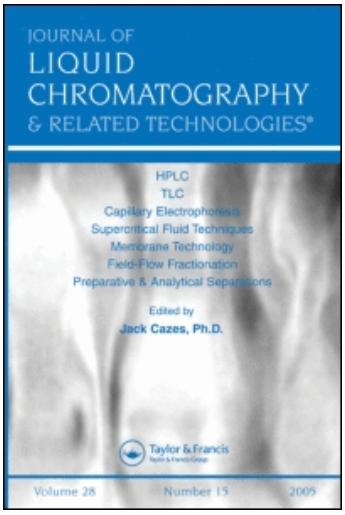

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HPLC Analysis of Carbohydrates Important to Beer Brewing Using an Aminopropyl Stationary Phase

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HPLC ANALYSIS OF CARBOHYDRATES IMPORTANT TO BEER BREWING USING AN AMINOPROPYL STATIONARY PHASE

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ABSTRACT

A silica-based aminopropyl stationary phase has been evaluated for use in monitoring the sugars involved in the various steps of beer and ale production. After determining the chromatographic behavior of the relevant sugars, methods were developed for the analysis of malted barley, wort, fermenting and finished beers. The technique's usefulness is illustrated by documenting the changes in sugar concentration brought about by modification of brewing conditions.

INTRODUCTION

Fermentation is one of the oldest processes in which man has harnessed microorganisms, namely yeast, to accomplish a chemical transformation. The number of organisms which are used to accomplish bulk transformations has increased in recent years due to the advent of bio-engineering. Although fermentation as it relates to beer brewing has been

exploited for thousands of years, convenient and accurate methods of quantitatively evaluating beers and ales before, during, and after fermentation are only now becoming widely available.

Possibly the most useful method for evaluating sugars available to the modern scientist is high-performance liquid chromatography (HPLC). Minimal sample preparation, quick analysis, and accuracy of quantitation are all advantages of HPLC in the monitoring of carbohydrates in beer. Use of the aminopropyl phase with refractive index detection has been proven[1] to be the most effective method presently available for sugar analysis, offering excellent selectivity of the sugars involved , isocratic mobile phase utilization, minimal sample preparation, and ambient-temperature operation.

The three aspects of brewing which are suited to monitoring of sugars by HPLC are (1) production of simple sugars from barley malt starches, (2) utilization of sugars before and during fermentation, and (3) analysis of finished beers.

The production and utilization of sugars occurs as a result of complex enzymatic processes, the former due to amylase activity in partially germinated barley and the latter due to yeast metabolism. Monitoring of the sugars involved is an assay of a complex enzymatic system, one whose products are due to the activity of a number of enzymes. Each of the enzymes in the system has different optimum conditions under which it operates. By altering the conditions , the activity of the enzymes changes. As a result, the amounts of sugars produced and their proportions will change, and this in turn will alter the resulting beer's taste.

The analysis of finished beers gives the producer the ability to quantify subjective judgements of taste and sweetness. Since raw materials such as barley and yeast will inevitably vary in composition and behavior,

respectively, the ability to make objective evaluations of a product will eliminate much of the possible variation.

EXPERIMENTAL

Equipment

All chromatograms were run on a Rainin Instrument Company (Woburn, Massachusetts) HPLC system consisting of a Rainin HPX pump equipped with a 5 ml pump head and an analytical electronic pressure module. A 4.6 x 250 mm Dynamax Microsorb 5 μm Amino column equipped with a 15 mm guard cartridge was used for all sugar analyses. A Knauer Model 98 refractive index detector was used in all chromatographic runs.

Data was collected, processed, manipulated, and incorporated into this paper using Rainin Instrument Company's Dynamax HPLC Method Manager software and an Apple Macintosh Plus personal computer.

Materials

EM Science (Gibbstown, N.J.) Omnisolve acetonitrile and water were used for all mobile phases, which were degassed by sonication prior to use. Brewing materials were purchased from The Oak Barrel (Berkeley, California). Malt extracts were of the liquid type. Yeasts were of the dry-packed type and were used immediately after opening of the package. Analytical sugar standards were purchased from Sigma Chemical Co. (St. Louis, Missouri).

