Diastatic Power (Rapid Method)

Subcommittee Members: M. Joyce, *Chairman*; J. Cuti; R. Femmer; C. Judd; B. Johannes; S. Lie, European Brewery Convention (EBC); H. Numata; M. Rehmanji; D. Schaefer; A. Sugishita; W. Swenson; M. Wachter; K. Ziemba; and D. Thomas (*ex officio*).

Keywords: Malt infusion, Reducing sugars, α-Amylase

CONCLUSIONS

1. The Henry method (5) for diastatic power showed high repeatability and reproducibility coefficients of variation for all five sample pairs.
2. The Malt-6 diastatic power method (1) showed acceptable repeatability and reproducibility coefficients of variation for all five sample pairs.
3. There was no significant difference between the means of Malt-6 infusion (1) and the Henry method extraction used for α-amylase analyses.

RECOMMENDATIONS

1. The subcommittee recommends accepting the Henry method, as given in the Appendix, as an alternative method for the determination of diastatic power. The method can be used for the rapid determination of diastatic power levels in malts with levels below 200 diastatic power units. The method should first be standardized against Malt-6.
2. The subcommittee recommends accepting the Henry method extraction as an alternative procedure for Malt-7 α-Amylase determination (1).
3. Discharge the subcommittee.

This is the third year report of the subcommittee formed to investigate the use of the Henry method for diastatic power analysis (5). This method is attractive because it uses a shortened extraction and incubation procedure and is performed spectrophotometrically. For Malt-7 α-amylase assay (1), Malt-6 infusion (1) was compared with the Henry method extraction. If the results were similar, both assays could be determined on the same infusion. The third charge of the subcommittee was to calibrate the Henry method to Malt-6 diastatic power units.

PROCEDURE

Five sample pairs of malt were analyzed for diastatic power by Malt-6 and the Henry method. Each collaborator was asked to construct a glucose standard curve for the Henry method. The infusion from Malt-6 (0.5% sodium chloride extraction) and the extraction from the Henry method (6.0 m*M* ammonium hydroxide extraction) were used for the Malt-7 α-Amylase method.

The Youden unit block experimental design (4) was used for both diastatic power and α-amylase. A *t* test of the means (3) was used for comparing the α-amylase results. Linear calibration equations were calculated for each collaborator based on their glucose standard curves and Malt-6 diastatic power (as-is) results (1).

RESULTS AND DISCUSSION

Diastatic Power

Twelve collaborators submitted Malt-6 results for the five sample pairs. The results are presented in Table I. The repeatability and reproducibility coefficients of variation were acceptable. Twelve collaborators submitted Henry method results for the five sample pairs. Outliers, identified using Dixon's outlier test (1), were found in all sample pairs. The results are presented in Table II. The results of Tables I and II are summarized in Table III. The repeatability and reproducibility coefficients of variation for the sample pairs were 4.6–12.2 and 13.6–15.1, respectively. The reproducibility coefficients of variation decreased slightly compared with last years results (2). Comparison of the repeatability and reproducibility coefficients of variation between the Malt-6 and Henry method results indicates better precision with Malt-6. The Henry method is recommmended as a rapid method without the precision of Malt-6.

Diastatic Power-Linear Calibration

Twelve collaborators submitted glucose standard curves for the Henry method. Linear calibrations were calculated plotting g/L of reducing sugars (glucose) against Malt-6 diastatic power (as-is) for each collaborator. The correlation coefficients for the 12 collaborators were 0.917–0.999. The linear calibration equations and correlation coefficients for the individual collaborators are

TABLE I
Diastatic Power from Malt-6 (°ASBC, dry basis)

