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TECHNICAL REPORT: AMT-TRFB0621-2

TITLE: FAST SCREENING OF OLIGO- AND POLY-SACCHARIDES IN BEER

MARKET SEGMENT: FOOD/BEVERAGE

AUTHOR:

Conner McHale, Technical Support Specialist

Data Courtesy of: Merlin K. L. Bicking, Ph. D., Senior Analytical Scientist (ACCTA, INC)

ABSTRACT

A fast screening of oligo- and poly- saccharides in beer is performed during various parts of the brewing process using evaporative light scattering detection. This includes samples throughout the mashing process, fermentation, and the finished product. Monitoring the sugar composition can signal the status of fermentation and aid in quality control. Hydrophilic interaction liquid chromatography (HILIC) mode was selected for the best resolution and speed using superficially porous particle technology (SPP). Analyzing beer sugar profiles using high pressure liquid chromatography can significantly help brewers with lot-to-lot repeatability, quality of their product, and troubleshooting techniques.

INTRODUCTION

Beer is one of the most widely consumed beverages in the world. This beverage is simply made up of water, malted grains (malted barley, wheat, corn, sorghum, rye), hops, and yeast. As simple as this may sound, beer has a very complex chemical composition and has a wide range of flavors. Monitoring yeast behavior, hop profiles, sugar composition, and water purity are just a few of the important components to make a delicious beer. Common instrumentation used to measure these components includes UV-Vis spectrophotometers, gas chromatographs, high pressure liquid chromatography (HPLC), and mass spectrometry. Using these instruments are not required, however, they can in aid quality assurance significantly improving repeatability, taste, and yields.

A fast screening of oligo- and poly-saccharides in beer is performed throughout various parts of the brewing process including the final product. This can help brewers better understand the sugar behaviors in their beer and to know when the fermentation process is complete.

EXPERIMENTAL

Fast screenings of oligo- and poly-saccharides in beer are performed using HPLC coupled with an evaporative light scattering detector. The evaporative light scattering detector (ELSD) is a type of LC detector. The detector is very useful when the compounds of interest do not have UV-Vis chromophores. This includes many types of compounds such as sugars, lipids, and polymers.

- How does it work?

After the eluent passes through the column, it reaches the ELSD. The eluent passes through the heated nebulizer, mixes with the nebulizer gas (nitrogen or air), and then forms an aerosol. Once nebulized, the eluent heads through a heated drift tube and the mobile phase

KEY WORDS:

oligosaccharides, polysaccharides, HILIC, superficially porous particles, beer, HPLC

evaporates. The solid particles travel through a flow cell containing a light source and a photomultiplier for detection. It is important to note that the ELSD can only detect compounds that are less volatile than the mobile phase used. A representation of a common ELSD detector can be seen in figure 1. The ELSD has the benefit of increased sensitivity and the ability to run in gradient mode compared to a refractive index detector. This gives an advantage when trying to choose the proper detection for sugar analysis. Hydrophilic interaction liquid interaction

