Technical Committee and Subcommittee Reports

2010–2011 Report of the Technical Committee

Committee Members: D. Sedin, *Chair*; C. Benedict; S. Brendecke; L. Chadwick; J. Cornell; M. Eurich; A. Fritsch; R. Jennings; K. Lakenburges; A. Porter; C. Powell; and B. Foster (*senior advisor*)

Activity in 22 subcommittees was conducted by the ASBC Technical Committee and Subcommittee chairs during 2010–2011. As a result, 10 methods are being recommended for inclusion in the ASBC *Methods of Analysis* (MOA):

- Reduced Solvent Usage for IBU Analysis, chaired by Ruth Martin (Sierra Nevada Brewing Company)
- ATP, chaired by Caroline Pachello (MillerCoors)
- Deoxynivalenol Analysis by ELISA, chaired by Andrea Stern (Malteurop)
- Miniature Fermentation Assay, chaired by Alex Speers (Dalhousie University)
- Sortimat, chaired by Theresa Chicos (Rahr Malting Co.)
- Iso-alpha-acids in Beer and Wort by HPLC, chaired by Loy Barber (Anheuser-Busch InBev)
- IBU in Wort by Segmented Flow Analysis, chaired by Mark Payne (Skalar)
- IBU in Wort by Spectrophotometer, chaired by Katie McGivney (New Belgium Brewing Co.)
- β-Glucan in Wort by Segmented Flow Analysis, chaired by Aaron MacLeod (Canadian Grain Commission)
- Free Amino Nitrogen in Wort by Segmented Flow Analysis, chaired by Aaron MacLeod (Canadian Grain Commission)

The following methods will continue for another year of collaborative study:

- Alpha-Amylase Automated Flow Using Potassium Ferricyanide, chaired by Theresa Chicos (Rahr Malting Co.)
- GC-FID Analysis for Beer Volatiles, chaired by Joe Palausky (Boulevard Brewing)

A review of one section of the MOA will be initiated:

- Beer, chaired by Karl Lakenburges (Anheuser-Busch InBev)

The following five methods are being recommended for collaborative study in 2011–2012:

- X-Alpha-Gal for Differentiation of Ale/Lager Yeast Strains, chaired by Wendy Box (University of Nottingham)
- Reduced Solvent Usage Hops-6A, chaired by Joyce Carr (John I Haas)
- Wort Viscosity, chaired by Aaron MacLeod (Canadian Grain Commission)
- Wort Amino Acids by HPLC, chaired by Aaron MacLeod (Canadian Grain Commission)
- Spectrophotometric Analysis of IBU Utilizing Solid-Phase Microextraction, chaired by Tom Shellhammer and Philip C. Wietstock (Oregon State University)

As in previous years, the following eight standing subcommittees continue:

- Soluble Starch, chaired by Rebecca Jennings (Rahr Malting Co.)
- Check Services, Jim Munroe (retired Anheuser-Busch), Sue Casey (ASBC), Stephen Kenny (Washington State University IAREC), and John Barr (North Dakota State University)
- Coordination of New and Alternate Methods of Analysis, chaired by Karl Lakenburges (Anheuser-Busch InBev)
- International Methods, chaired by Dana Sedin (New Belgium Brewing Co.)
- Craft Brewers, chaired by Luke Chadwick (Bell's Brewery)
- Sensory Science, chaired by Annette Fritsch (Boston Brewing Company)
- International Hop Standards Subcommittee, chaired by Bob Foster (MillerCoors)
- Packaging Methods, chaired by Scott Brendecke (Ball Corporation) and Chaz Benedict (Hach Ultra Analytics)

Jim Munroe (retired member, formerly of Anheuser-Busch) continues to provide statistical input and recommendations to the Check Services program. Sue Casey, Stephen Kenny, and John Barr continue in their roles as Check Service managers for Beer Analysis, Hop Analysis, and Malt and Barley Analyses, respectively. Rebecca Jennings has been actively working with the Check Services Committee to provide updates to the malt program. Their hard work and dedication are greatly appreciated!