Brewing and Sampling Procedures

All brewing and mashing procedures used ultrapure water in order to minimize variations in pH and ion content exhibited by tap water. Mashing procedures were carried out on a one liter scale, using a hot plate. Temperatures were maintained by careful monitoring of a submerged thermometer. Barley for mashes was finely ground in a coffee mill prior to mashing. All malt extracts, yeasts, and barleys were measured by weight.

Fermentations were carried out at room temperature in 500 ml round bottom flasks. Each flask was sealed against entry of outside air by a rubber septum vented with a short length of teflon tubing to allow carbon dioxide to escape. The exit end of the tube was submerged in a flask of water, to prevent air from entering the flask. The fermentation endpoint was determined by the cessation of carbon dioxide evolution. Adjustment of pH in wort (the sugar-rich nutrient solution to which yeast is added) prior to addition of yeast was accomplished, if necessary, with 0.1 *N* KOH or 0.1 *N* HCl.

Samples (2 ml) were collected from fermenting worts by syringe once every 24 hours. Acetonitrile (0.6 ml, final concentration ~25%) was then immediately added to the sample. This served two purposes: the acetonitrile would halt any fermentation in the sample, and also precipitate large proteins or starches in the sample prior to injection. Samples were filtered through a 0.45 μm syringe filter before injection. Calibrations of glucose, sucrose, maltose and maltotetraose were performed daily.

RESULTS AND DISCUSSION

In Germany, brewers are constrained by a centuries-old law which states that beer may be made only with barley malt, hops, water, and yeast.

Obviously, the selection and quality of German beers has not suffered as a result of this decree. Even using only barley malt as a source of sugars, differences in raw materials and the manner in which they are processed allows for a wide range of beers to be produced.

Sugar Production - Mashing

The sugars in barley malt are stored as starch, long chains of simple sugars (predominantly glucose), joined by one of two types of linkages. Amylose is a long, straight chain of glucose joined by a(1-4) linkages. Amylopectin is also composed of glucose molecules, but in addition to a(1-4) linkages, amylopectin contains a(1-6) branches. The two types of starch require different enzymes to cleave them into their constituent sugars. a- and b-amylases both attack 1-4 linkages, but have slightly different activity. a-amylase cleaves a(1-4) linkages at random, producing glucose, maltose, maltotriose, and higher oligomers of glucose. However, a-amylase will not cleave maltose into its constituent glucose molecules. b-amylase breaks a(1-4) linkages also, but it works from the end of the starch chain, cleaving alternate 1-4 linkages, thereby producing maltose exclusively. a(1-6) glucosidase is the enzyme involved in breaking the 1-6 linkages in amylopectin to produce glucose and larger oligomers which can then be broken down further by a- or b-amylase. The balance of starch types and the enzymes which degrade them are factors which will determine the composition and concentration of sugars after mashing (enzymatic breakdown) of barley malt.

Each of these enzymes has a pH, temperature, concentration of substrate, etc., at which it will be most active. Since the system under study is so complex, small changes in these factors will have a noticeable influence on