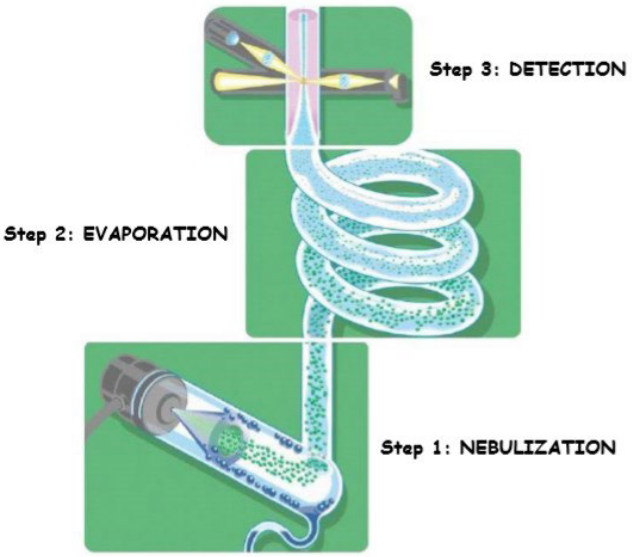

Figure 1: image from SEDEX Model 90LT ELSD manual

chromatography (HILIC) mode along with ion exchange are the preferred modes used for sugar analysis due to the polarity of the compounds. These columns can produce a wide variety of selectivity and retention due to their differences in the stationary phases. HILIC is an alternative HPLC mode primarily used to separate polar compounds. This is also known as aqueous normal phase liquid chromatography. The HALO 90 Å Penta-HILIC, 2.7 µm, 4.6 x 50mm column produces fast, high resolution results for oligo- and poly-saccharides in beer. This HILIC stationary phase works very well with these compounds due to the interactions with the -OH groups and utilizes superficially porous particle technology (SPP), allowing fast run times with high efficiency. The Penta-HILIC ligand can be seen in figure 2.

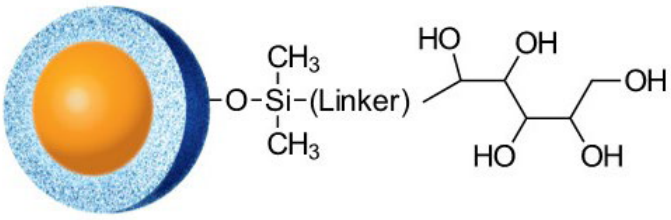

Figure 2: The HALO® Penta-HILIC ligand structure

There are some challenges when performing HPLC sugar analysis. This includes anomer splitting for certain compounds which will increase peak widths along with long retention times. Because of this a shorter column dimension is recommended along with a shorter column diameter in order to improve sensitivity and also reduce solvent consumption. Higher column oven temperatures (65˚C) have also shown a significant improvement in peak shapes compared to lower temperatures (35˚C). For example, figure 3 shows a separation of dextrose equivalent (DE) sugars which were ran at two different temperatures. Degree of polymerization (DP) increases as retention times increase. The 65 °C temperature significantly improves anomer splitting allowing for sharper peak shapes and faster retention times.

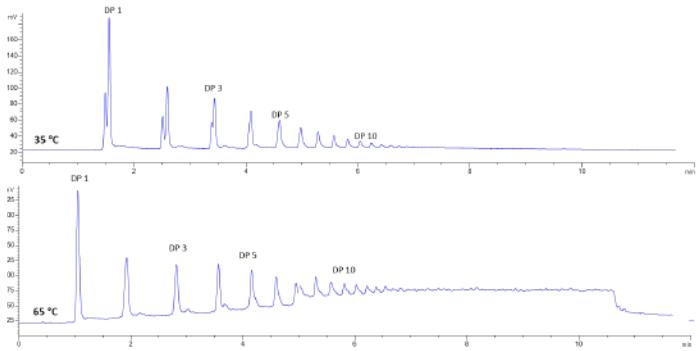

Figure 3: Dextrose equivalent sugars ran at two different temperatures to avoid anomer peak splitting. DP indicates the degree of polymerization (e.g., the number of glucose units).

TEST CONDITIONS
Column: HALO 90 Å Penta-HILIC, 2.7 µm, 3.0 x 50 mm
Part Number: 92813-405
Mobile Phase A: Water
B: Acetonitrile

Gradient:	Time	%B
	0.0	92
	8.0	52

Flow Rate: 0.75 mL/min
Temperature: 65 °C
Detection: ELSD, 40°C, 45 psi
Injection Volume: 2 µL
Data Rate: 10 Hz, 2 sec filter
Data Courtesy of Merlin K. L. Bicking, Ph. D. (ACCTA, Inc.)

An Agilent 1290 HPLC with diode array detection (DAD) and evaporative light scattering detector (ELSD) were used with HPLC grade acetonitrile (B) and deionized (DI) water (A). Two gradients were used for analysis: 92-42 %B in 10 minutes and 92-54 %B in 8 minutes. A flow rate of 0.75 mL/min with a column oven temperature at 65 ˚C using a 2 µL injection was implemented for all runs. The ELSD used a 10 Hz data rate, 2 sec filter, 40 ˚C, at 45 psi.