The ASBC Board of Directors instituted a grant program in 2010 to be administered by the ASBC Technical Committee. The student grant program was very successful in its first year. Mark Zunkel (a recently graduated student from Martina Gastl's group at Weihenstephan) has developed a beer flavor database. The Flavor Database is being finalized for inclusion in the MOA. Bryan Donaldson (a recently graduated student from Charlie Bamforth's group at UC Davis) has reviewed beer degassing methods (related to analytical analyses) and provided recommended updates to the MOA. The updates will be added to the MOA soon. Both Mark and Bryan presented their projects at the ASBC Annual Meeting. One grant will be provided for 2011–2012 to Philip C. Wietstock, a member of Tom Shellhammer's group at the University of Oregon. Philip and Tom have developed a new method for the analysis of IBU in beer and wort.

The Coordination of New and Alternate Methods of Analysis Subcommittee submitted a survey to members on May 20, 2011. Karl Lakenburges (subcommittee chair) worked closely with the Technical Committee to design the questions, and a number of topics were polled for interest in future subcommittees. The results were presented at the 2011 ASBC Annual Meeting. Based on the polling results and feedback at the annual meeting, multiple methods have been recommended for collaborative study in 2011–2012.

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The ASBC Technical Committee fall meeting was graciously hosted in St. Louis by Anheuser-Busch InBev on September 13.

I would like to thank the subcommittee chairs for their hard work and dedication in conducting their respective collaborative studies during the past year. I would also like to recognize the many subcommittee members who participated this past year. Finally, I would like to recognize the dedication and hard work put forth by the Technical Committee.

Coordination of New and Alternate Methods of Analysis
(*Karl Lakenburges, karl.lakenburges@anheu4ser-busch.com*)

This is a standing subcommittee whose function is to collect, from various sources including polling members, new and alternate methods of analysis that may be useful for the industries our Society serves. These methods are reviewed to establish their merit and utility.

Soluble Starch
(*Rebecca Jennings, rjennings@rahr.com*)

This is a standing subcommittee whose goal is to coordinate a testing program for soluble starch that will ensure a consistent supply of quality soluble starch for the Society. To further this goal, the subcommittee monitors process methodology utilized in the production of starch, investigates improved methods for starch quality testing, and evaluates potential new suppliers of starch.

Craft Brewers
(*Luke Chadwick, lchadwick@bellsbeer.com*)

The mandate of the Craft Brewers Subcommittee is to connect with the craft brewing members of ASBC and explore opportunities to make the Society more relevant to these individuals. Additionally, the subcommittee will develop and pursue strategies to bring craft brewers who are not members of the Society into ASBC.

Sensory Science
(*Annette Fritsch, annette@fritschsensory.com*)

This is a standing subcommittee. It was formed on the recommendation of the Technical Committee to bring more focus to sensory science in ASBC and provide a forum for sensory scientists in the brewing industry to share and discuss current methodologies and propose new methodologies for collaborative testing. The current focus is on updating the beer flavor wheel(s), methods for shelf-life testing, and decision trees for sensory evaluation.

Alpha-Amylase Automated Flow Using Potassium Ferricyanide
(*Theresa Chicos, tchicos@rahr.com*)

There has been concern over the use of iodine for the determination of α-amylase across a wide spectrum. It has been suggested that ASBC look into creating an alternative method for the determination of α-amylase using automated flow analysis. However, instead of using iodine and β-limit dextrin, the method would utilize potassium ferricyanide.

Deoxynivalenol Analysis by ELISA
(*Andrea Stern, Andrea.Stern@malteurop.com*)

Laboratories are looking for accurate methods that are easy to use and that produce results in a quick and efficient manner. There are methods in practice that do just this for the determination of deoxynivalenol (DON). DON is a vomitoxin produced by *Fusarium* that can lead to brewing performance issues. It has been suggested that ASBC look into creating an alternative method for the determination of DON using an ELISA method and a rapid method by Diagnostix called EZ-Tox. Both of these methods are enzyme immunoassays incorporating "homogeneous assay technology." The difference is the EZ-Tox method yields results in about 5 min compared to the 15 min needed for a regular ELISA method. This subcommittee will look at both methods for addition to the Malt section of the MOA.

