharides between 1800 and 4000 Daltons range from 23.1 to 32.2%. The amount of carbohydrate in each of the larger-size groups is small. The size group of 4000–6000 Daltons is a narrow size range. Therefore, its composition of about 1 to 4% is a significant part of the total composition. The largest range of sizes measured, with a molecular weight ≥ 40,000, was 1 to 3.5% of the total.

**Amyloglucosidase, Pronase, and β-Glucanase Treatments**

The data from these experiments are shown in Fig. 4. Each bar

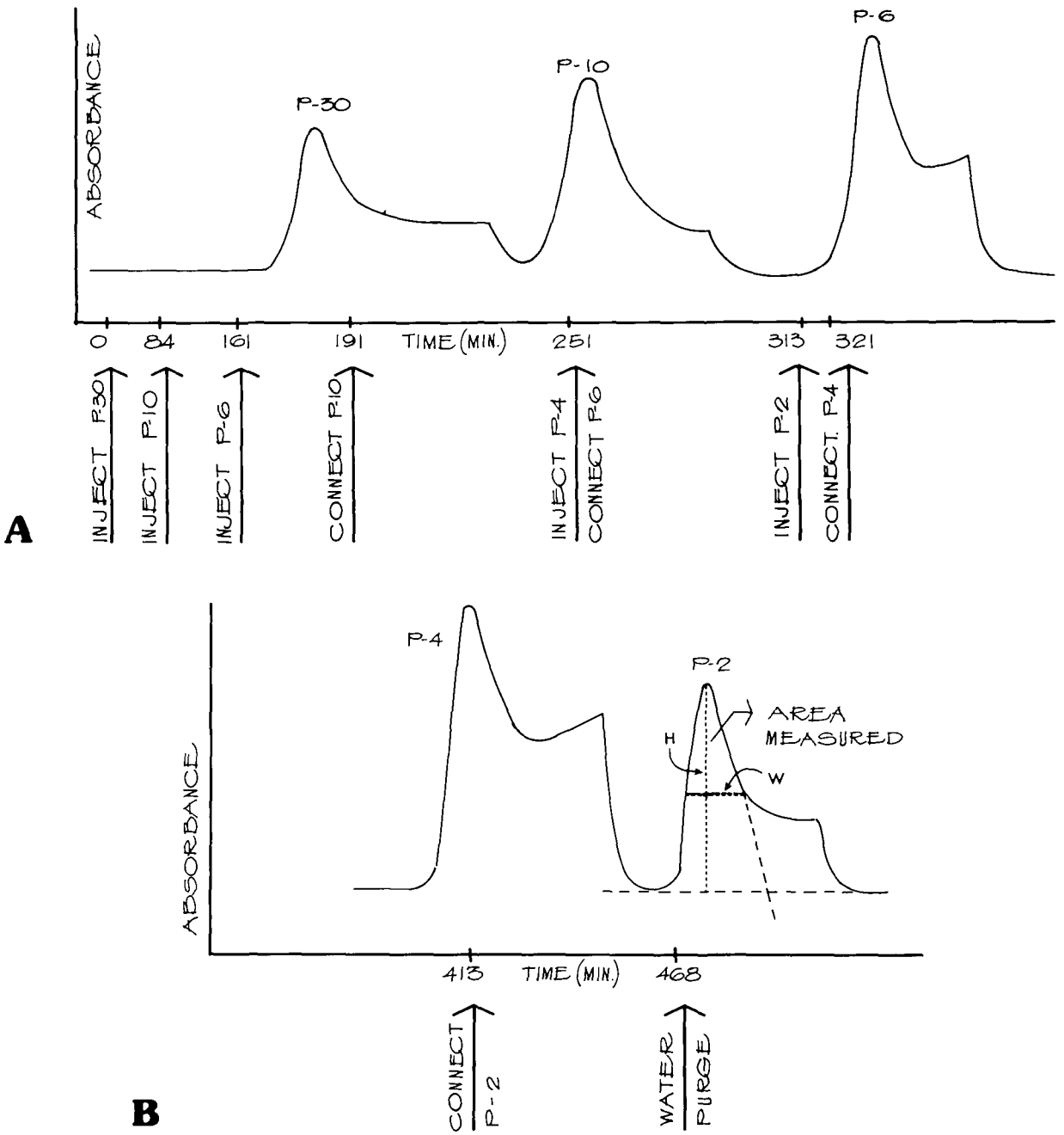


Fig. 3. Operating sequence and recorder chart for multicolumn chromatography: a) appearance of excluded volume curves from injections onto columns P-30, P-10, and P-6; b) appearance of excluded volume curves from injections onto columns P-4 and P-2.

illustrates the per cent of the total carbohydrate represented by a size range, before and after enzyme treatment. Amyloglucosidase digests 86% of the polysaccharide in the range of 1800 to 4000 Daltons, and 98% of that in the 4000 to 6000 range. Smaller

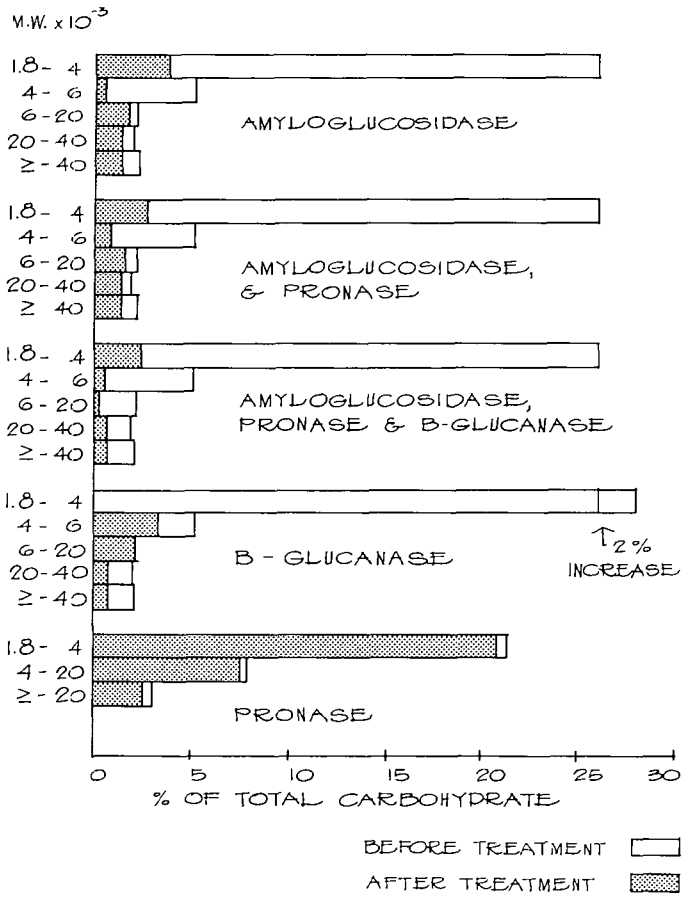


Fig. 4. Size distribution of beer polysaccharides after enzyme treatment.

percentages of the larger size ranges were hydrolyzed, but they are significant. Of the range $\geqslant$ 40,000 Daltons, 38% was degraded. Pronase digestion effected a reduction in some of the larger fractions. This was undoubtedly due to hydrolysis of polypeptide material attached to carbohydrate; no increase in reducing groups was detected. The carbohydrate fragments then eluted in the region of smaller molecular-weight ranges. $\beta$-glucanase degraded 35% of the size range of 4000 to 6000 Daltons. The larger size ranges were more susceptible; 73% of the 20,000–40,000 and 71% of the $\geqslant$ 40,000 molecular-weight ranges were degraded. This particular enzyme seemed to release smaller polysaccharide fragments, some of which were apparently in the 1800 to 4000 molecular-weight range. This group actually showed an increase. The most extensive degradation seemed to occur when amyloglucosidase was followed by pronase and then $\beta$-glucanase. Yet, some polysaccharide of unknown composition remains.

