

# Trends in Wort Carbohydrate Utilization

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A gas chromatographic method suitable for any type of low-molecular-weight carbohydrate analysis has been utilized to determine the individual wort sugars in corn adjunct wort from a Western Canadian brewery. The fluctuations in each sugar during primary lager fermentation have been graphed. "End fermented" wort has been shown to contain some maltotriose, a small amount of maltose, and the nonfermentable carbohydrates, including maltotetraose, maltopentose, and dextrans.

Qualitative and quantitative carbohydrate analyses of wort have become increasingly more important to the brewer to detect differences in the composition of wort from brew to brew, mainly to predict or ensure the degree of attenuation (10). For a number of years, however, reliable analytical work on wort or beer carbohydrate composition depended on the time-consuming separation of individual carbohydrates by paper chromatography followed by elution, and the application of a colorimetric and quantitative chemical assay (15, 16, 25). Most often, therefore, only fermentable carbohydrate (3, 10), or total carbohydrate by the anthrone method (9, 17, 21) or by specific gravity determination (5), is followed. The desire to eliminate tedious paper chromatographic methods has led to interesting developments in gas-liquid chromatographic (GLC) analysis (22). The application of this newer method to carbohydrates, however, awaited the contribution of Sweeley et al. (24), who prepared and chromatographed a number of carbohydrates in the form of volatile trimethylsilyl derivatives. Since that time, a number of other papers have appeared (for review, see references 20 and 22). For the brewing industry, the reports by Marinelli and Whitney (12, 13), Otter and co-workers (18, 19), and Clapperton and Holliday (4) have been most significant.

The application of this method for individual sugar analysis, except in quality control of initial wort, or final beer, has been more limited. For example, the work of Harris and co-workers (7, 8) with paper chromatography has not been extended to show the disappearance of individual sugars in wort during commercial fermentation by using the more sensitive and more rapid GLC method, even though

Griffin (5, 6) has used the method with the top fermenting ale yeast, *Saccharomyces cerevisiae*, growing in a stirred laboratory fermenter.

A gas-liquid chromatography system in the present study has been used to qualitatively and quantitatively determine the fermentable carbohydrate levels in beer wort throughout the course of a Western Canadian commercial lager fermentation. We have made use of this method to apply the results toward a study on the effect of yeast environment on flocculation of *S. carlsbergensis* (manuscript in preparation).

## MATERIALS AND METHODS

**Fermentation.** A typical closed 8,600-gallon (39,000-liter) brewery fermentor was used, maintained at 57 F (14 C) throughout primary fermentation until the cooling stage was reached. Samples were taken from a lower port and a higher port (upper) near the center of the tank. Only the lower port samples have been discussed and plotted.

**Gas chromatography (12, 13).** All GLC analyses were performed with a Hewlett Packard model 5750 B chromatograph equipped with a thermal conductivity detector at 355 C with a 120-mA bridge current.

The column used was copper (2 ft by ¼ inch [60.96 by 0.635 cm] outside diameter) packed with 3% SE-52 Silicone gum rubber on 60/80 mesh Chromosorb W AW-DMCS (Chromatographic Specialties, Brockville, Ontario). The carrier gas was 80 ml of helium per min. The oven program from 150 to 350 C was carried out at a rate of 6 to 10 degrees per min with the injection port at 375 C. An attenuation of 1 was usually used. The strip chart recorder was a Hewlett Packard model 7127A run at 0.5 inch (1.27 cm)/min.

The method described by Marinelli and Whitney (12, 13) was used to prepare trimethylsilyl derivatives but with the incorporation of phenyl-β-D-glucopyranoside as an internal standard for quantitation. To each vial containing 0.5 ml (approximately 60 mg solids) of wort was added 1.0 ml of pyridine (silylation grade,

Pierce Chemical Co., Rockford, Ill.) containing 5 mg of phenyl- $\beta$ -D-glucopyranoside per ml. Then 0.9 ml of 1,1,1,3,3,3-hexamethyldisilazane and 0.1 ml of trifluoroacetic acid (Aldrich Chemical Co., Milwaukee, Wis.) were added. The vials were shaken for 30 s and then allowed to stand at 20 to 22 C for 15 min with occasional shaking. Reactions are quantitative using this method (20). When insoluble components were seen, the reaction mixtures were warmed prior to injection. Injections of 10 to 50  $\mu$ liters were done in duplicate by using a Hamilton syringe (Hamilton Co., Whittier, Calif.). Peak areas were calculated by cutting and weighing xeroxed peaks. The retention times were obtained by injection of standard solutions of sugars found in wort. Relative response values were also calculated for all sugars of lower molecular weight than maltotriose.

