Fermentable sugar profile as an alternative to Apparent Attenuation Limit for selection in Barley Breeding

R.L.Fox, S.J.Logue and J.K.Eglinton

University of Adelaide, Dept. of Plant Science, PMB 1, Glen Osmond, SA, 5064, Australia.

Introduction

Fermentability is a measure of the ability of yeast to convert sugars to ethanol during brewing and is becoming an important parameter in asssessing malt quality. Traditionally, fermentability has been measured using Apparent Attenuation Limit (AAL) where an excess amount of yeast is allowed to ferment under controlled conditions. In this procedure specific gravity is measured before and after fermentation and used as the basis for calculating AAL.

This standard method however is time consuming, involves using large volumes of wort and is therefore not suited to the large number of samples generated in a barley improvement program. Consequently, only lines in the most advanced stages of quality evaluation are tested for fermentability.

An alternative to this method is the use of high performance liquid chromatography (HPLC) which can measure the levels of individual fermentable (glucose, fructose, sucrose, maltose, maltotriose) and non-fermentable sugars (maltotetraose and larger linear and branched maltodextrins) in wort. It is anticipated that correlation equations could be used to predict AAL from the sugar profile. HPLC offers the advantage of using less sample, and providing a more accurate and less time consuming method of evaluating fermentability.

In this study the applicability of using this method as an alternative to the traditional attenuation method is investigated using trials from the barley breeding program. The genetic basis of AAL and component polysaccharides were also investigated through the QTL analysis of the Galleon*Haruna nijo mapping population.

Materials and Methods

Materials

Samples from two different trials were used in this study including 15 lines from 4 sites from the 1999 season SARDI Stage 4 trial and 70 lines from the 1998 Galleon*Haruna nijo mapping population. Samples were micomalted in a Phoenix Biosystems Micormalting Unit, using the protocol described in the 1999 Waite Barley Quality Evaluation Laboratory (WBQEL) Report (WBQEL, 1999).

Each sample was mashed using a small scale variation of the standard EBC hot water extract (HWE) method using 10g of malt (MacLeod et al., 1991). An aliquot of wort was taken and frozen for HPLC analysis and the remainder fermented using a small scale Apparent Attenuation Limit (AAL) method (as described below). A portion of fermented wort was also frozen for HPLC analysis for the SARDI Stage 4 trial samples.

Methods

Apparent Attenuation Limit (AAL)

A small scale AAL method was used which involves incubating EBC HWE wort with yeast at 25°C for 24 hours (WBQEL, 1999).

Beta amylase assay

Beta amylase is determined through the measurement of Diastatic Power (DP), which is the combined activity of alpha and beta amylase. DP is assessed by measuring the amount of enzymic activity in breaking down starch to reducing sugars in 10 minutes. The beta amylase component is calculated from the total DP minus alpha amylase. Alpha amylase is assayed individually by heat treating the sample which denatures beta amylase and allows the heat stable alpha amylase component to be reassayed as for DP (WBQEL, 1999).

Fermentable Sugar Analysis

Sugar profiles were analysed using a DX-500 HPLC system (Dionex) fitted with a SIL-10AD autoinjector (Shimadzu) and an ED-40 pulsed amperometric detector (Dionex). Fermentable and nonfermentable sugars were separated on 250 * 4 mm Carbopac PA1 and PA10 columns respectively, using linear sodium acetate gradients at flowrates of 0.6 mL/min. Data was analyzed with Peaknet Chromatography Workstation version 4.3 (Dionex).

Glucose, fructose, sucrose, maltose, maltotriose, maltotetraose and maltohexaose were used as standards. Wort samples were centrifuged and diluted in deionised water before injection. 1:200 dilution of the wort was made for analysis of fermentable sugars and a 1:5 dilution for the analysis of non-fermentable sugars and samples were transferred to 96 well ELISA plates for automated injection.