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H	I	J
1	86	88	116	113	139	141	158	153	199	209
2	89	88	114	112	142	147	159	161	203	217
3	88	89	115	113	149	151	158	166	213	226
4	89	88	115	112	145	146	160	161	208	217
5	86	85	110	111	139	147	148	151	204	215
6	80	82	120	114	136	139	152	154	195	227
7	87	91	115	116	137	137	152	152	220	221
8	86	88	115	115	143	142	159	159	200	215
9	94	98	118	116	152	149	160	161	213	217
10	82	84	110	107	138	135	146	153	192	203
11	87	92	113	114	146	142	155	154	192	201
12	96	95	128	121	156	144	172	160	210	222
Mean	87.5	89.0	115.8	113.7	143.5	143.3	156.6	157.1	204.1	215.8
Grand mean	88.3		114.7		143.4		156.8		210.0	

TABLE II
Reducing Sugars from the Henry Method (g/L)

Collaborator	Sample Pair A	B	Sample Pair C	D	Sample Pair E	F	Sample Pair G	H	Sample Pair I	J
1	0.28	0.27	0.36	0.34	0.44	0.43	0.48	0.50	0.70	0.73
2	0.23	0.25	0.34	0.31	0.44	0.36	0.43	0.43	0.57	0.62
3	0.32	0.33	0.42	0.40	0.49	0.50	0.57	0.56	0.71	0.76
4	0.31	0.34	0.42	0.36	0.49	0.49	0.51	0.52	0.75	0.77
5	0.25	0.36	0.34	0.38	0.56[a]	0.61[a]	0.83[a]	0.77[a]	1.01[a]	0.86[a]
6	0.23	0.26	0.32	0.35	0.32	0.41	0.52	0.43	0.61	0.91
7	0.32	0.34	0.47[a]	0.46[a]	0.53	0.54	0.61	0.59	0.96[a]	0.98[a]
8	0.27	0.28	0.36	0.35	0.41	0.43	0.46	0.45	0.58	0.62
9	0.37[a]	0.38[a]	0.49	0.42	0.58	0.55	0.75[a]	0.63[a]	0.80	0.90
10	0.34	0.25	0.38	0.36	0.49	0.47	0.52	0.51	0.61	0.75
11	0.24[a]	0.17[a]	0.24[a]	0.24[a]	0.29[a]	0.34[a]	0.37	0.35	0.54	0.53
12	0.28	0.26	0.28	0.30	0.39	0.42	0.52	0.47	0.72	0.73
Mean[b]	0.283	0.294	0.371	0.357	0.458	0.460	0.499	0.481	0.659	0.732
Grand mean[b]	0.289		0.364		0.459		0.490		0.695	

[a]Denotes outlier at $P \leq 0.01$, based on totals and/or differences (1).
[b]Calculated excluding outliers.

TABLE III
Statistical Summary of Results[a]

Sample Pair	No. of Labs	Grand Mean	Repeatability s_r	cv_r	r_{95}	Reproducibility s_R	cv_R	R_{95}
Reducing sugars from Henry method								
A/B	10	0.289	0.035	12.2	0.099	0.041	14.3	0.115
C/D	10	0.364	0.025	7.0	0.071	0.050	13.6	0.139
E/F	10	0.459	0.031	6.8	0.087	0.068	14.8	0.190
G/H	10	0.490	0.022	4.6	0.062	0.069	14.1	0.194
I/J	10	0.695	0.064	9.2	0.180	0.105	15.1	0.293
Diastatic power from Malt-6								
A/B	12	88.3	1.523	1.7	4.263	4.457	5.1	12.479
C/D	12	114.7	1.845	1.6	5.167	4.139	3.6	11.590
E/F	12	143.4	3.603	2.5	10.090	5.648	3.9	15.813
G/H	12	156.8	3.687	2.4	10.322	5.877	3.7	16.455
I/J	12	210.0	5.318	2.5	14.891	8.556	4.1	23.958

[a]All calculations were made based on reference 4.