International Hop Standards Subcommittee
(*Bob Foster, Robert.Foster@millercoors.com*)

This subcommittee has existed for 13 years as the International Subcommittee for Isomerized Hop Alpha-Acids Standards (ISI-HAS) and is a standing subcommittee whose goal is to produce, purify, and verify isomerized and unisomerized hop standards for the brewing, hops, and related industries.

GC-FID Analysis for Beer Volatiles
(*Joe Palausky, jpalausky@boulevard.com*)

This subcommittee was formed to evaluate a potentially more accessible and lower cost gas chromatographic method (relative to the method using mass spectrometry) to measure esters and alcohols.

Packaging Methods
(*Scott Brendecke, sbrendec@ball.com*)

This is a standing subcommittee. It was formed to evaluate packaging methodology, review packaging methods within the MOA, and act as a liaison between ASBC and other packaging related organizations.

International Methods
(*Dana Sedin, dsedin@newbelgium.com*)

The function of this standing subcommittee is to encourage collaboration between ASBC and international brewing organizations. The primary focus is shared method collaboration with both BCOJ and EBC.

Wort Viscosity
(*Aaron MacLeod, aaron.macleod@grainscanada.gc.ca*)

Based on interest from polling conducted in 2011, this subcommittee will evaluate automated methodology for the measurement of wort viscosity.

Wort Amino Acids by HPLC
(*Aaron MacLeod, aaron.macleod@grainscanada.gc.ca*)

Based on interest from polling in 2011, this subcommittee will evaluate high-performance liquid chromatography for the measurement of amino acids in wort.

X-Alpha-Gal for Differentiation of Ale/Lager Yeast Strains
(*Chris Powell, chris.powell@nottingham.ac.uk*)

This method is based on the current X-alpha-gal technique for differentiation of ale/lager strains. The current method involves growing yeast colonies on solid medium containing the compound X-alpha-gal, an analogue of melibiose containing indol. Yeasts that can utilize melibiose (lager strains) possess the enzyme α-galactosidase, which cleaves X-alpha-gal and releases indol, which turns colonies blue/green. The current method yields blue/green colonies for lager strains and white colonies for ale strains after growth for 3–6 days (6 days is recommended by ASBC Method Yeast-10). The new method also involves the use of X-alpha-gal, but the preparation removes the use of some of the more toxic compounds associated with the original protocol. Furthermore, the method re-

lies on suspension of cells in liquid rather than solid medium, and results can be obtained in 30 min rather than 6 days. This is a significant time saving, and therefore, the new method is much more useful for real-time assessment of yeast cultures.

Reduced Solvent Usage Hops-6A

(*Joyce Carr, Joyce.Carr@johnihaas.com*)

This is an update to the current methodology utilizing significantly less solvent for analysis (ASBC Method Hops-6A). This method is based on a poster presented at the 2010 ASBC Annual Meeting by Joyce Carr and Tim Kostelecky.

Spectrophotometric Analysis of IBU Utilizing Solid-Phase Microextraction

(*Tom Shellhammer, tom.shellhammer@oregonstate.edu*)

This is a new method developed at Oregon State University in Tom Shellhammer's lab. The method utilizes solid-phase extraction followed by spectrophotometric detection for rapid and accurate bitterness analysis. The method correlates well with the IAA method using HPLC, thus providing a more accurate assessment of bitterness compared with the standard spectrophotometric method. The method utilizes methanol and water as solvents and can be run by any laboratory with a spectrophotometer.

Reduced Hazardous Solvent Use for Determination of Isohumulone Bitterness Units

Subcommittee Members: R. Martin, *Chair*; M. Anderson; D. Collazo; J. Davis; F. Hamp; S. Huestis; B. Jaskula-Goiris; J. Jordan; R. Juzeler; S. Krug; K. Lee; K. McGivney; S. Mulqueen; N. Perry; R. Schmidt; G. Spedding; K. Taylor; S. Taylor; and R. Foster (*ex officio*)

Keywords: IBU

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the spectrophotometric determination of isohumulone bitterness units (IBU), using reduced sample size and solvent volume, ranged from 1.9 to 5.3% and 5.4 to 9.1%, respectively, and were judged acceptable.
2. A *t* test was performed on the results of current and modified IBU methods and showed no significant difference in mean values at the 95% confidence level.