Results and Discussion

Fermentable and non-fermentable sugars were successfully separated. A typical profile for the fermentable sugars is illustrated in figure 1.

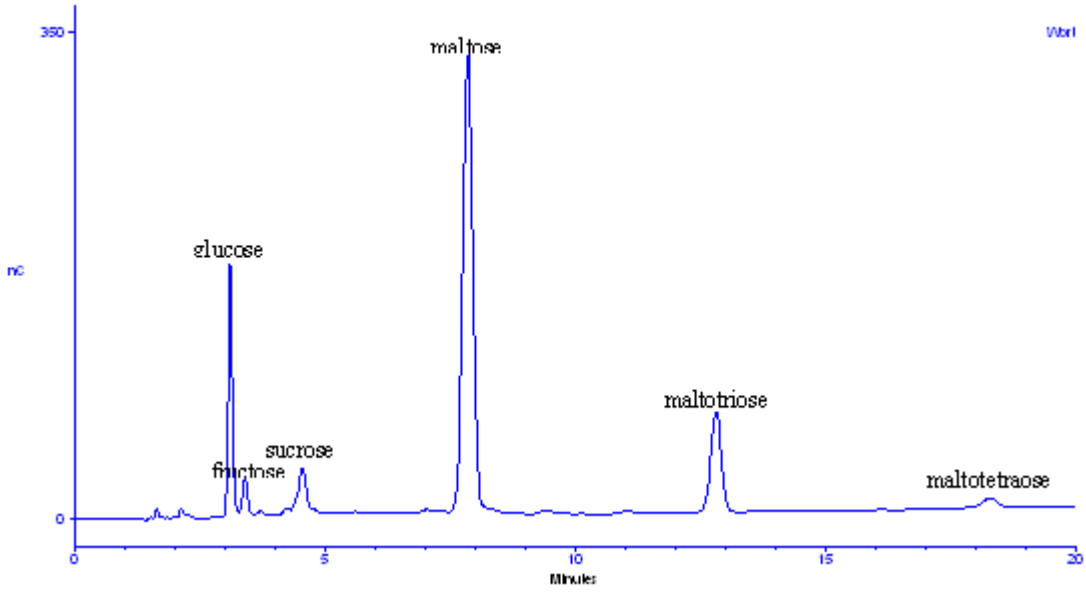

Figure 1 Separation of wort sugars by HPAEC with pulsed-amperometic detection

Figure 2 graphically summarises the results from the Stage 4 trial. This analysis showed that sugar profiles between varieties tended to be small for a given site, although there was more between site variation.

When these results were compared to the corresponding AAL results it was observed that lines with low fermentability like Schooner tended to have higher levels of maltotetraose than higher fermentability lines such as Sloop and SBWI-1.

As well as assessing unfermented wort, fermented wort from the stage 4 trial was also collected and sugar levels assessed. These results confirmed that the AAL method successfully ferments samples to completion with residual sugar levels approaching the lower detection limits of the HPLC (results not shown).