**Yeast dry weight determinations.** Wort samples were centrifuged by using a Sorvall SS-1 super-speed angle centrifuge (Ivan Sorvall Inc., Norwalk, Conn.) at a relative centrifugal force greater than  $5,400 \times g$ . Supernatant fluid was discarded, and cells were washed twice and resuspended in 0.05 M phosphate buffer (pH 6.4) in  $\frac{1}{2}$  the volume of the original sample. Triplicate 2-ml samples of the resuspensions and of the resuspending buffer were transferred to pre-weighed aluminum foil pans. Dishes were dried to constant weight at 105 C, and cell mass/ml of wort was calculated.

**Chemical determinations.** Total N in wort was determined by the Kjeldahl method as adapted by Bremner (2). Prior heating of samples in 0.5 ml of concentrated  $H_2SO_4$  was used to prevent excessive foaming during digestion (1). The distillate was titrated with standard  $H_2SO_4$  to an end point of pH 4.9 by using a Radiometer automatic titrator type TTT1 (Radiometer, Copenhagen, Denmark).

Protein nitrogen in wort was estimated by using the method of Lowry (11) with crystalline (X3) egg albumin (Nutritional Biochemical Corp., Cleveland, Ohio) as standard. Results were read at 750 nm (9) on a Spectronic 20 colorimeter (red phototube and filter).

Triplicate anthrone tests for total carbohydrate in wort and cell samples were carried out by the method outlined by Herbert et al. (9), adapted for more reproducibility from Morris (17). Standard curves were prepared for each essay. A Beckman B spectrophotometer at 625 nm rather than a colorimeter was used as advised by Herbert et al. (9).

## RESULTS AND DISCUSSION

Table 1 shows a typical carbohydrate composition of a Western Canadian corn adjunct brewers wort. Fermentable carbohydrates in the table were calculated by GLC analysis. Total carbohydrate was calculated by anthrone, and both figures were used to determine the amount of nonfermentable carbohydrate. The major sources of fermentable sugar are glucose, maltose, and maltotriose. The levels of individual carbohydrates in Canadian lager beer do not

completely correspond to English ale (14). Primary fermented wort in Table 1 is actually beer after the initial 5 to 6 day fermentation and cooling cycle. At this time, all fructose, sucrose, and glucose, most of the maltose, and 80% of maltotriose have disappeared from the wort (94% of all fermentable sugar). Maltotetraose, maltopentose (detected but not quantified by this GLC method), and the larger-molecular-weight, nonfermentable dextrins remaining after primary fermentation are not attacked by *S. carlsbergensis*, but were measured by anthrone.

In Fig. 1, the trends of total fermentable carbohydrate, wort nitrogen levels, and yeast mass in suspension have been recorded. The decrease in total nitrogen is almost entirely a decrease in low-molecular-weight nitrogen compounds (amino acids, small peptides, and inorganic nitrogen). Protein nitrogen, for example, as measured by the Lowry method (11), shows little decrease during the fermentation, with some of this decrease due to utilization of Lowry-positive amino acids. Yeast mass throughout this time increases dramatically (450%) in the wort. In the later stages the decrease in mass is due to the phenomenon of flocculation, as yeast sink by the lower sampling port to sediment at the bottom.