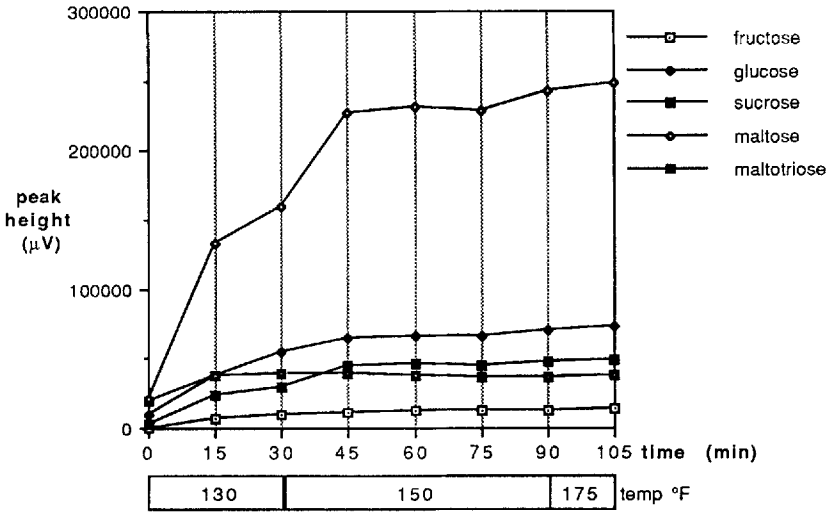

Figure 1. Mashing of American Pale 2-row malt

the product composition. In the mashing step, ground malted barley is added to water and held at a particular temperature at which the glucosidic enzymes will function well. By sampling the slurry during mashing, it is possible to monitor the net progress of all the enzymatic activity occurring. Figure 1 shows the progress of a mashing from beginning to end. The process is not 100% efficient, so a significant amount of starch, which is relatively insoluble, may not be converted to the soluble simple sugars which are easily monitored by HPLC.

The graph illustrates the effect that increasing temperature has on starch conversion over time. The mash is initially held at 130°F to allow breakdown of complex proteins for use later by yeast. This temperature is conducive to conversion of starch to simpler sugars, though the rate of conversion, particularly for maltose, is enhanced greatly by increasing the

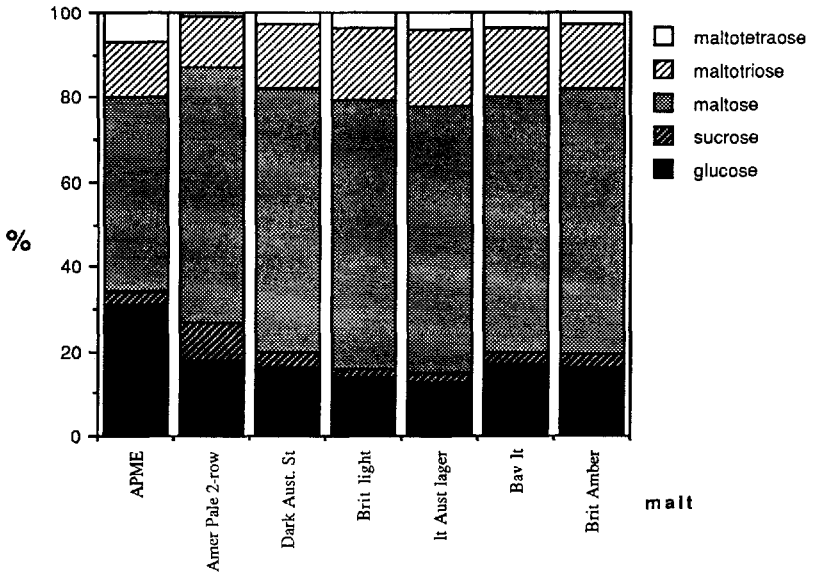

Figure 2. Proportions of simple sugars in malt extracts

temperature to 150°F. The final increase to 175°F finishes the process and denatures the glucosidic enzymes. By studying the concentration profile of each type of sugar, one could potentially obtain a qualitative estimate of the types of enzymes and starches which are present in the malt.

For those who brew on a small scale (5-10 gallon batches) for personal consumption ("home brewing"), it is often easier to use the commercially available malt extracts. These dark syrupy liquids are the concentrated products of a mash prepared by the manufacturer. A survey of the sugars in six malt extracts is shown in Figure 2. The composition of the American Pale 2-row malt mashing is included for comparison.