TABLE IV
Collaborator Linear Calibration Equations for Rapid Diastatic Power Method

Collaborator	No. of Labs	Correlation Coefficient	Linear Equation	Standard Error of Y Estimate
1	10	0.992	$y = 245.52x + 21.18$	5.11
2	10	0.980	$y = 319.90x + 7.68$	8.67
3	10	0.995	$y = 299.08x - 13.33$	4.46
4	10	0.986	$y = 262.05x + 5.42$	7.48
5	10	0.917	$y = 146.26x + 44.78$	17.68
6	10	0.950	$y = 202.65x + 43.64$	14.31
7	10	0.999	$y = 195.23x + 21.37$	2.45
8	10	0.998	$y = 354.41x - 14.81$	2.76
9	10	0.963	$y = 213.37x + 13.75$	11.55
10	10	0.974	$y = 264.30x + 37.07$	9.45
11	10	0.960	$y = 281.42x + 37.97$	10.63
12	10	0.974	$y = 218.96x + 46.02$	9.54

TABLE V
α-Amylase from Malt-6 Infusions (20°C, DU, dry basis)

Collaborator	Sample Pair A	B	Sample Pair C	D	Sample Pair E	F	Sample Pair G	H	Sample Pair I	J
1	41.4	44.1	38.5	38.8	37.6	36.3	41.0	41.5	42.3	42.9
2	49.5	51.1	45.1	42.9	41.7	43.3	49.2	49.4	50.7	52.7
5	39.5	39.6	35.8	33.9	33.9	34.9	38.4	36.3	40.2	42.6
6	42.0	44.0	42.5	43.0	52.5	53.0	48.5	50.5	57.5	59.0
7	43.0	43.9	39.9	39.6	38.9	39.0	41.8	41.3	43.1	43.4
8	41.5	42.9	38.5	36.3	35.9	35.0	39.9	39.5	42.1	42.8
9	41.5	46.1	39.1	38.1	37.0	33.1	37.2	37.4	39.9	40.7
10	42.1	44.0	39.5	36.6	34.5	34.4	39.7	38.3	42.8	41.4
11	46.2	48.3	41.7	39.9	37.5	37.8	43.1	41.0	46.6	43.7
12	37.2	39.1	32.6	30.2	28.2	29.8	35.5	33.1	37.9	37.9
Mean	42.39	44.31	39.32	37.93	37.76	37.65	41.43	40.83	44.31	44.71
Grand mean	43.35		38.63		37.71		41.13		44.51	

summarized in Table IV. The standard error of Y estimate (diastatic power, as-is) ranged from 2.45, when a correlation coefficient of 0.999 was obtained, to 17.68, when a correlation coefficient of 0.917 was obtained.

α-Amylase

Ten collaborators submitted results for the α-amylase comparison. The results using Malt-7 and the Henry method extraction are presented in Tables V and VI, respectively. The statistical summary of results is presented in Table VII. Repeatability and reproducibility coefficients of variation for the five sample pairs from both extraction procedures were acceptable. A comparison of α-amylase means for both methods is presented in Table VIII. A t test indicated no significant difference between the two extraction methods.

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*, 7th ed. Malt-6 Diastatic power, -7 α-Amylase; Statistical Analysis-4 Youden block collaborative testing procedures. The Society: St. Paul, MN, 1976.

TABLE VI
α-Amylase from the Henry Method Extraction (20°C, DU, dry basis)

Collaborator	Sample Pair A	B	Sample Pair C	D	Sample Pair E	F	Sample Pair G	H	Sample Pair I	J
1	44.6	46.4	40.8	41.0	39.3	38.4	43.3	41.9	45.4	46.4
2	48.8	50.0	41.9	42.3	39.7	39.8	44.2	44.5	48.8	51.4
5	42.9	45.9	39.3	40.9	35.6	37.4	41.2	38.4	47.1	44.9
6	35.0	36.0	33.0	31.5	44.5	45.5	39.0	38.5	45.5	49.0
7	42.5	49.5	42.9	42.3	41.0	41.2	44.3	44.2	43.7	43.6
8	45.3	46.3	40.3	38.4	40.0	40.3	41.9	40.8	44.5	45.8
9	42.1	43.4	38.6	35.7	33.5	33.0	39.7	38.2	41.0	44.0
10	42.1	45.0	39.9	37.2	35.7	35.1	40.5	39.0	45.9	44.0
11	53.3	53.9	48.5	45.6	43.3	45.2	49.1	50.1	52.4	52.0
12	36.0	38.9	29.5	27.4	26.1	27.8	33.1	32.1	36.8	36.7
Mean	43.26	45.53	39.47	38.23	37.87	38.37	41.63	40.77	45.11	45.78
Grand mean	44.40		38.85		38.12		41.20		45.45	