RECOMMENDATIONS

1. The subcommittee recommends the modified spectrophotometric bitterness method be used for determining bitterness in beer and be included in the ASBC *Methods of Analyses* (1).
2. Discharge the subcommittee.

This was the first year of the subcommittee's evaluation of the modified spectrophotometric isohumulone bitterness units (IBU) method, which uses half the amount of solvent compared with the current spectrophotometric IBU method.

The committee was formed in response to an ASBC poster presented at the Brewing Summit held in Providence, RI, in 2010 (2). Hops were tested using ASBC methodology (Hops-6A and -14A analyses), but the methodology was modified by reducing the sample size and solvent usage by 50% compared with the original methods. Using a smaller sample size and less organic solvent yielded the same precision as the original ASBC methods.

In this first year, a ruggedness test was performed with select collaborators to test the accuracy and feasibility of the modified IBU method.

PROCEDURE

Three sample pairs were sent to each collaborator. Each pair was similar but had distinct production dates and was selected to cover a range of IBUs. The sample set included a low, medium, and high dry-hopped ale.

The samples consisted of two low-hopped beers (D1/D2), two medium-hopped beers (E1/E2), and two high-hopped beers (F1/F2). Participants were asked to follow the methods as closely as possible. Any deviations or modifications to the procedure were to be noted. Results were evaluated using the Youden unit block design (1) and the paired *t* test, assuming unequal variance at the 95% confidence level.

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RESULTS AND DISCUSSION

Results for the current and modified spectrophotometric IBU methods were received from 18 collaborators for sample pairs D1/D2, E1/E2, and F1/F2. The results are summarized in Tables I

TABLE I
Isohumulone Bitterness Units Measured Using the Current Spectrophotometric Method

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**D1**	**D2**	**E1**	**E2**	**F1**	**F2**
1	28.0	25.5	37.5	37.5	68.0	66.5
2	28.0	26.0	36.5	37.0	64.5	64.5
3	27.0	25.0	36.0	36.0	64.0	64.0
4	30.5	34.5	38.5	43.0	73.0	75.0
5	30.0	28.5	41.0	41.0	69.0	69.0
6	29.5	27.0	38.0	38.5	67.0	66.5
7	29.0	29.5	38.5	39.0	70.0	72.0
8	28.0	26.0	36.0	37.5	65.5	61.0
9	32.0	28.5	46.0[a]	44.0[a]	73.5	76.0
10	26.0	25.5	38.0	38.0	68.0	67.5
11	33.0[a]	22.5[a]	32.0[a]	31.5[a]	59.5	60.0
12	22.0[a]	19.5[a]	29.0[a]	36.0[a]	60.0	66.5
13	29.5	27.0	39.0	39.5	67.0	67.0
14	29.5	29.0	39.0	40.5	68.5	69.0
15	32.5	29.0	42.0	43.5	70.0	71.5
16	27.5	25.5	35.0	36.0	65.0	63.0
17	29.0	27.5	39.0	39.5	69.0	69.5
18	28.0	31.5	27.0[a]	27.0[a]	39.0[a]	40.0[a]
Mean	29.00	27.90	38.14	39.04	67.15	67.56
Grand mean[b]	28.45		38.59		67.36	

[a] Outlier at $P \leq 0.05$ based on totals and differences.
[b] Calculated excluding outliers.