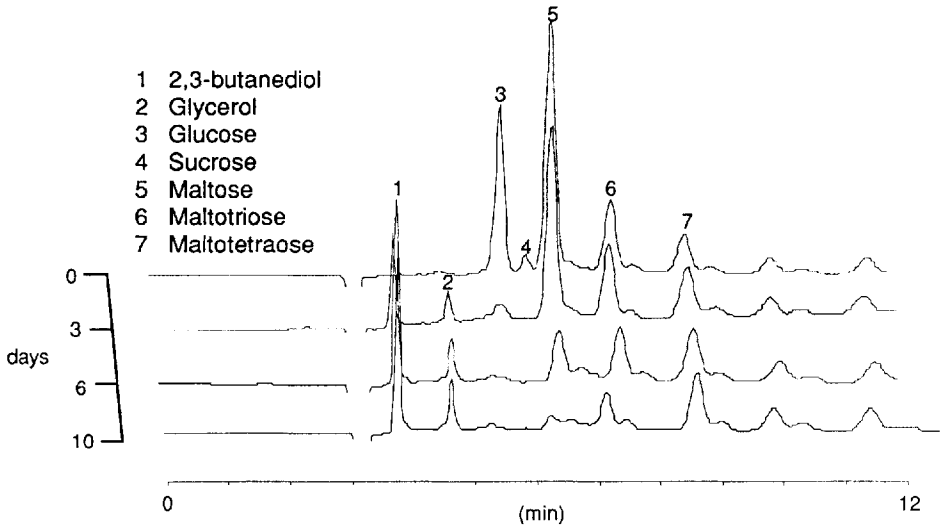

Figure 3. Progress of fermentation of Australian Pale malt extract by Red Star Lager yeast

Fermentation of Sugars

The simple sugars produced by mashing, along with proteins, vitamins, and minerals also present in the grain, serve as the fuel for yeast metabolism and reproduction. Although only two species of yeast are used for beer brewing (*Saccharomyces carlsbergensis* is used for lager beers, *S. cerevesiae* for ales), many strains of each exist. These strains exhibit considerably different behavior in their sugar metabolism and by-product formation.

The order of sugar uptake by yeast is as follows: glucose, fructose, sucrose, maltose, maltotriose. Normally there is very little free fructose in malt, and only slightly more sucrose. Thus, these sugars, along with the more abundant glucose, are depleted quickly. Maltose, which is the most abundant sugar in malted grain wort, takes longer to deplete and may not be completely

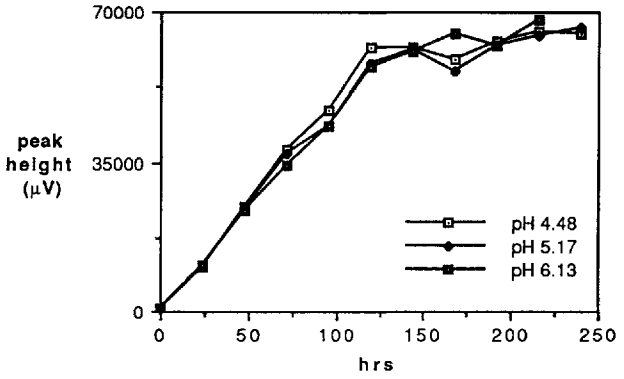

Figure 4. Comparison of 2,3-butanediol formation in lager fermentation at different pH values.

utilized before fermentation is ended. Yeast can utilize maltotriose and sometimes even maltotetraose, but higher glucose oligomers are indigestible until broken down into simpler sugars.

Figure 3 details chromatographically the progression of fermentation of Australian Pale malt extract by Red Star® brand lager yeast. As is expected, the glucose and sucrose in the wort are consumed almost immediately, while maltose requires somewhat longer to be depleted. Maltotriose is utilized by the yeast only sparingly, and maltotetraose not at all.

Figure 3 also shows the production of glycerol and 2,3-butanediol, which are side products of fermentation. Although both compounds are undesirable, 2,3-butanediol is the more problematic, as it imparts a heavy, butterscotch-like flavor to beer. Yeasts which either produce little 2,3-butanediol or consume it as fuel are very desirable. Brewing conditions are usually manipulated to give the lowest amounts of 2,3-butanediol while maintaining yeast metabolism. Figure 4 compares 2,3-butanediol production by the same yeast strain at three different pH values.

