

Application of Spectrophotometric Analysis of Soluble Nitrogen in a Barley Improvement Program

R.L.Fox, S.J.Logue, S.Roumeliotis and A.R.Barr

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

Introduction

The nitrogen content of malt is an important factor in determining its suitability for brewing. Nitrogen exists in a number of different forms in malt, from long chain, complex proteins to simple proteins and amino acids. The portion of this nitrogen that is solubilized into wort in a traditional laboratory mash is known as the soluble nitrogen, and represents about 40% of the total nitrogen (Haslemore, 1995). The ratio between the amount of water soluble nitrogen produced by a laboratory mash and the total nitrogen in the malt, is referred to as the Kolbach Index (KI). This gives an indication of the degree of modification of proteins during the malting process, as the greater the breakdown of proteins during malting, the more nitrogenous compounds that will be soluble.

Traditionally, soluble nitrogen has been measured using the Kjeldahl method (EBC, 1998). This method is time consuming, involves using a number of toxic and hazardous chemicals and is difficult to apply to the large number of samples which are generated in a barley breeding program. Consequently, soluble protein is usually only determined at the most advanced stages of quality testing.

A simpler and more convenient approach is to measure soluble nitrogen using the American Society of Brewing Chemists (ASBC) spectrophotometric method (ASBC, 1992), which requires very little wort and can be applied to a large number of samples. The method involves diluting a small sample of wort in sodium chloride and measuring the absorbance at 215 and 225 nm. Soluble protein is then predicted using linear regression against a calibration equation developed using a set of Kjeldahl analyses.

In this study the ASBC spectrophotometric method was assessed for its suitability in a barley improvement program. A number of approaches for generating calibration sets, including the ASBC recommended method, and both site-specific and site-combined calibrations, were compared to see which performed better in determining soluble protein.

Materials and Methods

Sample selection

Barley varieties were chosen to yield as wide a range of soluble protein results as possible. Samples were micromalted in a Phoenix Biosystems Micromalting Unit, using the protocol described in the 1997 Waite Barley Quality Evaluation Laboratory (WBQEL) Report (WBQEL, 1997).

a) ASBC calibration

A total of six barley varieties were selected from 1996 season Waite breeders trials (Table 1).

b) Site-specific and site-combined calibrations

Six barley varieties were selected from each of seven sites for both 1997 and 1998 seasons of the South Australian Research and Development Institute (SARDI) Stage 4 trials (Table 1).

Table 1. Barley varieties and sites used for calibration development (all varieties were grown at all sites within each year).

ASBC Calibration (Sol. Protein Range 3.44-5.18%)	Site-specific and –combined calibration (1997) (Sol. Protein Range 3.48-6.17%)		Site-specific and -combined Calibration (1998) (Sol. Protein Range 3.59-6.30%)	
Variety	Variety	Site	Variety	Site
Schooner	Arapiles	S. Springs	Arapiles	Borrika
Schooner*	Schooner	Port Clinton	Schooner	Bute
Chebec	Namoi	Warooka	Namoi	Arthurton
WI 3086	Sloop	Wharminda	Sloop	Mundulla
Franklin	WI 3107	Lameroo	WI 3107	Lameroo
89/126/C*08	WA 5040	Mangalo	Franklin	Mangalo
		Brentwood		Brentwood

*Laboratory analysis control, supplied by Joe White Maltings Ltd.

Measurement of soluble protein

For each calibration sample, wort was prepared using a small scale variation of the standard EBC fine grind hot water extract (HWE) method using 10g of malt (MacLeod *et al.*, 1991). HWE was determined using a Anton Paar AMV 200 densitometer. Each sample was analysed in duplicate for soluble nitrogen using both the Kjeldahl (EBC Analytica, 1998) and ASBC spectrophotometric (ASBC, 1992) methods. The Kjeldahl method applied was an adaption of the EBC Soluble Nitrogen of Malt: Kjeldahl Method where a Tecator Kjeltac 1030 auto distillation unit was used. For the ASBC method, a 1/50 dilution of wort was made with sodium chloride and the absorbance was determined using a single beam spectrophotometer zeroed on sodium chloride at 215 and 225 nm.

Calibration Development

A number of regression equations were derived from the calibration samples, including:

1. An equation derived from the ASBC method, composed of the six varieties which were selected from 1996 season Waite field trials.
2. Site specific equations, in which the six varieties from the SARDI Stage 4 trials were plotted independently for each site. This generated seven equations for 1997 season trials and seven for 1998 season trials.
3. Site-combined equations, where results from all sites were pooled for both the 1997 and 1998 data, generating one equation for 1997 and one for 1998.