SAMPLE PREPARATION

Beer samples were collected through collaboration with 3rd Act Craft Brewery (Woodbury, MN). Samples were collected throughout various parts of the brewing process and stored cold. Samples were adjusted to pH 2 with phosphoric acid in order to increase stability. Samples were then centrifuged

and the supernatant was removed. With HPLC it is best practice to remove any particulates in the sample in order to avoid plugging and contamination. Because of this, samples were then filtered through a 0.2 µm filter.

Dilution with water and 40% organic solvent (1:1 acetonitrile: methanol) was found to give the best results in terms of sensitivity. A 1:25 dilution was made with mashed samples, 1:10 or 1:5 for fermented samples, and 1:10 or1:5 or undiluted for finished product samples.

RESULTS:

Several mash samples were analyzed throughout the mashing process. This process is a pre-fermentation step that involves combining a mixture of grains and steeping them in water for a period of time at elevated temperatures, similar to making a cup of tea. Mashing allows the enzymes in the malt to break down the starch in the grain into sugars, typically maltose, to create a malty liquid called wort.1 The chromatographic overlay can be seen in figure 4. The first sample (4A) is collected during the initial mash process at 147˚F, second sample (6A) at 158˚F mid-mash, and 180˚F at the end of the process. (7A) Sugar concentrations will increase overtime as demonstrated in figure 4.

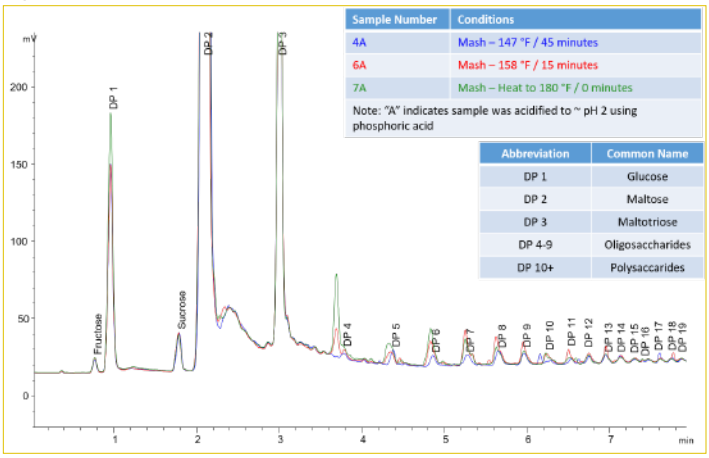

Figure 4: Mashing process of malted grains monitored

Once the mash process is complete, fermentation takes place, which is when yeast reacts with the sugars converting them to ethanol. Fructose and sucrose were added to the beer before fermentation took place to aid with fermentation. After the first day of fermentation, sucrose is completely converted to carbon dioxide and ethanol while maltose and other fermentable sugars are also decreasing in intensities. This can be seen in figure 5. A plot of the fermentable sugars is seen in figure 6, showing decreasing concentrations over time.

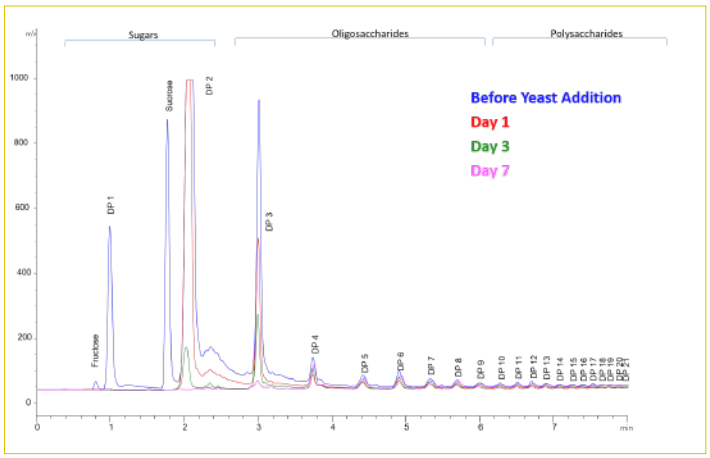

Figure 5: Oligo-and poly-saccharides are monitored throughout the beer fermentation process