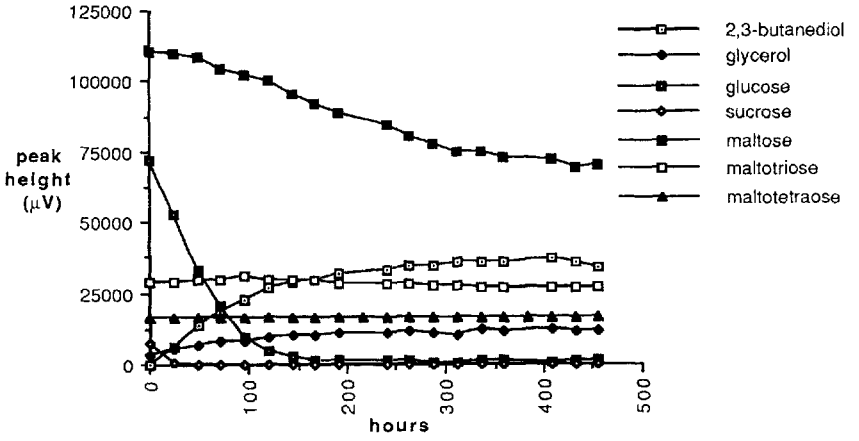

Figure 5. Fermentation with Red Star Ale yeast

Yeast strains vary considerably in their metabolism. Figures 5, 6, 7 and 8 show the fermentation of the same malt extract by four types of ale yeast. These fermentations demonstrate the considerable differences in sugar utilization and by-product formation between yeast strains.

Red Star brand ale yeast (Figure 5) exhibits very low metabolism and can utilize only glucose and sucrose completely. The rate of maltose uptake is very low and only 30% of the availablemaltose was consumed prior to the end of fermentation. This behavior is typical of a "non-attenuative" yeast; one in which the ability to consume the available sugars, and particularly higher oligomers, is limited.

Whitbread ale yeast (Figure 6) is considerably more attenuative, consuming 90% of the available maltose. With this increased metabolism comes an increased amount of 2,3-butanediol.

Vierka ale yeast (Figure 7) is extremely attenuative. It exhibits a considerable lag between addition of yeast to the wort and the onset of

