Enhancing the Assessment of Wort Fermentability in a Barley Breeding Program

Stefan Harasymow and Allen Tarr

Department of Agriculture Western Australia, Locked Bag No. 4 Bentley Delivery Centre, South Perth, Western Australia, 6983
http://www.agric.wa.gov.au; sharasymow@agric.wa.gov.au; atarr@agric.wa.gov.au

Abstract

Wort fermentability describes the capacity of barley malt to produce fermentable sugars that are utilised by yeast to produce alcohol during fermentation, and is an important parameter when determining malt quality. A small-scale version of an industry standard procedure has been developed to determine fermentability when smaller quantities of malt are available. A standard mashing bath has been modified with an additional externally cooled heat exchanger assembly to provide uniform and standard fermentation conditions. Using a dried yeast strain, fermentability values can be determined on a batch size of 16 samples in 48 hours. The method provides comparable results to the industry method and has potential for application in quality evaluation in a barley breeding program.

Key Words

Malting, wort fermentability, AAL, yeast

Introduction

Wort fermentability or Apparent Attenuation Limit (AAL) is an important quality attribute to consider when determining the malting performance of barley varieties. The high fermentability of recently released malting barley varieties in Australia has caused concern for some domestic brewers for the impact on the production of low and mid-strength style beer. The fermentability of the wort, is the proportion of the wort dissolved solids (extract) which can be fermented, and is expressed as a percentage (Briggs et al, 2004). The Australian malting and brewing industry use standard procedures, namely European Brewery Convention (Analytica - EBC, 1998) and Institute of Brewing (IOB - Methods of Analysis, 1997) to measure fermentability. These procedures require larger quantities of malt than is usually available from small-scale micromalting carried out in the early stages of a barley breeding program. A small-scale version of the standard EBC procedure is currently being developed and collaboratively evaluated by the Australian Barley Chemists Group (ABCG).

Variation in AAL may be influenced by the equipment used in the assay, in addition to the effect of different yeast strains and incubation conditions. Utilising a dried yeast strain as an alternative to fresh yeast has perceivable advantages including not requiring equipment to culture and propagate yeast, convenience of use, long shelf life and certified purity allowing comparisons between different laboratories. The storage and rehydration conditions of the dried yeast are especially important for preserving cell viability. Slow rehydration kinetics in the low to mid water activity range and higher rehydration temperatures have been shown to increase cell viability in *Saccharomyces cerevisiae* (Poirier et al, 1999). It was previously reported that a dried yeast strain (Mauribrew Lager 497), was tested as being suitable for the determination of fermentability and had similar performance to fresh yeast strains obtained from commercial Australian breweries (Evans and Hamet, 2005).

The aim of this study was to investigate a small-scale fermentability procedure using Mauribrew Lager 497 dried yeast strain, improve the stability of fermentation conditions and compare results from the small-scale method with the EBC method.

Methods

Malt

Malt samples used were from commercial batches (Joe White Maltings and Barrett Burston Maltings) and the 15th EBC standard control malt. The samples covered a range in AAL values and were a sub-sample taken from the Australian Barley Chemists Group study into a small-scale fermentability method.

Yeast

Mauribrew Lager *Saccharomyces cerevisiae* strain Y497 (batch 497111) yeast was purchased from Mauri Yeast Australia (Toowoomba, QLD). The 500g vacuum packs were divided into smaller quantity snap lock

bags and stored desiccated at 4°C prior to use. On each day of analysis, yeast were "revived" by transferring sufficient quantity into a sterile beaker with a moistened filter paper lid, covered in aluminium foil, and stored at room temperature for approximately 5 hours. The humid conditions are preferred to reduce yeast mortality as a result of rehydration shock when pitching into wort.

Mashing

The mashing procedures were carried out in a 20 head IEC mash bath (Industrial Equipment and Control P/L, Melbourne, Australia), following the standard EBC temperature profile (mashing parameters had been programmed on an EEPROM chip by the manufacturer). Samples were analysed in duplicate in randomised order (EBC and small-scale procedure on different occasions). The EBC procedure was carried out as per EBC Extract method 4.5.1 (EBC, 1998) with the omission of returning the first filtrate portion during filtration. In the modified EBC small-scale procedure, 20g malt was used with grist to water ratios in the same proportion as in the EBC method. Modified mash beakers were used in the small-scale procedure as previously reported (Harasymow et al, 2003). The processed mash from each procedure was filtered through 330mm postlip paper (Hollingsworth and Vose Co Ltd, Winchcombe, England) and 604 Rundfilter paper (Schleicher and Schuell Microscience GmbH, Dassel, Germany) directly into 500mL and 250mL Schott reagent bottles for the EBC and small-scale procedures respectively. The reagent bottles and contents were weighed, sealed with an aluminium foil cap and placed in a boiling water bath for 15 minutes, then cooled to room temperature in an ice bath and adjusted to their original weight with deionised water. A 30mL sample was taken to determine the original wort specific gravity on a DMA 5000 density meter (Anton Paar GmbH, Graz, Austria).

AAL Determination

The fermentation incubation was carried out in the IEC mash bath fitted with a custom made heat exchanger coil (Fraser Fabrications P/L, Malaga, Western Australia) and Frigomix 1495 / Thermomix 1441 refrigerated water bath system (B.Braun, Melsungen AG, Germany). The IEC mash bath was used to maintain uniform stirring and temperature control for the fermentation test procedure.