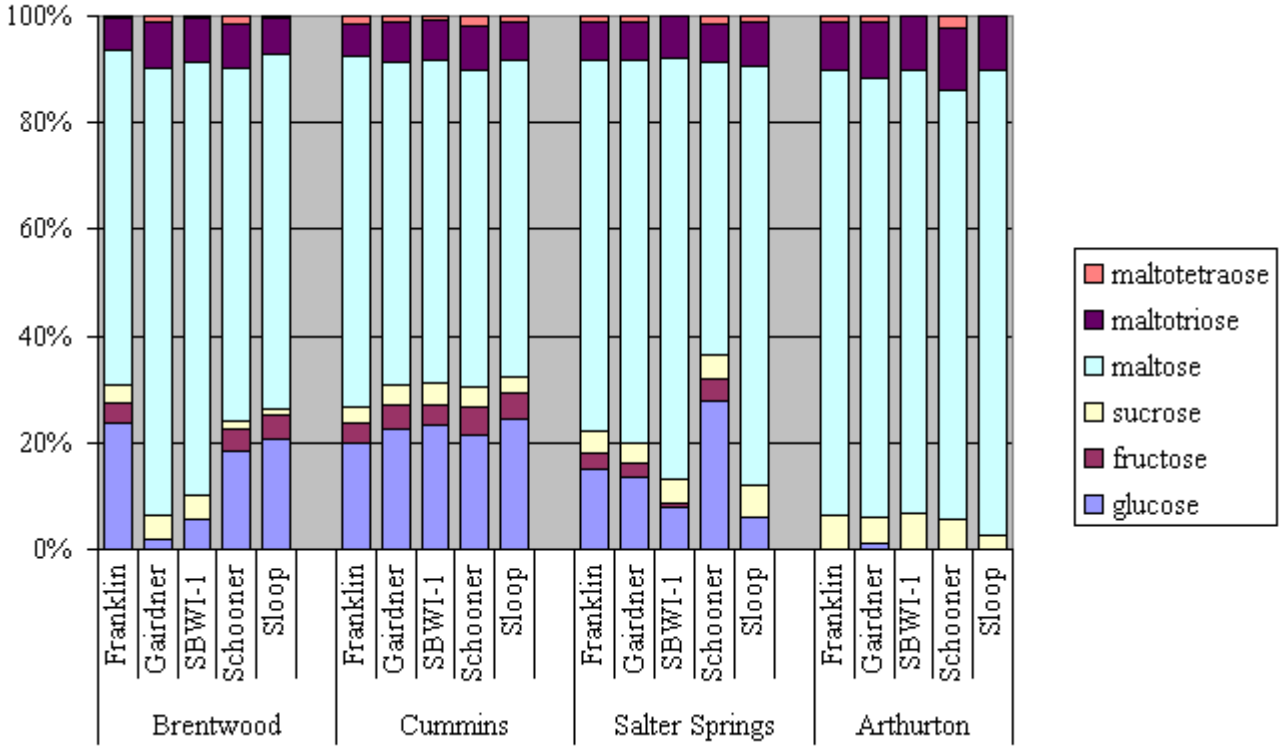

Figure 2. Fermentable sugar profile of five SARDI Stage 4 entries grown at four sites. Results are expressed as the percentage of fermentable sugars (glucose, fructose, sucrose, maltose and maltotriose) and the non-fermentable dextrin maltotetraose, in wort.

Correlation analysis showed that there was no simple relationship between AAL and the assessed sugars. For both the SARDI trial and the Galleon*Haruno Nijo mapping population individual and total sugars were plotted against AAL. In all cases no correlation was seen. Adjusting the fermentable sugar results for malt protein saw no improvement in regression results and likewise no simple relationship was seen between fermentable sugars and beta-amylase. Table 1 illustrates the regression results obtained for the SARDI Stage 4 trial.

Similar poor correlations have also been observed in previous studies (Swanston et al 1999). One possible explaination for the poor relationship seen between fermentable sugars and AAL may be related to the use of specific gravity in assessing fermentability in the AAL method because a number of different components of the wort may be contributing the result. An alternative may be to measure actual alcohol levels in the final wort.

	AAL vs Sugar	**Sugar vs B-amylase**	**AAL vs Sugar adj MP**
Glucose	0.002	0.02	0.016
Fructose	0.009	0.09	0.010
Sucrose	0.17	0.005	0.12
Maltose	0.05	0.003	0.03

Maltotriose	0.02	0.08	0.04
Maltotetraose	0.162	0.10	0.178
TOTAL	0.02	0.01	0.02

Table 1 Correlation results (r^2) for the Stage 4 trial. Relationships between AAL and individual sugars, individual sugars and beta amylase and AAL and individual sugar (adj MP) are shown.