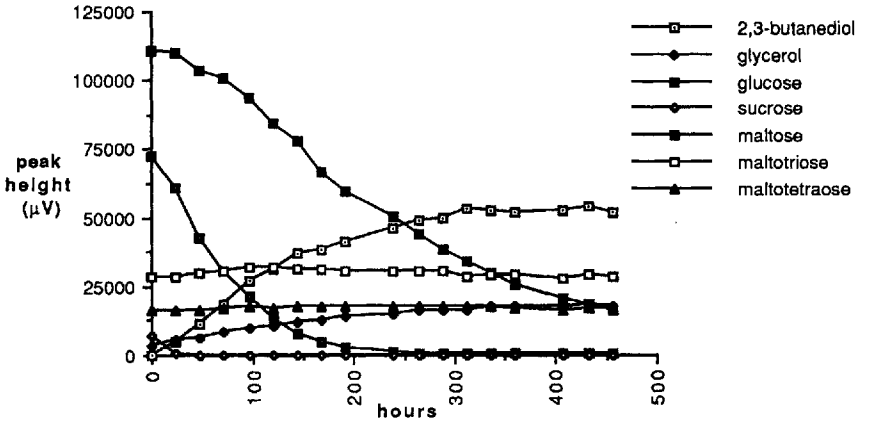

Figure 6. Fermentation with Whitbread Ale yeast

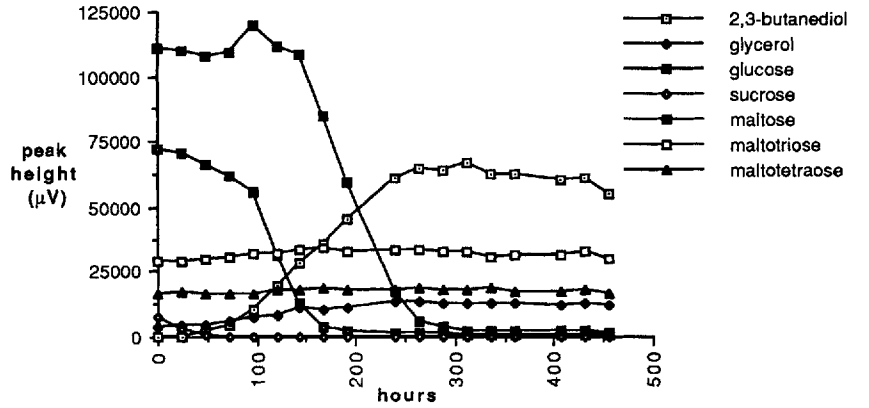

Figure 7. Fermentation with Vierka Ale yeast

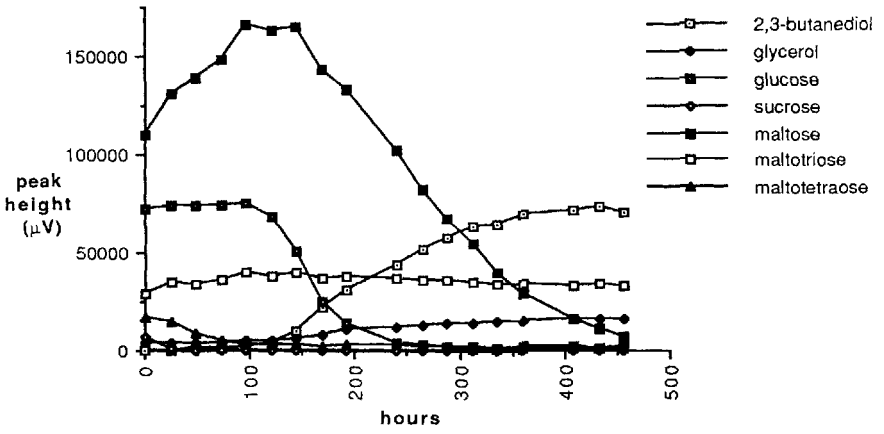

Figure 8. Fermentation with Arauner ale yeast

fermentation. Note also the high level of 2,3-butanediol, though this yeast is able to reduce the concentration of 2,3-butanediol from its peak before fermentation ends.

Arauner ale yeast (Figure 8) not only has an initial lag before fermentation commences, but also appears to have some glucosidic activity. The maltose concentration increases initially, and the maltotetraose levels decrease, suggesting that the higher oligomers of glucose present in the wort are being cleaved to maltose. This yeast also creates a great deal of 2,3-butanediol, perhaps in part because of the extremely large amount of maltose available, almost all of which is consumed.

Final Product Analysis

Analysis of finished beers affords the opportunity for correlation between sensory impressions and a quantitative measure of the components.

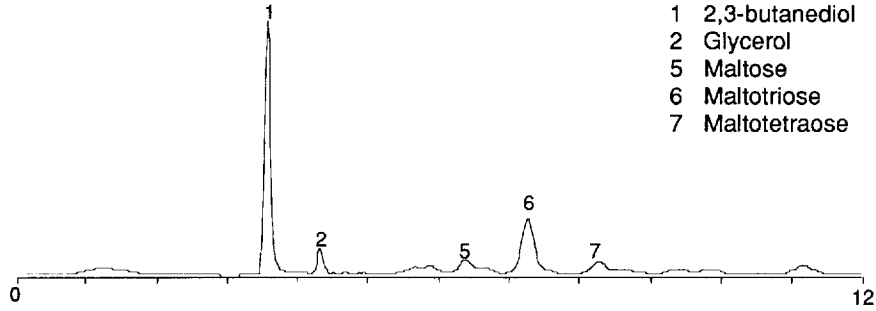

Figure 9. Analysis of a light, dry beer. Column: Dynamax Microsorb 5µm Amino, 4.6x250 mm. Mobile Phase: 60/40 acetonitrile/water, 1.0 ml/min. Detection: Refractive Index. Scale: 106500 µV