TABLE VII
Statistical Summary of α-Amylase Collaborative Results[a]

Sample Pair	No. of Labs	Grand Mean	Repeatability s_r	cv_r	r_{95}	Reproducibility s_R	cv_R	R_{95}
Malt-6 infusion								
A/B	10	43.35	0.835	1.9	2.339	3.500	8.1	9.800
C/D	10	38.63	0.844	2.2	2.362	3.703	9.6	10.368
E/F	10	37.71	1.152	3.1	3.224	6.367	16.9	17.829
G/H	10	41.13	0.991	2.4	2.775	4.994	12.1	13.983
I/J	10	44.51	1.115	2.5	3.121	6.081	13.7	17.028
Henry method extraction								
A/B	10	44.40	1.334	3.0	3.735	5.316	12.0	14.885
C/D	10	38.85	1.109	2.9	3.104	5.355	13.8	14.995
E/F	10	38.12	0.738	1.9	2.066	5.396	14.2	15.109
G/H	10	41.20	0.762	1.9	2.135	4.515	11.0	12.642
I/J	10	45.45	1.391	3.1	3.895	4.315	9.5	12.083

[a] All calculations were made based on reference 4.

2. American Society of Brewing Chemists. Report of Subcommittee on Diastatic Power (Rapid Method). *Journal* 48:143, 1990.
3. American Society of Brewing Chemists. Report of the Subcommittee on Statistical Analysis. *Journal* 44:138, 1986.
4. Guidelines for collaborative study procedures. *J. Assoc. Off. Anal. Chem.* 71:161, 1988.
5. Henry, R. A rapid method for the determination of diastatic power. *J. Inst. Brew.* 90:37, 1984.

APPENDIX

DIASTATIC POWER (RAPID METHOD)

The method provides a rapid means of measuring malt diastatic power activity based on absorbance at 415 nm (3). The method is described as a rapid method without the precision of Malt-6. The extraction method described can be used as an alternative extraction method for Malt-7 α-Amylase (1).

Reagents

For Digestion of Starch Solution

(a) *Acetate buffer solution.* Dissolve 68 g of sodium acetate ($CH_3COONa \cdot 3H_2O$) reagent grade in 500 ml 1*N* acetic acid and dilute solution to 1 L with distilled water.
(b) *Sodium hydroxide solution*, 0.5*N*.
(c) *Special starch.* Starch manufactured specifically for diastatic power determinations (designated Soluble Starch Special for Diastatic Power Determination) is available from the ASBC.

For Reducing Substances, Henry Method

(d) *Alkaline diluent solution.* Dissolve 14.70 g of trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) and 1.47 g of calcium chloride ($CaCl_2 \cdot 2H_2O$) separately in distilled water. Then add 20.00 g of sodium hydroxide, mix, and dilute solution to 1 L with distilled water.
(e) *PAHBAH working solution.* Add 5.00 g of solid *p*-hydroxybenzoic acid hydrazide (PAHBAH) to 1 L of alkaline diluent working solution (reagent d); mix well. Make up each day as required. The solution is stable overnight in the dark at 4°C. Do not use if reagent becomes yellow.

For Preparing Malt Infusion

(f) *Ammonium hydroxide, 6.0 mM.* Pipette 65 ml of ammonium hydroxide (29% NH_3), reagent grade, in distilled water, and dilute solution to 1 L. This will be the stock 1.0*M* solution used to prepare 6.0 m*M* ammonium hydroxide. Pipette 6 ml of the 1.0*M* stock ammonium hydroxide into distilled water and dilute solution to 1 L.

