

STANDARDISATION OF A SMALL-SCALE HOT WATER EXTRACT METHOD FOR APPLICATION IN BARLEY BREEDING PROGRAMS

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INTRODUCTION

Hot water extract (HWE) is one of the key quality attributes considered when determining the malting performance of barley. The international malting and brewing industries utilise standard procedures for measuring HWE, including European Brewery Convention (Analytica - EBC 1998), Institute of Brewing (IOB - Methods of Analysis 1997), and American Society of Brewing Chemists (ASBC - Methods of Analysis 1992). EBC and ASBC procedures are similar and use a multi-temperature programmed mashing profile, while the IOB procedure uses a constant temperature infusion mashing profile. In Australia, maltsters and brewers generally use the EBC procedure to determine extract levels on commercial samples. Specific quality goals for developing Australian commercial barley varieties include a high extract carbohydrate potential with a desired level in excess of 82% EBC fine grind, dry basis (MBIBTC, 2001).

Malting quality testing protocols vary in public Australian barley breeding programs especially in early generation testing where significant quantities of grain may not be available. Different methodologies are used including NIR and small-scale assays developed in-house. A review of the barley quality evaluation laboratories in 1995 by the Grains Research and Development Corporation (GRDC) included a recommendation of implementing standardised methodology for micromalting and quality testing of advanced material (Enright *et al.*, 1995). The Australian Barley Chemists Group (ABCG) incorporating members of barley quality evaluation laboratories in Perth, Adelaide, Horsham, Wagga Wagga and Toowoomba, meet formally on an annual basis to discuss testing protocols and quality issues. The objective of method standardisation is to develop small-scale methods to provide breeding programs and industry groups with MBIBTC equivalent results from within the breeding programs. In 1999, a diastase method was standardised by the ABCG and correlated to the EBC industry method (Fox *et al.*, 1999). Due to sample quantity limitations in some stages of a barley breeding program, a small-scale EBC HWE procedure has been developed and collaboratively evaluated by the participants of the ABCG.

MATERIAL AND METHODS

The first phase was to benchmark each of the existing laboratory's small-scale procedure. Five commercial malt samples including the EBC 14th standard malt were analysed by each laboratory on three separate days for fine grind and coarse grind extracts. A supplied standard sucrose sample was included to monitor densitometer performance.

The second phase of the study was to (I) refine the standardised test method and (II) to provide a method for standardising the densitometers.

Ten blind duplicate malt samples and a sucrose density check sample were supplied to each laboratory. Each sample was analysed by the standardised protocol and specific gravity

results reported. Each laboratory determined malt moisture by using the EBC method 4.2 (Analytica EBC, 1998).

I. Standardised small-scale protocol

Equipment

- Mash baths were either IEC (Industrial Equipment and Control Pty. Ltd., Melbourne) or BRF (Crisp Instrumentation, Wainford Maltings, Suffolk, UK)
- Buhler Miag disc mill, 0.2mm fine grind setting (Braunschweig, FRG).
- Small-scale aluminium mash beakers (custom made approx 47mm ID).
- Magnetic stirrer bars – approx 30mm x 6mm with collars.
- Density meter – DMA58 or DMA5000 model (Anton Paar GmbH, Graz, Austria).
- Filter paper – Schleicher and Schuell #597 ½ (fluted), 185mm diameter.
- Miscellaneous equipment – electronic balance (0.01g accuracy), beakers, glass funnels.

Method

- 1) Weigh 10.0g (0.2mm fine grind) malt into mash pot and equilibrate at 45°C for 5 min.
- 2) Add 40mL distilled water at 45°C, cover and mash for 30 min.
- 3) Ramp temperature to 70.0°C at 1°C per minute.
- 4) Add 20 mL distilled water at 70.0°C and mash for a further 60 minutes.
- 5) Rapidly cool to 25°C and make up contents to 90.0g.
- 6) Filter, return first 20 mL filtrate and continue to filter to completion.
- 7) Read density within 2 hours of filtration.

II. Density meter standardisation

Densitometer standardisation was carried out using three sucrose standards, designed to cover a similar specific gravity range to wort samples. Each instrument was calibrated using high purity water and air from a desiccated source. The density of sucrose samples was determined from duplicates in randomised order.

The third phase of the study was to determine the small-scale method precision and correlation with the standard EBC procedure. Five commercial malt samples (including the EBC 14th standard check malt) were selected to cover a range in HWE. Replicated, randomised and coded samples were distributed to collaborators. Each laboratory analysed the samples by both the small-scale and the standard EBC methods on two separate days. Each laboratory also determined malt moistures. The calculated extract values were derived from the reported specific gravity and moisture data. The densitometers were calibrated as per the previous exercise. Instrument performance was monitored using a standard sucrose sample.

RESULTS AND DISCUSSION

An analysis of the first phase results by ANOVA (Genstat, VSN International Ltd, Oxford, UK) showed considerable variation in reported values (data not presented) with only two of the five laboratories being within the EBC 14th standard malt tolerance limit. The survey of the in-house procedures highlighted differences in mash baths and vessels, grist:liquor ratios, mashing profiles, protocols and method to calculate the final extract value. The standard sucrose results also showed variation between densitometers. The outcome of phase one resulted in an agreement of a standardised protocol closely resembling a one fifth scale EBC procedure. Each laboratory undertook to acquire similar equipment before proceeding with the next phase of the study.