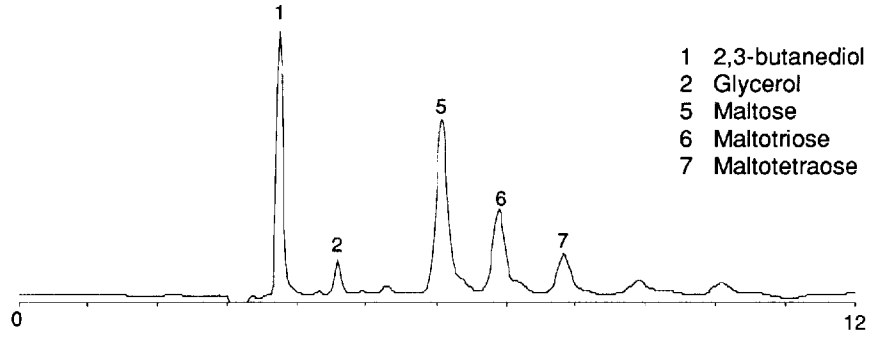

Figure 10. Analysis of a well-balanced beer. Column: Dynamax Microsorb 5µm Amino, 4.6x250 mm. Mobile Phase: 60/40 acetonitrile/water, 1.0 ml/min. Detection: Refractive Index. Scale: 106500 µV.

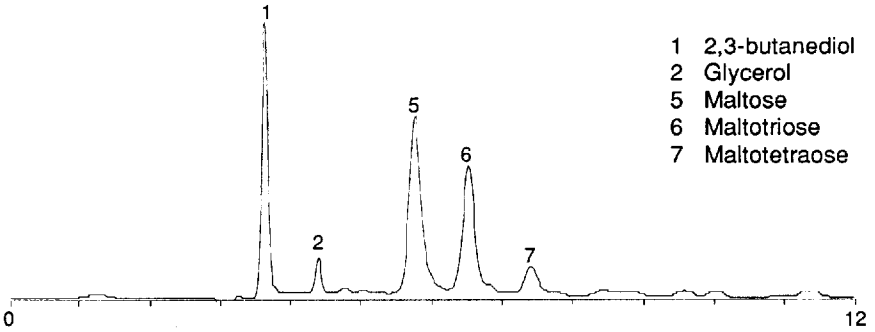

Figure 11. Analysis of a barely wine style ale. Column: Dynamax Microsorb 5µm Amino, 4.6x250 mm. Mobile Phase: 60/40 acetonitrile/water, 1.0 ml/min. Detection: Refractive Index. Scale: 168350 µV

Figures 9, 10, and 11 show three very different tasting beers, and the chromatograms give reasons for some of the taste differences.

Figure 9 shows the chromatogram of Corona, an extremely light, relatively dry beer. There is very little sugar of any sort in this beer but a fair amount of 2,3-butanediol. This particular beer is often accompanied by a slice of lime, which helps "cut" the flat taste imparted by the diol.

The chromatogram in Figure 10 was obtained by analysis of Pilsner Urquell, a very highly regarded European beer. This beer has a superior balance of sweetness and bitterness. Although the amount of 2,3-butanediol is comparable to the beer in Figure 9, there is considerably more sugar of all types, particularly maltose, which is virtually absent from the previous beer. Although maltose is only one-third as sweet as sucrose, its presence provides both body and sweetness.

Figure 11 comes from Old Nick's barley wine style ale. Barley wine style ales are very heavy and sweet. Note that the scale on this chromatogram is 60% greater than the two previous, so the quantity of the components shown is approximately twice that of the beer in Figure 10. As one would expect, this beer is very sweet and syrupy.

CONCLUSION

Because fermentation takes days or weeks to be completed, monitoring of the process allows one to ascertain progress and delineate the point at which the fermentation is effectively finished. Although pH, specific gravity, and temperature can be checked easily, these parameters are only secondary indicators of the fermentation process. The ultimate purpose of fermentation as used by man is the breakdown of a complex substrate into a simpler, more usable form. It follows that in addition to the monitoring of these secondary

indicators, a careful observation of the primary products would be even more desirable. HPLC using an amino phase as detailed here offers a simple, relatively inexpensive, fast, and accurate means of monitoring the primary products of yeast fermentation.

(1) Plaga, A., Stumpfel, J., and Fiedler, H. "Determination of carbohydrates in fermentation processes by high-performance liquid chromatography", Appl. Microbiol. Biotechnol. **32**(1), 45, 1989