Apparatus

(a) *Spectrophotometer*, capable of measuring absorbance at 415 nm.
(b) *Quartz cuvettes*, 10-mm path length.
(c) *Balance.* See Malt-4, apparatus b.
(d) Glassware:
Volumetric pipettes, 5, 10, and 20 ml (Class A).
Volumetric pipette, 200 ml (fast).
Micropipettes, 0.2, 1.0 ml, or adjustable micropipette.
Flasks, Erlenmeyer, 50 ml.
(e) *Infusion flasks*, 500 ml. Erlenmeyer flask or glass-stoppered bottle is suitable.
(f) *Test tubes*, 25 × 150 mm.
(g) *Filter paper.* See Malt-4, Apparatus d.
(h) *Funnels*, 20 cm. See Malt-4, Apparatus f.
(i) *Mash beaker.* See Malt-4, Apparatus i.
(j) *Mill, fine grind.* See Malt-4, Apparatus k.
(k) *Stopwatch*, or clock that indicates seconds.
(l) *Water bath*, 20°C (±0.2°C).
(m) *Water bath*, boiling water.

Digestion of Starch Solution

Preparation of Special Starch Solution, Malt-7

Preparation of Malt Infusion

Grind separately no more than 10.5 g of malt according to the method for fine grinding of malt for determination of extract (Malt-4, Preparation of sample for mashing, fine grinding). Collect finely ground malt into mash beaker. Carefully brush malt particles remaining in mill into mash beaker. Without delay, place mash beaker with its contents on balance, adjust weight of ground malt to 10 g (±0.05 g), and transfer to infusion flask. Add 200 ml of 6.0 m*M* ammonium hydroxide (reagent f), close flask, swirl, and note time. Let infusion stand for 10 min at 20°C (±0.2°C), agitating it by rotation at 2-min intervals. Infusion flask must not be mixed by inverting, and the quantity of grist left adhering

TABLE VIII
Comparison of Malt-6 and Henry Extraction Methods[a]

Variable	α-Amylase				
	Sample Pair A/B	Sample Pair C/D	Sample Pair E/F	Sample Pair G/H	Sample Pair I/J
Malt-6, $\bar{X}$	43.35	38.63	37.71	41.13	44.51
Henry method, $\bar{X}$	44.40	38.85	38.12	41.20	45.45
Mean difference	1.05	0.22	0.41	0.07	0.94
t value[b]	0.72	0.15	0.22	0.05	0.55
Degrees of freedom	19	19	19	19	19

[a]All calculations were made according to reference 2.
[b]No significant difference at the 95% confidence level.

to the inner walls of the flask as a result of agitation must be as small as possible. Gentle swirling of contents of flask without splashing against walls will give sufficient mixing. At the end of 10 min, filter infusion through a 32-cm fluted filter in a 20-cm funnel. Return filtrate to filter after first 5 min of filtration. Collect filtrate for an additional 15 min. Place watch glass over funnel and suitable cover around stem and over receiver to reduce evaporation losses during filtration.

Diastasis

When filtration of infusion is completed, pipette 0.2 ml of filtrate to a 50-ml Erlenmeyer flask and bring to 20°C. Add 20 ml of buffered starch solution at 20°C to the flask from a fast-flowing pipette and start the stopwatch the instant addition begins. Mix by rotating the flask during addition. Maintain starch-infusion mixture at 20°C (±0.1°C) for exactly 10 min from the time addition of starch solution was begun. After 10 min, add 1.2 ml of 0.5*N* sodium hydroxide (reagent b) rapidly and mix by swirling flask.

Preparation of Blank Correction Solution

Prepare blank solution by adding 1.2 ml of 0.5*N* sodium hyrdoxide (reagent b) to 0.2 ml filtrate before adding 20 ml of starch solution. Otherwise, treat blank solution in exactly the same way as starch solution actually undergoing diastasis.