The presence of pronase-susceptible carbohydrate substrate warranted further investigation of polypeptide-bound polysaccharide. This was pursued by using a P-2 column long enough to resolve dextrins with a degree of polymerization (D.P.) $\leqslant$ 9, according to the method of Dellweg *et al.* (1). As seen in Fig. 5, a, extensive amyloglucosidase digestion left polysaccharide residues which are normally part of the analyses of dextrins. A substantial portion of these components is bound to polypeptide. In Fig. 5, b, the chromatographic pattern resulting from pronase digestion reveals a breakdown of these components. (Note that this sample size is doubled).

In another investigation related to peptide-bound polysaccharide, beer protein was chromatographed on a Sephadex G-100 column. A protein component was collected which eluted shortly after the excluded volume. It contained twice as much carbohydrate as protein and showed little tendency to foam. This component would be included in the polysaccharides measured in the excluded volumes of the Bio-Gel columns. Thus, there are different kinds of evidence that a part of the beer polysaccharide is bound to polypeptide.

The nature of the peptide-carbohydrate linkage is not known; *cf.*, the serine-glycosyl bond found in many glycoproteins. To determine if there was a heat-driven conjugation of polypeptide and polysaccharide during malt kilning, worts from unkilned (lyophilized) and kilned Larker malts were compared. The polysaccharide size distributions are shown in Table V. There is no evidence for such a conjugation. The wort from kilned malt does

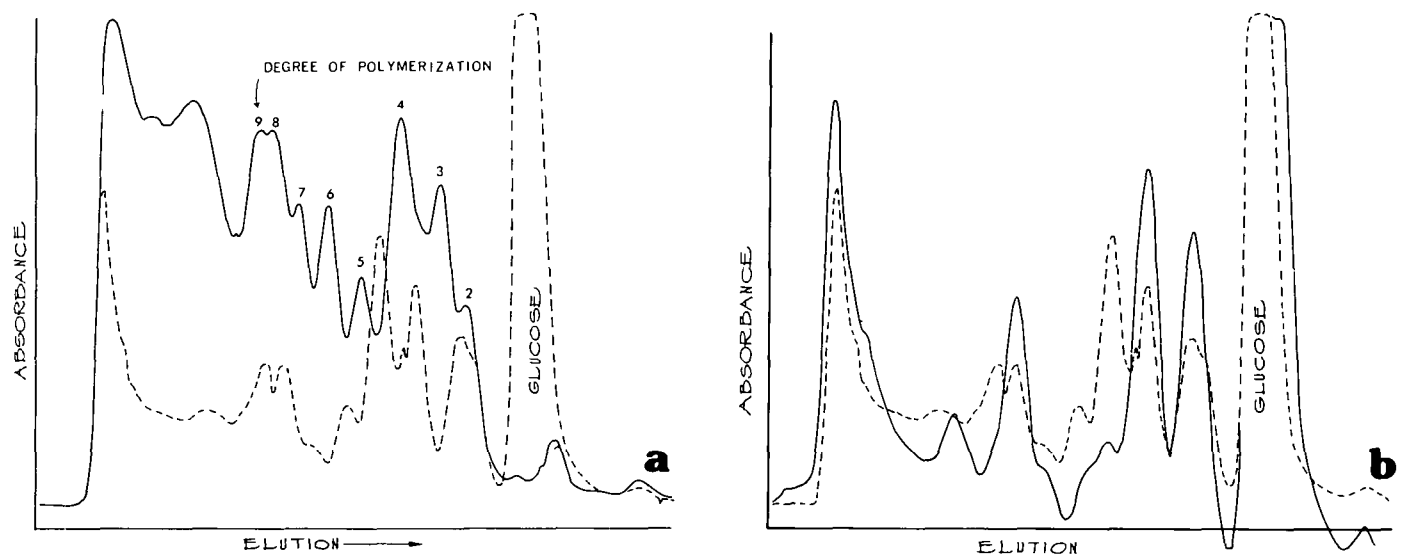


Fig. 5. Chromatography of beer polysaccharides on Bio-Gel P-2: a) untreated ——, amyloglucosidase-treated - - -; b) amyloglucosidase-treated - - -, pronase-treated (two times sample size) ——.

not show a relative shift to higher molecular-weight distribution of the polysaccharides.

