Rapid Malt Modification Analyses in a Production Malthouse: Friabilimeter and Calcofluor Methodologies[1]

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ABSTRACT

Sixty-nine production malt samples with various levels of modification were analyzed by Friabilimeter and Calcofluor-stain methods and compared to conventional ASBC malt analyses. A statistical comparison of the results proves that the two newer methods correlate well with conventional malt modification measurement and to each other, and can be used effectively to provide rapid and accurate malt modification data. However, the data show that these two methods differ considerably with respect to malt total and soluble protein analyses and, therefore, to malt extract quantification as described in the Bishop regularity principle of malt extract prediction. Specifically, the Friabilimeter method was highly correlated to total protein ($r = -0.64$) and malt extract ($r = 0.60$) but was not correlated to soluble protein ($r = 0.09$) in the malt. The Calcofluor method, however, was not statistically correlated with total protein, ($r = -0.15$), was correlated with soluble protein data ($r = 0.34$), and was somewhat less correlatable to percent malt extract ($r = 0.36$). These differences indicate that the Friabilimeter and Calcofluor-stain methods can be used to yield rapid and accurate information about general malt modification, but can result in different information regarding malt protein content and solubilization.

Throughout the years, new technology and methods have been developed that yield important information about the quality of barley malt in commercial malting processes. These "new" techniques are usually compared to time-honored conventional methods of analyses, and can generally be grouped into three main types: microscopy, chemical analysis, and physical testing. The first category, microscopic examination of barley malt, although nearly 100 years old (4), can require expensive light, transmission, or scanning electron microscopes and has limited usefulness. Chemical analyses, the second category, are generally used worldwide as the standard methods for malt quality assessment (2,9,11). However, the principal disadvantage of most of these standard methods is the considerable time (usually several days) between malt sampling and complete analysis. Third, physical testing methods of malt analyses are usually the simplest and most rapid tests to perform and, therefore, are most desirable to the practical maltster. These methods, which include simple biting, rubbing out, smear tests, or acrospire growth measurements are immediate assessments but can suffer from personal subjectivity and poor precision and accuracy.

For these reasons, new techniques are continually being sought that combine the reliability of microscopic and chemical analyses with the applicability of physical testing methods. Two of these newer methods, Friabilimeter analysis (6) and Calcofluor staining visualization (1), are compared to each other and to a series of conventional malt analyses of the American Society of Brewing Chemists (2).

MATERIALS AND METHODS

Malt Samples

Sixty-nine samples of malt (two-rowed barley, cultivar Moravian III) taken from production kilns and malt blends were selected to yield a wide range of malt analyses from the Adolph Coors Company malthouses over a period of several weeks.

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Standard Malt Analysis Methods

All conventional malt analyses were performed according to the ASBC *Methods of Analysis* (2).

Calcofluor-Stain Method

The Carlsberg Seed Fixation and Malt Modification Analyser systems were used in this study. Analysis of Calcofluor modification and homogeneity were performed according to Aastrup et al (1). Calcofluor method calculations were made according to Carnielo et al (5).

Friabilimeter Method

A Friabilimeter designed by Chapon was obtained from Franz-Pfeuffer Apparatebau, West Germany. Percent friability, half-glassy and all-glassy malt analyses were performed according to Chapon et al (6). Friable malt is that which is crushed and passes through the screen of the Friabilimeter during 8 min of operation. Half-glassy malt is that portion of malt remaining in the Friabilimeter screen which passes through a 5/64-in. slotted screen. All-glassy malt is that portion of malt remaining in the Friabilimeter that is retained on a 5/64-in. slotted screen.

Statistical Analysis

A completely randomized design was utilized for malt sample collection. All data were entered into a Hewlett-Packard 9845T computer for basic statistics, correlation coefficients, and multiple linear regression analysis. Determination of statistical significance was made according to a standard statistical method (8).

RESULTS AND DISCUSSION

Table I shows the mean values and standard deviations for all of the malt analysis parameters examined in this study. It is interesting to note that percent friability, Calcofluor modification and homogeneity yield mean values similar to fine-grind extract but with much higher standard deviations.