TABLE II
Isohumulone Bitterness Units Measured Using the Modified Spectrophotometric Method

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**D1**	**D2**	**E1**	**E2**	**F1**	**F2**
1	29.0	26.0	37.5	38.5	68.5	68.5
2	28.5	29.0	37.0	37.5	65.0	65.5
3	27.5	25.0	35.5	36.5	64.5	63.5
4	36.5	30.0	46.5[a]	47.5[a]	79.0	79.0
5	31.0	28.5	41.0	41.5	69.0	69.5
6	27.5	29.0	37.0	37.0	65.0	67.0
7	31.0	30.0	41.0	40.0	72.5	71.0
8	27.0	23.0	35.5	35.0	60.0	59.5
9	32.0	29.0	40.5[a]	29.5[a]	72.0	67.5
10	26.5	26.5	38.5	38.5	70.0	69.0
11	33.5	30.0	39.0[a]	44.5[a]	75.0	71.5
12	26.5	24.5	36.5	39.0	66.5	64.5
13	27.0	26.0	36.0	37.5	62.0	62.0
14	30.5	31.0	40.0	42.0	69.5	70.0
15	33.0	28.0	44.5[a]	43.5[a]	75.0	69.5
16	27.0	25.0	36.0	36.5	64.5	64.0
17	28.5	26.5	39.0	38.5	66.5	66.5
18	27.5	28.5	35.5	35.0	45.5[a]	40.0[a]
Mean[b]	29.44	27.52	37.57	38.07	68.50	67.53
Grand mean[b]	28.48		37.82		68.02	

[a] Outlier at $P \leq 0.05$ based on totals and differences.
[b] Calculated excluding outliers.

TABLE III
Statistical Summary of Results[a]

Method Sample Pair	No. of Labs	Grand Mean	Repeatability			Reproducibility		
			S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Current								
D1/D2	16	28.45	1.59	5.58	4.45	2.20	7.75	6.17
E1/E2	14	38.59	0.83	2.16	2.33	2.13	5.52	5.97
F1/F2	17	67.36	1.62	2.40	4.53	1.62	6.16	11.62
Modified								
D1/D2	18	28.48	1.52	5.33	4.25	2.59	9.10	7.26
E1/E2	14	37.82	0.72	1.91	2.02	2.06	5.43	5.76
F1/F2	17	68.02	1.37	2.01	3.83	4.74	6.97	13.28

[a] Calculations were made according to ASBC Method Statistical Analysis-4 (1).

TABLE IV
***t* Test with Two Samples, Assuming Equal Variances, for Current and Modified Spectrophotometric IBU Methods[a]**

	Current Method	Modified Method
Mean	43.79028	44.60704
Variance	287.884	286.8316
Observations	108	108
Pooled variance	287.3578	
Hypothesized mean difference	0	
df	214	
t statistic	–0.35406	
$P(T \leq t)$ two-tail	0.723641	
t critical two-tail	1.971111	

[a] IBU = isohumulone bitterness units.

and II, respectively. Outliers were identified using Dixon's ratio test as described in the Youden unit block procedure (1).

Test results for the sample pairs identified in Tables I and II as outliers were excluded from data analyses for the six collaborators. One outlier did not analyze the samples as soon as they were received, and the results were significantly lower.

A statistical summary of the current and modified IBU data for both analytical methods is presented in Table III. Repeatability and reproducibility coefficients of variation for the current IBU method ranged from 2.2 to 5.6% and 5.5 to 7.8%, respectively, and were judged acceptable. Repeatability and reproducibility coefficients of variation for the determination of IBU using reduced sample size and solvent volume ranged from 1.9 to 5.3% and 5.4 to 9.1%, respectively, and were judged acceptable. Based on the paired *t* test for differences in means, no statistically significant differences were found between the current and modified methods for testing IBU levels, and the modified IBU procedure was judged an acceptable method (Table IV).

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*. Beer-23A Beer bitterness (BU); Statistical Analysis-5 Comparison of test methods. The Society, St. Paul, MN, 2009.
2. Carr, J. E. Reduction of hazardous solvent usage in the hops laboratory. Abstr. P-66 Brewing Summit 2010. The Society, St. Paul, MN, 2010.