QTL analysis of AAL was carried out using data from the Galleon*Haruno Nijo Mapping Population using QGENE (Nelson 1997). This identified two genomic regions significantly associated with fermentability. One QTL is positioned on the long arm of chromosome 4H, coincident with the *Bmy*1 locus, and the second is present on chromosome 3H.

Mapping results showed that level of maltotetraose (the smallest of the non-fermentable dextrins) is significantly associated with the *Bmy*1 locus (Figure 3). It is concluded that the thermolabile Sd2L ß-amylase from Galleon results in incomplete starch degradation and increased levels of maltotetraose, yielding lower values for fermentability. The more thermostable Sd2H β-amylase from Haruna nijo results in more effective hydrolysis, yielding higher levels of fermentable sugars.

The second QTL for fermentability was identified on chromosome 3H, and does not coincide with the map position of enzymes involved in either starch synthesis or hydrolysis, but does correspond to the location of the *denso* dwarfing gene. Analysis of the non-fermentable sugar profiles of the Galleon x Haruna nijo population shows the level of small branched (α-1,6 linked) dextrins is also significantly associated with the *denso* locus on 3H. It is likely that this locus does not have a direct biochemical role in determining malt quality, but exerts pleitropic effects through control of plant development and adaptation. Both the denso locus and the ari-eGP dwarfing gene on chromosome 5H have previously been reported to influence fermentability (Swanston et al 1999).

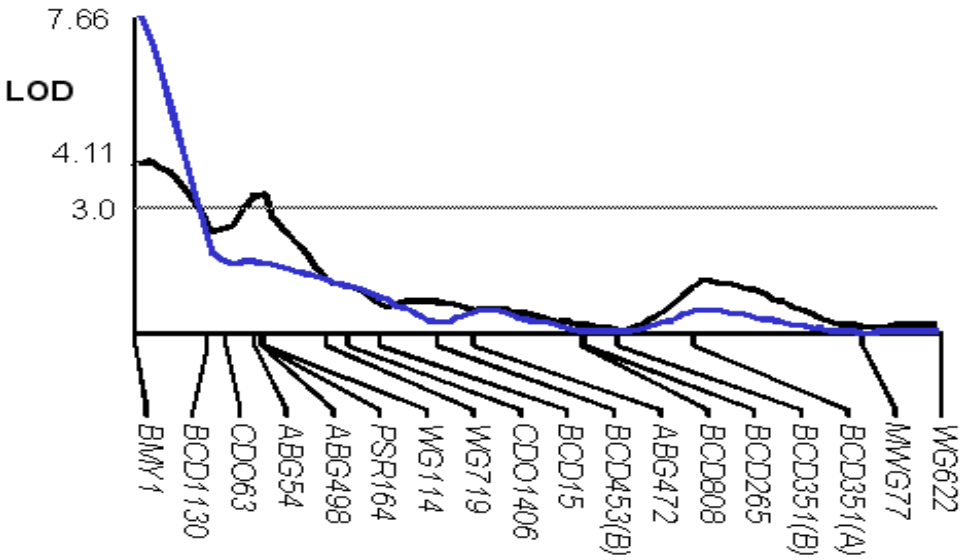

Figure 3: Interval analysis of wort fermentability measured as AAL (LOD 4.11) and wort maltotetraose concentration (LOD 7.66) in the Galleon x Haruna nijo mapping population. Both traits show significant QTL coincident with the *Bmy*1 locus encoding ß-amylase.

Conclusion

Detailed analysis of fermentable sugar profiles is an important method for researchers to better understand the biochemical basis of wort fermentability, which is now a key quality parameter. However, the lack of a simple relationship between levels of fermentable sugars and current quality parameters precludes the application of HPLC as a replacement for AAL in routine quality evaluation.

The QTL mapping results support previous studies which have demonstrated the role of alternative Bmy1 alleles in influencing the levels of wort fermentabiulity (Eglinton et al 1998), validating the use of Bmy1 selection for improved wort fermentability.

Acknowledgement

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