Determination of Reducing Substances, Henry Method Procedure

A) With pipette, add 0.2 ml (±0.01 ml) of digested starch solution to a test tube (25 × 150 mm) containing 5 ml (±0.05 ml) of PAHBAH working solution (reagent e). Mix well and immerse test tube in boiling water bath for exactly 4 min (using a stopwatch). Level of boiling water should be slightly above level of mixture in test tube. After 4 min in bath, remove test tube and place in 20°C water bath for 5–10 min. Add 10 ml (±0.05 ml) of distilled water. Mix well (vortexing is recommended), and read absorbance at 415 nm.

B) Pipette 0.2 ml (±0.01 ml) of blank correction solution into a test tube (25 × 150 mm) containing 5 ml (±0.05ml) of PAHBAH working solution (reagent e). Immerse test tube in boiling water for 4 min and cool for 5–10 min in a 20°C water bath. Add 10 ml (±0.05 ml) distilled water. Mix well (vortexing recommended) and read absorbance at 415 nm.

Standardization of Reducing Sugars

Oven dry reagent grade anhydrous dextrose ($C_6H_{12}O_6$) for 4 hr at 103°C. Weigh 0.2000, 0.4000, 0.6000, 0.8000, and 1.0000 g of dextrose. Record exact weight of dextrose weighed for linear calibration equation. Dissolve each in distilled water and dilute volume to 1 L. Pipette 0.2 ml of each dextrose solution into a separate test tube (25 × 150 mm) containing 5 ml (±0.5 ml) of PAHBAH working solution (reagent e). Prepare a similar blank with 0.2 ml of distilled water. Immerse test tubes in boiling water bath for 4 min and cool for 5–10 min in a 20°C water bath. Add 10 ml (±0.05 ml) of distilled water and mix well (vortexing is recommended) and read absorbance at 415 nm. Perform a linear calibration (Statistical Analysis-1) by calculating the regression line equation ($Y = a + bX$) for the data, using the absorbance at 415 nm as the independent variable (X) and the actual glucose weights as the dependent variable (y). Use this equation to calculate reducing sugars from absorbance values.

Linear Calibration

The alternate procedure for diastatic power is calibrated against Malt-6 as follows:

(a) Select 5–10 samples with a range of diastatic power values below 200. Samples selected for calibration should reflect diastatic power values slightly broader than would be encountered during routine analysis.
(b) Determine the diastatic power of each malt using Malt-6.
(c) Measure the absorbance of each malt at 415 nm using the alternate Henry method procedure outlined above. Subtract the blank correction absorbance for each malt.
(d) Calculate reducing sugars produced (dextrose, g/L) from the linear calibration equation outlined above in Standardization of Reducing Sugars.
(e) Perform a linear calibration (Statistical Analysis-1) by calculating the regression line equation ($Y = a + bX$) for the data using the reducing sugars produced (dextrose, g/L) as the independent variable (X) and diastatic power (as-is) from Malt-6 as the dependent variable (Y). Use this equation to calculate diastatic power (as-is) from reducing sugars produced (dextrose, g/L).

Calculation

A two-stage calculation is used to determine diastatic power. The first stage determines standardized reducing sugars produced using the Henry method. Calculate the reducing sugars produced (dextrose, g/L) with the following formula:

Stage 1: Absorbance reading at 415 nm to reducing sugars.

$$\text{Reducing Sugars (dextrose, g/L)} = a + b[A_{415}],$$

where a = y intercept from the regression equation calculated under linear calibration, b = response factor (slope) from the regression equation calculated under linear calibration, and A_{415} = absorbance of malt at 415 nm.

Example

$a = 0.007$
$b = 0.894$
$A_{415} = 0.433$
Reducing sugars (dextrose, g/L) = $0.007 + 0.894[0.433] = 0.39$

Stage 2: Diastatic power (as-is) from reducing sugars.

$$\text{Diastatic power (as-is)} = a + b \text{ reducing sugars (dextrose, g/L)},$$

where a = Y intercept from the regression equation calculated under linear calibration and b = response factor (slope) from the regression equation calculated under linear calibration, reducing sugars (dextrose, g/L) obtained from stage 1 calculation.