Figure 2 is a representation of the fermentable carbohydrates in a typical chromatogram of corn adjunct brewers wort. By using peak weights (areas) and the calculated relative response values in Table 2, Fig. 3 was graphed showing the levels of each carbohydrate

TABLE 1. Carbohydrate analysis of Western Canadian corn adjunct wort

Sugars	Fermen- table car- bohydrate (%)	mg of carbohy- drate/100 ml of wort		% of each utilized
		Wort	Primary fer- mented wort	
Fructose	1.34	126	0	100
Glucose	15.71	1,473	0	100
Sucrose	1.89	177	0	100
Maltose	66.95	6,278	275	95.6
Maltotriose	14.10	1,322	270	79.6
Fermentable carbohydrate		9,376	545	94.2
Nonfermentable carbohydrate		2,374	2,355	0.8
Total carbohy- drate		11,750	2,900	75.3

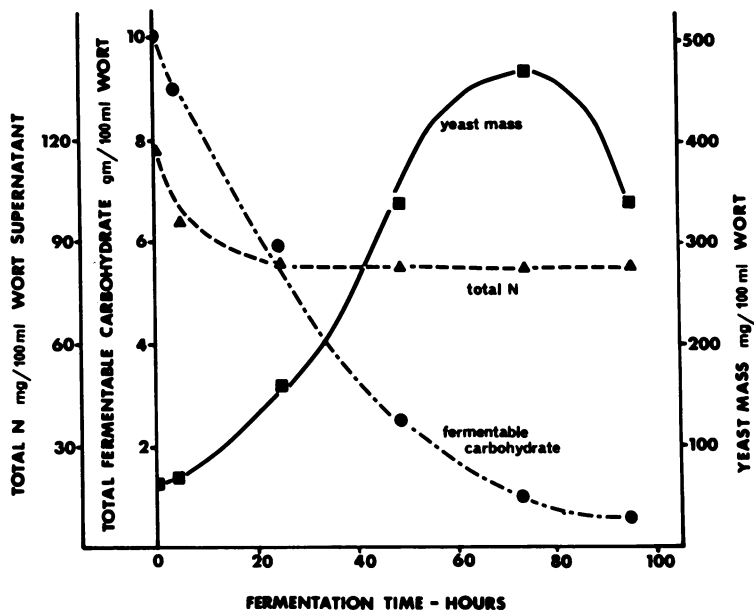


FIG. 1. Total fermentable carbohydrate, total nitrogen, and suspended yeast cell mass in wort throughout primary fermentation.

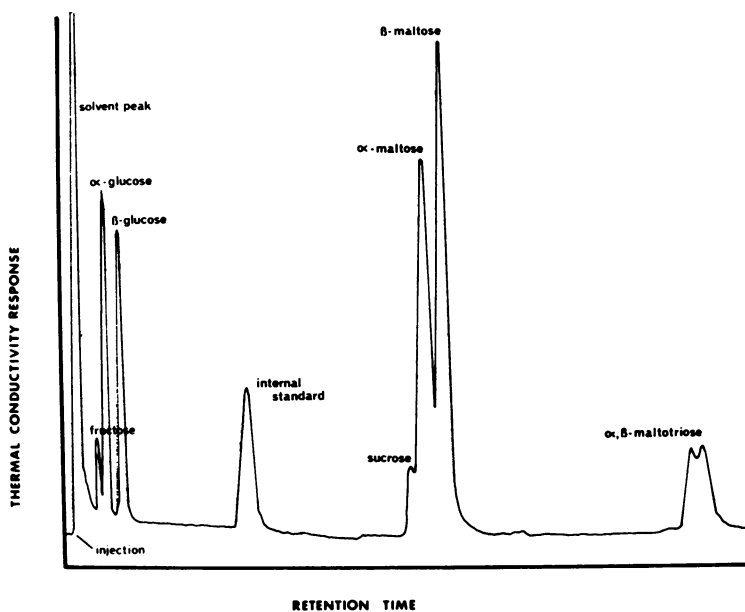


FIG. 2. Simulated gas chromatogram of corn adjunct brewers wort.

throughout primary fermentation. Figure 3 reflects the ease of following environmental changes in a medium by this method compared to previous studies (7, 8).