The linear correlation of Friabilimeter and Calcofluor-stain parameters to conventional malt characteristics (Tables II and III) produced both expected and unexpected results. Fine-grind extract, as expected, yielded significant and positive correlation coefficients (r values) to percent friability, percent Calcofluor modification, and percent Calcofluor homogeneity. Also as

TABLE I
Malt Analysis Data Summary ($n = 69$ samples)

Method	Method Reference[a]	Mean	SD
Extract, fine grind, dry basis (%)	(2)	81.0	0.5
Diastatic power (° L)	(2)	121	8.6
α-Amylase (20° D.U.)	(2)	37.9	3.9
Total protein (%)	(2)	12.0	0.6
Soluble protein (%)	(2)	5.4	0.3
Soluble/total protein (%)	(2)	44.9	2.9
Friability (%)	(6)	79.6	7.1
Half-glassy malt (%)	(6)	16.7	4.4
All-glassy malt (%)	(6)	3.7	2.8
Calcofluor modification (%)	(1)	94.7	5.8
Calcofluor homogeneity (%)	(1)	84.9	11.2

[a](2) Official Methods of Analysis of the American Society of Brewing Chemists, (6) Friabilimeter analysis, and (1) Calcofluor malt modification.

expected, the calculated r values were significant and negative in correlating percent extract with the quantity of unmodified remnants in Friabilimeter analysis, namely percent all-glassy and percent half-glassy malt. Calculated statistical significance was higher for all Friabilimeter parameters (Table II) than those of the Calcofluor-stain method (Table III) when correlated with percent fine-grind extract. One possible reason for this difference is discussed later.

Diastatic power data did not correlate with any of the Friabilimeter or Calcofluor-stain parameters. In fact, the only significant correlations with diastatic power in this study were to total protein ($r = 0.38$, significant at the 95% confidence interval [CI]), and S/T protein ratio ($r = 0.37$, 95% CI). It should be noted that these results are for a single variety of barley over a relatively narrow protein range and, therefore, may account for the lack of correlation to diastatic power.

The statistical analysis of the α-amylase data produced highly significant correlation coefficients for all Friabilimeter (Table II) and Calcofluor-stain parameters (Table III). This finding is not surprising, as it is well documented that *de novo* synthesis of α-amylase occurs during germination in barley malting (7) and, therefore, should relate to parameters that measure the degree or efficiency of germination and malt modification. Indeed, the breakdown of endosperm cell walls appears to be a prerequisite to the diffusion of *de novo* synthesized enzymes (12,13).

An important, and unexpected, difference between the two new methods is shown by the correlations of Friabilimeter and Calcofluor-stain data to total protein in malt. It was expected that a higher level of storage matrix proteins in the endosperm of barley would make barley malt kernels harder and more difficult to modify and crush in the Friabilimeter; hence, the negative relationship between total protein and friability and the positive correlation to the quantity of half-glassy and all-glassy malt (Table II). However, within the range of total protein examined (11.2–13.7%), there is no statistical relationship between total malt protein content and Calcofluor modification or homogeneity (Table III). This lack of correlation agrees with results reported by Horgan et al (10) for Australian barley. Two inferences can be drawn from this data. First, endosperm cell wall β-D-glucan hydrolysis, as quantified by Calcofluor staining, proceeds at a rate which is, apparently, independent of total endosperm protein content.

The second relationship evidenced by this data may explain why there is a lower correlation between Calcofluor-stain parameters and fine-grind extract, as noted earlier. A protein-extract relationship, first described by Bishop in 1930 (3) and known as the Bishop regularity principle, may apply here. It expressed a quantifiable relationship of mass balance, which simply states that as one constituent of barley malt increases (protein), all other constituents of the whole kernel (e.g., extractable carbohydrates) must, by difference, decrease. The percent friability of the malt decreases with increasing total protein content, as does the total extract yield. However, the Calcofluor-stain method, while reflecting the degree of modification of the endosperm cell walls, does not correlate to total extract production as well as the Friabilimeter data. The reason for this lowered correlation to total extract yield is probably attributable to the lack of correlation to total malt protein content. Therefore, the effects of malt protein content, rate of endosperm modification, and prediction of final malt extractability are measured in slightly different manners by the Friabilimeter and Calcofluor-stain malt analysis methods.

The degree of protein solubilization is also evidenced in different ways by the two methods examined, as shown by the correlations of Friabilimeter and Calcofluor data to percent soluble protein and soluble to total protein (S/T) ratio in Tables II and III. Friabilimeter analysis is not correlated to soluble protein content, and apparently is positively related to the S/T ratio through its negative correlation with total protein. The Calcofluor-stain technique, however, has a statistically significant relationship with protein modification, as evidenced by the positive r values with percent soluble protein and S/T ratio. The Calcofluor-stain method, therefore, shows no predictive value in total malt protein effects, but can monitor the hydrolysis of endosperm protein and cell wall β-D-glucan. On the other hand, the Friabilimeter method can also accurately reflect the degree of malt modification as well as the effects of total malt protein on modification and extractability.