Validation of calibrations

The performance of the calibration sets in determining soluble protein was assessed using the complete set of 1997 and 1998 season SARDI Stage 4 trial entries (approximately 14 entries x 2 reps x 7 sites for each season). The calibration sets developed from 1997 season samples were validated using samples from the 1997 Stage 4 trial, and likewise the 1998 season-derived calibrations were validated using 1998 season entries. The ASBC-derived calibration was validated using both 1997 and 1998 season samples.

Validation samples were micromalted and extracts produced as described above. Absorbances were measured at 215nm and 225 nm, and soluble protein was then predicted using the three sets of regression equations.

Statistical analysis

Simple correlation analysis was used to compare soluble protein results calculated by the spectroscopy method with Kjeldahl results. To assess whether the samples analysed by both the spectroscopy and the Kjeldahl method were ranking the lines in a similar order the Spearman Rank Correlation Coefficient was used. The statistical packages used were Jmp v3.0, Excel 5.0 and Agrobases97 for Windows.

Results and Discussion

The calibration developed using the ASBC method, with six malt samples, gave a good correlation between absorbance difference and Kjeldahl soluble protein ($R^2 = 0.973$, data not shown).

The results show that for both 1997 (Figure 1) and 1998 (Figure 2) seasons the site-specific correlations between absorbance difference and Kjeldahl soluble protein were excellent, with a range of R^2 values between 0.901-0.995 for 1997, and 0.932-0.997 for 1998. In Figure 3, the site-specific calibrations have been pooled for both the 1997 and 1998 data. The correlations for each year were very good ($R^2 = 0.826$ for 1997; $R^2 = 0.954$ for 1998). In practical terms, the estimated soluble protein would range from 5.07 – 5.43 for an absorbance value of 0.4, depending on whether you use the 1997, 1998 or combined data regression model (Table 2).

Table 2. Regression model details for SARDI 1997 & 1998 data and combined data from both years, and in parentheses the standard error of the slope and intercept.

	Combined Data	1997 Data	1998 Data
Slope	12.47 (0.37)	13.7 (0.71)	11.99 (0.42)
Intercept	0.34 (0.13)	-0.05 (0.24)	0.47 (0.14)

Table 3 shows the results obtained when the three sets of calibration equations (ASBC, site-specific and site-combined) were validated using the total SARDI Stage 4 trial set for the 1997 and 1998 seasons. All correlations were highly significant, with calibrations performing particularly well for the 1998 season. The ASBC and site-combined calibrations performed slightly better than the site-specific calibrations. However, the lower correlations obtained for the site-specific calibrations than the site-combined ones probably reflect the much smaller number of data points in the site-specific calibration sets (6 versus 42). Importantly, the

Spearman ranks showed that the calibrations were correctly ranking entries, regardless of the calibration equation used.

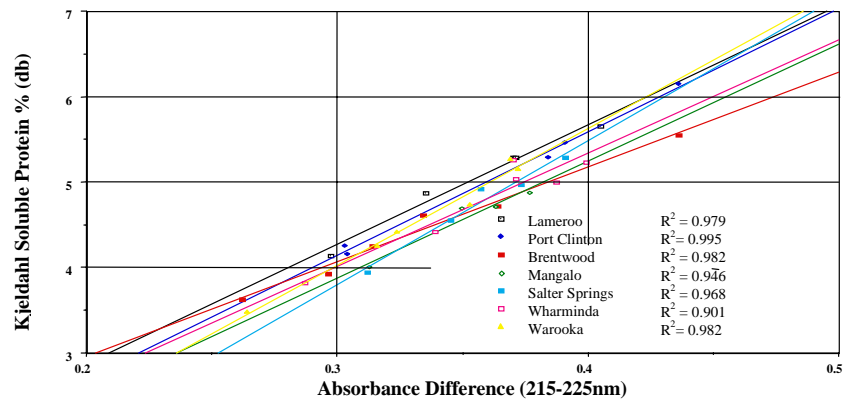


Figure 1. Fitted Linear Regression Lines for Individual Site of Absorbance Difference against Kjeldahl Soluble Protein for seven sites from 1997 SARDI Stage 4 trials.