Sucrose decreased rapidly to an undetectable level during the first 5 h of the fermentation. The sucrose probably is hydrolyzed by invertase

(sucrase), a yeast enzyme situated between the cell wall and cell membrane (23). However, Harris et al. (7) state that glucose is used first from wort, followed by fructose and then sucrose. In this experiment, the sucrose was hydrolyzed first. Fructose levels increase to the 5-h sampling period, reflecting hydrolysis of su-

TABLE 2. Retention times and relative response values (RRV) of wort sugars

Sugar	Retention time (min)	RRV <sup>a</sup>
Fructose	0.63	0.2030
$\alpha$ -Glucose	0.88	0.2308
$\beta$ -Glucose	1.13	0.2308
Sucrose	8.4	0.0865
$\alpha$ -Maltose	8.8	0.1521
$\beta$ -Maltose	9.3	0.1521
$\alpha, \beta$ -Maltotriose	15.3, 15.5	0.1521 <sup>b</sup>
Maltotetraose	22	
Phenyl- $\beta$ -D-glucopyranoside	4.38	Internal standard

<sup>a</sup> RRV/mg of sugar = (area sugar peak  $\times$  attenuation/area internal standard  $\times$  attenuation)  $\div$  mg of sugar in 0.5 ml of freeze-dried simulated wort. Amount of sugar (mg/100 ml) in unknown wort = (200  $\times$  area sugar peak (wort)  $\times$  attenuation/area internal standard  $\times$  attenuation)  $\div$  RRV/mg of sugar.

<sup>b</sup> The maltose response factor may be used for maltotriose and maltotetraose, since it is difficult to purchase these compounds in pure state (Marinelli, personal communication, 1972).

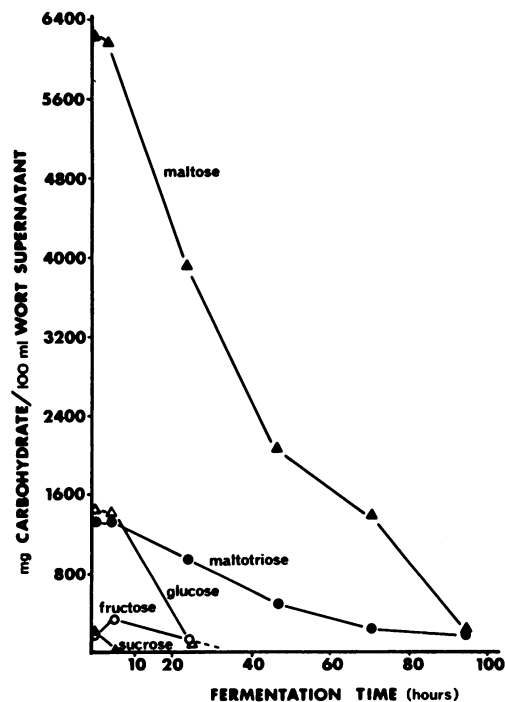


FIG. 3. Utilization of individual wort carbohydrates during primary fermentation.

crose; the rate of fructose utilization is, in fact, slower than that of glucose, even though all is used by 24 to 48 h.

Glucose, one of the major carbohydrates at

16%, is unchanged for the first 4 to 5 h, probably because its rate of utilization is comparable to its rate of formation from sucrose. However, by 24 to 48 h, measurable levels of glucose disappear. Maltose and maltotriose hydrolysis to glucose must therefore be slower than glucose utilization.

Maltose (66% of fermentable sugar) is not significantly attacked during the first few hours, presumably because maltase permease or the hydrolysis reaction are glucose repressed. A very rapid utilization is seen between 10 and 50 h, eventually resulting in 93 to 96% utilization. Maltotriose utilization is similar to maltose, eventually leading to 75 to 80% metabolism by the yeast. Residual maltose and maltotriose are attacked in subsequent fermentation and aging steps in the brewing process, since bottled beer from this process contains only nonfermentable carbohydrate and small amounts of maltotriose.

The ability to monitor wort carbohydrates or any other carbohydrates by gas chromatography is certainly an advantage to a quality control or research laboratory. The method is rapid, quantitative, and can be applied to a large variety of studies, including lactose in milk, maltose and glucose ratios in syrups, and enzymatic attack on carbohydrates. It has already been used to detect glucose, fructose, and sucrose levels in tomato, cabbage, apple, carrot, and potato tissues (22).

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