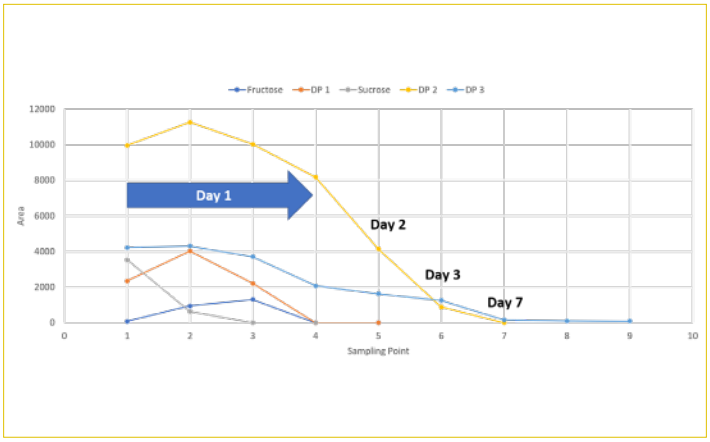

Figure 6: A plot of fermentable sugars decreasing over time during the beer fermentation process

After fermentation the beer is bottled, canned, or kegged and is then ready for consumption. Monitoring the oligo- and poly-saccharides in the finished product can give brewers information of flavor profiling and signs of batch-to-batch repeatability. The completeness of the fermentation process is easily evaluated with this technique, allowing the brewer to determine if they have used up all the available fermentable sugars. In the example below, a blueberry cream ale is analyzed in figure 7 (see next page). Fructose is present from the real blueberries added to the beer after fermentation.

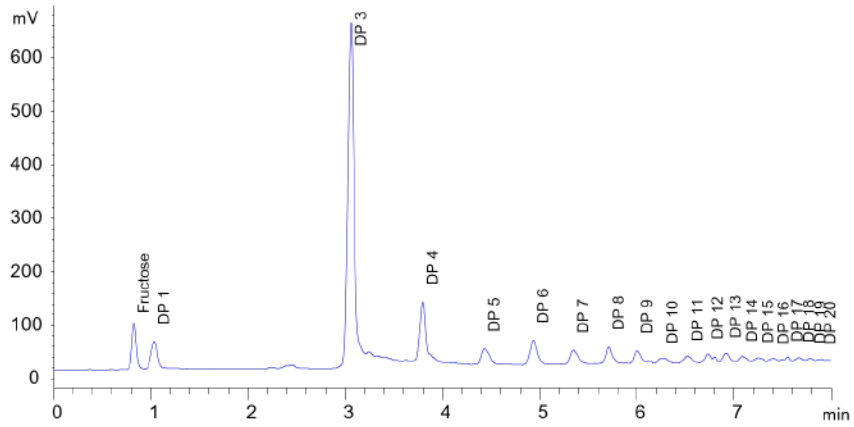

Figure 7: Analysis of blueberry cream ale from 3rd Act Brewery. Note that all the maltose (DP 2) has been fermented. Only small amounts of glucose remain.

CONCLUSION

A fast screening of oligo- and poly- saccharides in beer is performed with a HALO® Penta-HILIC column paired with an ELSD. The SPP particle technology along with the columns HILIC properties allows for fast and efficient separations. Screening these compounds provides information on fermentable sugars within the beer making process and can be very useful for the brewer. Cold sample storage at pH 2 preserves both mash and fermentation samples for later analysis. After several different sample preparation techniques, dilution in an aqueous-organic mixture provided the best results for sensitivity.

ACKNOWLEDGEMENTS:

1. Merlin K. L. Bicking, Ph. D., Senior Analytical Scientist (ACCTA, Inc)

2. 3rd Act Craft Brewery (Woodbury, MN)

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