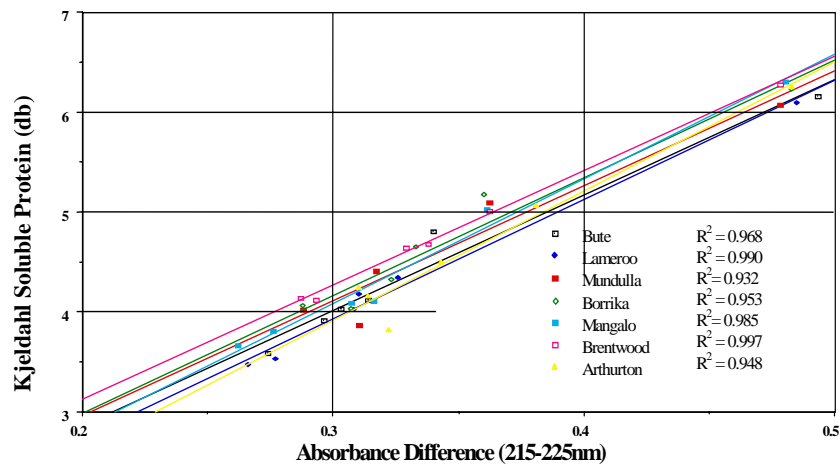


Figure 2. Fitted Linear Regression Lines for Individual Site of Absorbance Difference against Kjeldahl Soluble Protein for seven sites from 1998 SARDI Stage 4 trials.

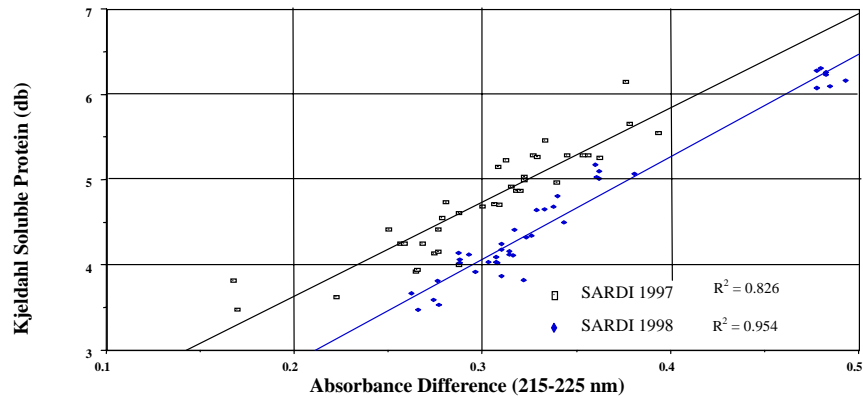


Figure 3. Fitted Linear Regression Lines for Pooled Sites of Absorbance Difference against Kjeldahl Soluble Protein for the SARDI Stage 4 trials in 1997 and 1998.

Table 3. Pearson and Spearman Rank Correlations for the validation of ASBC, site-specific and site-combined calibrations.

	1997	1997	1998	1998
	Spearman Rank	Pearson Correlation	Spearman Rank	Pearson Correlation
ASBC Calibration	0.817	0.745	0.948	0.976
Site-Combined calibration	0.821	0.745	0.948	0.976
Site-Specific Calibration	0.801	0.672	0.920	0.958

(All significant at $P < 0.001$).

It had been anticipated that the site-combined calibrations for the 1997 and 1998 seasons could be further combined to give a single, robust calibration. However it can be seen from Figure 3 that despite the 1997 and 1998 correlations having similar slopes, they have markedly different intercepts. This could be due to differences in the methods of analysis used in the two years, e.g. differences in micromalting regime or Kjeldahl method, differences in the cuvettes used or in the calibration of the spectrophotometer. Alternatively, there could be between-season environmental effects, which are not apparent between sites within a given season. The implications of this deviation are that there would be large errors associated with using the calibration developed in 1997 to predict soluble proteins on 1998 entries, and vice versa.

On the basis of these results, the most appropriate strategy would be to develop a new calibration for each site-season combination. However, this approach would only be justified for a site with a large number of entries, such as an early generation trial. In practice, for advanced trials, with small numbers of entries, a calibration using pooled data from a number of sites from a particular season should be adequate to predict soluble protein, but it will be necessary to develop a new calibration each season. In a Kjeldahl analysis, up to 18 samples can be analysed in a single batch. If 36 data points were used in the calibration (2 batches of Kjeldahl analysis), this represents an input of approximately 17% of the total number of SARDI Stage 4 entries (approximately 210 in a typical season), in terms of reference Kjeldahl analysis. However, if 18 data points were used (one batch), which should be an acceptable number of points for a calibration, this figure would be reduced to 8.5%.

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