Perhaps as a result of the differences shown by the two methods with respect to malt total and soluble protein content, the correlations between percent friability and Calcofluor modification and homogeneity are lower than might be expected (Table IV). The correlation between percent all-glassy malt and Calcofluor modification and homogeneity is high because of the obvious effect that slow or nongerminated barley has on both analytical techniques.

An additional aspect of Friabilimeter analysis, which may further reduce the correlation of Friabilimeter to Calcofluor data, is the presence of case-hardened malt as described by Thomas (14). Case-hardened malt is well-modified malt, as measured by Calcofluor stain and ASBC malt analyses, but is not crushed in the standard Friabilimeter assay, thus appearing as unmodified malt. The case-hardened malt phenomenon reflects kiln process conditions and may also relate to barley protein quantity or quality (14).

Stepwise regression of Friabilimeter and Calcofluor-stain data (Table V) shows the relative importance of each parameter that influences modification predictability of the new methods. Percent friability is most influenced by (or predictive of) percent fine-grind

TABLE II
Linear Correlation Coefficients of Friabilimeter Parameters ($n = 69$)

Parameter	% Friability	% Half-Glassy	% All-Glassy
% Extract, fine grind	0.60**[a]	−0.59**	−0.57**
Diastatic power	−0.23	0.20	0.16
α-Amylase	0.55**	−0.48**	−0.56**
% Total protein	−0.64**	0.63**	0.52**
% Soluble protein	0.09	−0.06	−0.17
% S/T protein[b]	0.54**	−0.50**	−0.52**

[a]** Statistically significant at the 99% confidence interval; all others not statistically significant.
[b]Ratio of soluble to total protein.

TABLE III
Linear Correlation Coefficients of Calcofluor-Stain Parameters ($n = 69$)

Parameter	% Calcofluor Modification	% Calcofluor Homogeneity
% Extract, fine grind	0.36*[a]	0.36*
Diastatic power	0.01	−0.09
α-Amylase	0.60**	0.63**
% Total protein	−0.15	−0.15
% Soluble protein	0.34*	0.48**
% S/T protein[b]	0.44**	0.57**

[a]*, **Statistically significant at the 95 and 99% confidence intervals, respectively; all others not statistically significant.
[b]Ratio of soluble to total protein.

TABLE IV
Linear Correlation Coefficients (r values) of Friabilimeter Versus Calcofluor-Stain Parameters ($n = 69$)

Parameter	% Calcofluor Modification	% Calcofluor Homogeneity
% Friability	0.55**[a]	0.59**
% Half-glassy	−0.43**	−0.49**
% All-glassy	−0.70**	−0.69**

[a]**Statistically significant at the 99% confidence interval.

TABLE V
Stepwise Regression of Friabilimeter and Calcofluor-Stain Parameters

Dependent Parameter[a]	Independent Parameters	Partial *F* Values
% Friability	% Extract, fine grind	795
	α-Amylase	518
	% Half-glassy malt	499
	% Total protein	219
	Regression residual	209
% Half-glassy malt	% Extract, fine grind	405
	% Friability	314
	α-Amylase	179
	% Total protein	102
	Regression residual	100
% All-glassy malt	% Extract, fine grind	243
	α-Amylase	168
	% Calcofluor modification	91
	Regression residual	74
% Calcofluor modification	% Calcofluor homogeneity	188
	α-Amylase	157
	% Extract, fine grind	75
	Regression residual	47
% Calcofluor homogeneity	% Calcofluor modification	193
	α-Amylase	192
	% Extract, fine grind	72
	Regression residual	44

[a]For each dependent parameter shown, the independent parameters are listed in decreasing order of statistical importance as indicated by the partial *F* values.

extract, α-amylase, percent half-glassy malt, and percent total protein, in descending order. Percent half-glassy malt content is most strongly related to percent fine-grind extract, percent friability, α-amylase, and total protein content. The quantity of all-glassy malt directly influenced extract, α-amylase, and Calcofluor modification measurements. Whereas Calcofluor modification and homogeneity exhibit strong calculated effects to each other, regression analysis also shows that α-amylase and malt extract quantity are predicted by the Calcofluor method. The calculated *F* values (Table V) are higher for the Friabilimeter data than for Calcofluor-stain parameters, again indicating slight differences between the two methods.

CONCLUSIONS

Both the Friabilimeter and Calcofluor-stain methodologies can provide simple and rapid measurement of malt modification. These methods produce highly significant correlations when compared to conventional methods of malt analysis. However, the two methods produce different results with respect to malt total and soluble protein analyses and the relationship of protein content to final malt extract prediction. This implies that the interpretation of data from the two methods can provide slightly different views of the complex phenomenon of malt modification, while allowing for the ultimate goals of rapid data turnaround and applicability to a production malting process.

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