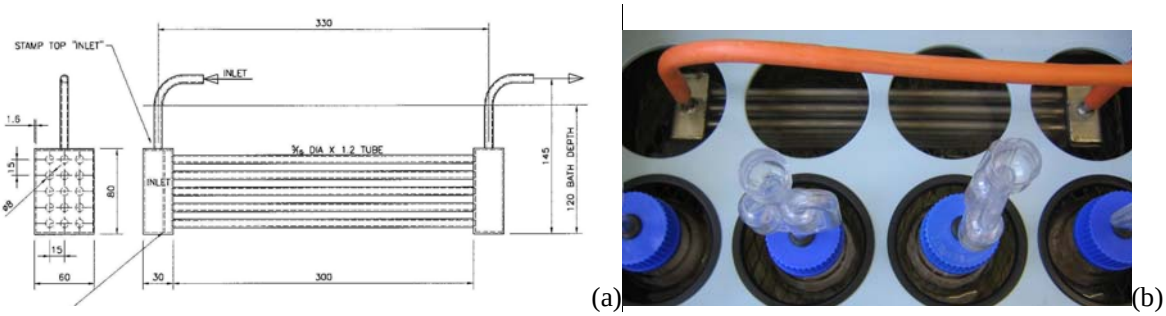
Wort volume was adjusted to 200mL for the EBC method and 100mL for the small-scale method. Samples were equilibrated to 25°C for 10 minutes in the mash bath and stirred constantly using a magnetic stirrer bar. The "revived" yeast was pitched into the wort samples at the rate of 1g/100mL and fermentation airlocks ("vintage" type) were fitted. Fermentation temperature was gradually reduced to 20°C over three hours and attenuation limit gravity determined at 24 hours and 48 hours after centrifuging an aliquot from the fermentation at 3000G for 10 minutes.

Statistical Analysis

Analysis of variance (ANOVA) analysis was performed using Genstat version 8.1.0.152 (VSN International Ltd, Hemel Hempstead, United Kingdom).

Results

The IEC 20 head mash bath is capable of accommodating 16 samples per fermentation batch, after the addition of the externally cooled heat exchanger assembly (Fig. 1).



(a) (b)

Figure 1. Engineering drawing (a) and photograph (b) of mash bath heat exchanger assembly.

The summary of experimental results comparing the small-scale and EBC fermentability procedures for each of the 8 malts tested are presented in Table 1. The results for the 15th EBC standard malt (sample 8) were within the range of values issued by the EBC Analysis Committee for both methods and fermentation times. The relatively wide tolerance range of ±2.5% further supports the notion that the fermentability test is influenced by a number of variables. A previous investigation (Evans and Hamet, 2005) found Mauribrew L497 dried lager yeast was capable of fully fermenting an EBC Congress wort under similar conditions (yeast pitched at rate of 0.5g/100mL) in 24 hours. This investigation found that 48 hours incubation was required to reach minimum gravity and there was a significant difference between 24 hours and 48 hours values (Table 2). The results also show that there is no significant difference between the small-scale procedure and that of the standard EBC method.

Table 1. Comparison of small-scale and EBC method fermentability performance at two intervals during fermentation for 8 commercial malt samples with Mauribrew 497 lager yeast.

Malt sample	Fermentation time (h)	Small-scale AAL(%)	EBC AAL(%)
1	24	79.0	78.3
	48	80.0	79.5
2	24	78.9	78.9
	48	80.0	80.0
3	24	75.1	75.2
	48	76.3	76.5
4	24	80.5	80.5
	48	81.8	81.6
5	24	73.6	73.5
	48	75.2	74.9
6	24	80.5	80.5
	48	81.6	81.6
7	24	79.9	80.1
	48	81.1	81.3
8*	24	78.9*	78.8*
	48	79.9*	79.9*
	LSD (P<0.05)	0.13	0.13

*15th EBC standard malt (tolerance 80.9 ± 2.5% AAL).

Table 2. Significance levels of treatment interactions for variate AAL%.

Source of variation	Significance level
Sample	***
Scale (Small-scale vs EBC)	ns
Time (24h vs 48h)	***
Sample.Scale	ns
Sample.Time	ns
Scale.Time	ns

*** (p<0.001), ns (p>0.05)

Conclusion

The reported small-scale AAL method can produce similar results to that obtained by the standard EBC method when carried out in a modified IEC mash bath. A 48 hour incubation was required to fully ferment wort using Mauribrew L497 dried yeast strain. The small-scale method has potential for application in evaluating promising crossbreds in barley breeding programs.

References

(1) Briggs, D.E., Boulton, C.A., Brookes, P.A. and Stevens, R. Brewing: Science and Practice. Woodhead Publishing Limited, Cambridge, England, 2004, pp. 510-512.

(2) European Brewery Convention Analytica – EBC. Verlag Hans Carl, Nurnberg, Germany, 1998.

(3) Evans, D.E. and Hamet, M.A.G. The Selection of a Dried Yeast Strain for Use in the Apparent Attenuation Limit Malt Analysis (AAL) Procedure. *J. Inst. Brew.*, **111**: (in press), 2005.
(4) Harasymow, S.E., Tarr, A.W., Diepeveen, D., Roumeliotis, S., Tansing, P., Black, C.K., Panozzo, J.F., Taylor, H., Fox, G., Skerman, Ferguson, A, R. and Inkerman, A.. Standardisation of a Small-Scale Hot Water Extract Method For Application in Barley Breeding Programs. Proceedings of the Eleventh Australian Barley Technical Symposium and the Fifty-third Australian Cereal Chemistry Conference, Glenelg, South Australia, 2003, CD ROM contribution 019.
(5) Institute of Brewing Methods of Analysis. The Institute of Brewing, London, England, 1997.
(6) Poirier, I., Marechal, P.-A., Richard, S., and Gervais, P., Saccharomyces cerevisiae viability is strongly dependent on rehydration kinetics and the temperature of dried cells. Journal of Applied Microbiology, 86: 87-92, 1999.