I. Standardised protocol results

The second phase results again indicated variability between laboratories for both the malt samples and sucrose standard, with only two of the five laboratories within the EBC 14th

standard malt tolerance limit. Some of the observed variation could be attributed to possible changes in the malt samples as the time frame of the experiment was extended due to operational delays in some of the laboratories. Discussion with collaborators indicated that the densitometer calibration technique might also have contributed to the variation in results.

II. Density meter standardisation

The procedure to calibrate the densitometer was not consistent across all laboratories. The “two point” air and water calibration may be influenced if dry air was not used. Each laboratory was set up with a desiccated air supply and a follow up exercise carried out. Standard sucrose results for this test were in close agreement except for one laboratory (data not presented). It was later identified that this instrument had a fault that needed to be rectified.

Analysis of the sucrose standard values in the third phase of the experiment (Table 1) indicated close agreement between the densitometer instruments and these are within the limits given in the ASBC guidelines (ASBC - Methods of Analysis 1992). HWE results were analysed by ANOVA and outliers greater than one percent extract difference were deleted. Significance levels for each of the treatment interactions are presented in Table 2.

Table 1. Sucrose standard results.

Lab	Mean SG	Est. HWE*
1	1.03617	79.6
2	1.03631	79.9
3	1.03615	79.6
4	1.03627	79.9
5	1.03611	79.5
6	1.03619	79.7
CV		0.24

CV – coefficient of variation (%)

* estimated HWE

Table 2. Significance levels of treatment interactions within procedures.

Source of variation	Small-scale	EBC
Lab	***	***
Day	ns	ns
Variety	***	***
Lab.Day	***	***
Lab.Variety	***	***
Day.Variety	ns	ns
Lab.Day.Variety	ns	ns

*** (p<0.001) ns – (p>0.05)

The results have been averaged across replicates and days and summarised in Table 3 and Table 4. A similar variability was achieved for each method over the HWE range (77.5% – 84.8%) used in this study. The standard deviations for both the small-scale and EBC method was 1.8%, which equates to coefficient of variation values of 2.2% and 2.3% respectively. Samples were ranked in order by the small-scale method with only two of the laboratories having a cross-over between very similar samples in the middle of the range. Two laboratories were also outside the tolerance limit for the EBC 14th Standard Malt.

Table 3. Summary of small-scale HWE results.

Small-scale Lab	Sample					Mean	SD
	S1 (EBC std)*	S2	S3	S4	S5		
1	83.6*	81.5	78.5	80.9	80.0	80.9	1.87
2	83.7*	80.8	78.6	80.8	79.7	80.7	1.88
3	84.3*	81.9	79.0	81.6	80.6	81.5	1.92
4	83.8*	82.0	78.9	81.2	80.1	81.2	1.87
5	82.5	80.1	77.5	80.6	79.4	80.0	1.81
6	83.3	81.3	78.3	80.9	79.8	80.7	1.84
Mean	83.5	81.3	78.5	81.0	79.9	Grand mean	80.8
SD	0.58	0.72	0.53	0.34	0.39	Mean SD	1.76

* within EBC 14th Standard Malt tolerance 84.14 ± 0.69 % HWE dry basis.

Table 4. Summary of EBC standard procedure HWE results.

EBC lab	Sample					Mean	SD
	S1 (EBC std)*	S2	S3	S4	S5		
1	84.1*	81.9	78.7	81.6	80.4	81.3	1.98
2	83.4	81.1	77.9	80.7	79.6	80.6	2.02
3	84.8*	82.5	79.4	82.2	81.2	82.0	1.99
4	83.5*	81.3	78.3	80.9	80.3	80.8	1.89
5	83.8*	81.3	78.8	81.1	80.0	81.0	1.84
6	83.5*	81.2	78.2	81.0	79.6	80.7	1.96
Mean	83.8	81.6	78.6	81.2	80.2	Grand mean	81.1
SD	0.53	0.52	0.52	0.55	0.58	Mean SD	1.84

* within EBC 14th Standard Malt tolerance 84.14 ± 0.69 % HWE dry basis.

A comparison of the results between the two methods is graphed in Figure 1. Individual points are mean values between replicates for each day. A high correlation ($r = 0.95$) was obtained between the two methods.

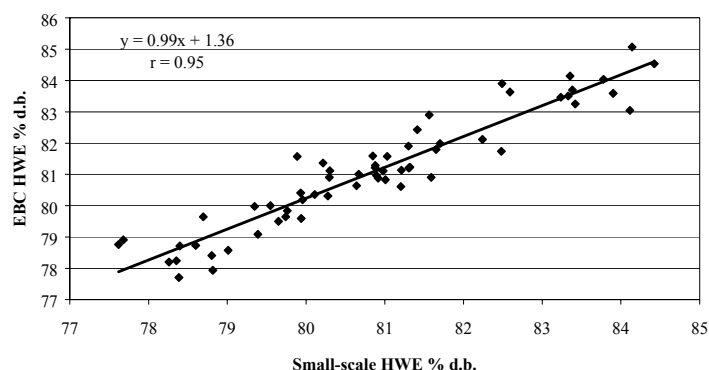


Figure 1. Correlation plot comparing HWE methods.

CONCLUSION

This study was able to demonstrate that the small-scale HWE method developed by the ABCG gave comparable results to the EBC standard HWE procedure. The procedure could significantly improve the measurement of HWE on material being evaluated through the barley breeding programs with no loss in precision. The exercise has also highlighted the importance of correct densitometer calibration and operation. The small-scale HWE method has been submitted to the methods committee of the Royal Australian Chemical Institute, Cereal Chemistry Division and has been approved and included as an official method of analysis (RACI Method 04-04, 2003).

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