**Digestion with Amylases**

Data presented in Fig. 6 show the results of treating the beer polysaccharides with various amylases, including another amyloglucosidase digestion for purposes of comparison. α-Amylase caused a 59% reduction of the dextrin in the 1800–4000 molecular-weight range. In the 4000–20,000 molecular-weight range, the reduction was 72%. About 11% of the sizes ⩾ 20,000 Daltons was hydrolyzed. β-Amylase had little effect on the size distribution; in combination with α-amylase there was a very slight increase in digestion in the size range of ⩾ 20,000 Daltons over α-amylase treatment alone.

The debranching enzymes, pullulanase and isoamylase, showed similar degradations in two of the size ranges. They both reduced the 1800–4000 molecular-weight range by 60%. In the 4000–20,000 Dalton range, pullulanase degraded 47% and isoamylase degraded 74%. Pullulanase degraded 22% of the size range of ⩾20,000 molecular weight, but isoamylase did not show any effect.

**Dextrins and Foam Stabilization**

A very limited investigation was conducted on the role of beer dextrin in foam stabilization. Beer dextrin and beer protein, each isolated as described above, were dissolved singly or together in carbonated water at beer concentrations. As expected, the protein foamed. The dextrin did not foam and it is significant that it did not increase the collapse time of the foam formed from the protein addition. No further investigation of the effect of the various fractions on foam was carried out.

## DISCUSSION AND CONCLUSIONS

The use of gel permeation chromatography and enzymatic degradations revealed certain properties of beer polysaccharides. The range of molecular weights was 1800 and greater.

The size range of 1800–4000 Daltons comprised approximately 20 to 30% of the total carbohydrate in several beers. It is mostly, but not entirely, dextrin. Amyloglucosidase, α-amylase, and debranching enzymes degraded 86, 59, and 60% of it, respectively. A small amount was degraded by pronase.

The narrowest size range studied, 4000 to 6000 Daltons, comprised 1 to 4% of the total carbohydrate in several beers. It was susceptible to partial degradation by amyloglucosidase and β-glucanase.

The size range of 6000–20,000 Daltons was about 0.5 to 2% of the total carbohydrate. It was mainly susceptible to amyloglucosidase attack, as about one-third was degraded. β-Glucanase digested only about 4% of the polysaccharide in this molecular-weight range.

In experiments where a P-6 column was not employed, a size range of 4000–20,000 Daltons was studied. Although these data are not listed in Table IV, this size range was about 1.5 to 4.5% of the total carbohydrate in several beers. It was extensively degraded by several amylases: amyloglucosidase, 72%; α-amylase, 72%; pullulanase, 47%; and isoamylase, 74%.

The size range of 20,000–40,000 Daltons showed a composition of 0.4 to 4.7% in the beers studied. This group may have more β-glucan than dextrin; β-glucanase degraded 73% and amyloglucosidase degraded 39%.

The largest size range studied was that in the excluded volume of a P-30 column, with a minimum molecular weight of 40,000. In many samples, this group contained the same amount of carbohydrate as that in the P-10 excluded volume. In these cases, there could be no distinction made between ⩾ 20,000 and ⩾ 40,000 Daltons and, consequently, the carbohydrate consisting of 20,000–40,000 Daltons was below the detection limits. The size range of ⩾ 20,000 Daltons showed susceptibility to α-amylase, pronase, and pullulanase, with 11, 12, and 22% digestion, respectively. Isoamylase did not degrade this fraction.

Finally, the range of ⩾40,000 Daltons, which comprised from 1 to 3.6% of the total carbohydrate in the beers studied, was extensively digested by amyloglucosidase and β-glucanase.

These results show that dextrins of considerable size still appear in wort after malting and mashing. As an example, the size range of 4000–20,000 Daltons is theoretically 25 to 125 hexose units. It contains considerable substrate for debranching enzymes. This supports the finding of Enevoldsen (5), who stated that debranching enzymes could be used to increase the fermentable sugar.

In addition to dextrin, there is β-glucan of considerable polymer length in beer. There appears to be polysaccharide material of up to 40,000 Daltons degradable by β-glucanase. Theoretically, 40,000 molecular weight is 250 glucose units. Before drawing such conclusions, it should be noted that the molecular weight data for gel permeation is based on globular proteins. It is not known if these partially-characterized beer polysaccharides have an axial ratio comparable to globular proteins.

The results from pronase treatment support work by Roberts (10), who found evidence for a heterogeneous mixture of carbohydrate-polypeptides in beer. The present work, and Roberts' work, open questions on the role of beer dextrins in foam stabilization. In the present study, no foam stabilizing effect by dextrin was found. At the time this work was being conducted, Roberts' work had not been presented. His results showed that

**TABLE V**
**Polysaccharide Size Distribution in Wort from Lyophilized and Kilned Larker Malt**

| | Per Cent of Total Carbohydrate | | | |
|---|---|---|---|---|
| Drying Method | 20,000 | 4000–20,000 | 1800–4000 | ⩽1800[a] |
| Lyophilized | 1.95 | 5.25 | 8.40 | 84.4 |
| Kilned | 1.02 | 0.86 | 4.83 | 93.3 |

[a]Difference between the total carbohydrate and P-2 excluded volume carbohydrate.

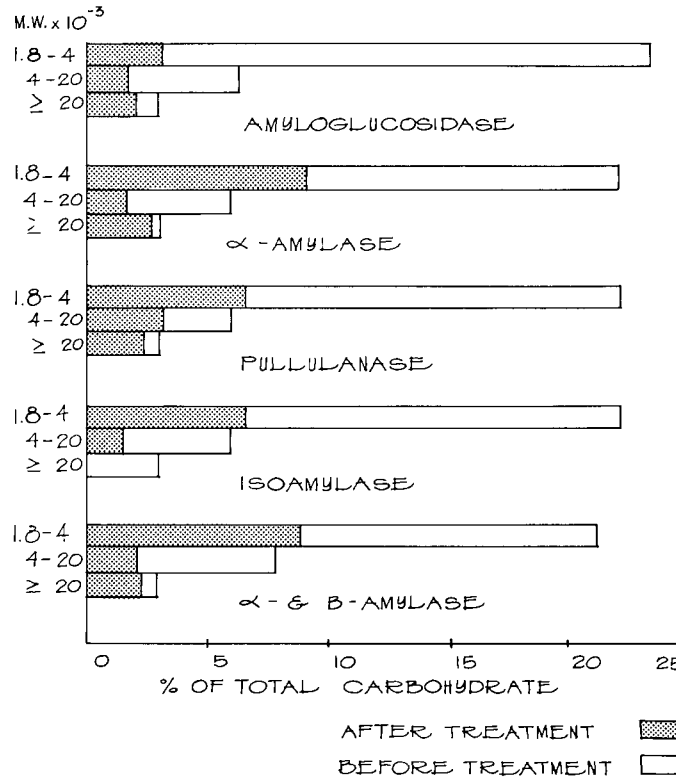


Fig. 6. Size distribution of beer polysaccharides after amylase treatments (includes another amyloglucosidase digestion for comparison).

polypeptide-bound carbohydrate is important in foam stabilization. Earlier work by Saruno and Ishida (11,12) showed that glycopeptide material isolated from beer had strong foaming tendencies. Dextrin preparations used in this study included a passage through a cation-exchange column, which presumably removed the materials studied by Roberts and by Saruno and Ishida. It is most likely that certain fractions of the protein- or polypeptide-bound carbohydrate, and not simple dextrin, are important in foam stabilization. This investigation also revealed the presence of some polypeptide-bound carbohydrate fractions which did not foam.

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[Received June 14, 1976.]