Adenosine Triphosphate (ATP) Rapid Testing for Water and Rinse Water Hygiene

Subcommittee Members: C. Pachello, *Chair*; E. Belden; D. Bendiak; M. Bradley; R. Eidman; S. Gallegos; T. Gojanovic (statistician); G. Kelly; R. Mancebo; L. Marques; A. Mercier; and C. Powell (*ex officio*)

Keywords: CIP assessment, Contamination, Microbial detection, Water assessment

CONCLUSIONS

1. For measuring adenosine triphosphate (ATP) in water containing bacteria, 48 of 50 data sets yielded a coefficient of variation of <10% and were considered to be acceptable.
2. Analysis of data between different laboratories using a linear regression model indicated that 69.2% of the variability in the data was captured by the model and was considered to be acceptable.

RECOMMENDATIONS

1. The current data suggest that the evaluated method can be used for the accurate assessment of bacteria in water samples.
2. Discharge the subcommittee.

The aim of this subcommittee was to evaluate the use of bioluminescence to detect ATP as a means of assessing water hygiene. This was the third year for the subcommittee, which was formed based on polling at the 2008 ASBC Annual Meeting. During the first year there were insufficient participants to initiate collaborative trials. However, polling was performed to ascertain the range of equipment and test methodology currently employed in breweries. Based on this information, it was decided that 3M Clean-Trace total ATP water tests and the Clean-Trace NG luminometer would be assessed. It should be noted that similar instruments and test reagents are available from a number of manufacturers. Although each brand may vary in sensitivity, precision, and cost, the main focus of this collaborative study was to assess the ability of ATP detection technology to detect yeast or bacteria in water samples. In the second year of the subcommittee, collaborative assessment indicated that the technique was reliable for the detection of water samples containing yeast. However, analysis of interlaboratory data generated from water samples containing bacteria, using a linear regression model, indicated that only 16.2% of the variability was captured by the model, and this was considered unacceptable (1). Consequently, it was recommended that further analysis should be performed to assess water samples containing bacteria. During the third year, an alternative protocol for culturing bacterial microbes was used in an attempt to standardize the metabolic state of the bacteria, leading to an improved correlation in ATP results between laboratories.

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PROCEDURE

A bacteria strain (*Lactobacillus brevis*) was sent to each of 10 collaborators. Participants were also provided with universal beer agar (UBA) plates obtained from Hardy Diagnostics that were prepared with low-hopped, lager-style beer and supplemented with Tween 80 for the purpose of cultivating the bacteria. Collaborators grew microbes anaerobically on UBA for 3–4 days at 28–30°C. Care was taken to ensure that cultures were not allowed to overgrow in order to maintain cell vitality. A saline solution containing bacteria, standardized as having an absorbance of 0.06 at 600 nm, was prepared, and 3 mL of the suspension was transferred to 40 mL of fresh MRS broth. The MRS broth was incubated at 28–30°C for exactly 24 hr. Following incubation, cultures were centrifuged at 800 RCF (relative centrifugal force) for 5 min to collect the bacteria. The supernatant was discarded, and cells were resuspended in 0.85% sterile saline and washed twice in sterile saline to remove any residual medium. The washed cells were resuspended in sterile distilled water to achieve a stock suspension with a target absorbance of approx. 0.06 at a wavelength of 600 nm. A series of dilutions (1:2, 1:5, 1:10, 1:40, and 1:100) was prepared using the stock solution to provide varying cell concentrations for ATP analysis.

TABLE I
Detection of Bacteria in Water Using Adenosine Triphosphate (ATP) Bioluminescence

	ATP Relative Light Units (RLU)									
Dilution	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1:2	302	2,942	195	189	2,098	735	631	159	263	129
	263	3,357	229	147	1,863	767	701	216	245	125
	238	2,874	260	125	1,839	801	680	167	306	130
1:5	182	282	101	58	913	270	266	55	110	55
	154	322	102	65	659	249	355	53	135	84
	185	303	86	70	952	274	263	101	141	123
1:10	45	223	41	30	590	120	73	42	69	49
	65	350	86	32	513	125	102	40	58	56
	80	212	81	36	527	131	134	37	81	31
1:40	30	118	30	17	133	60	38	30	20	21
	20	259	20	16	121	55	76	28	22	16
	25	106	29	17	89	35	75	30	22	17
1:100	18	88	17	11	53	10	23	20	13	10
	19	78	11	8	52	9	21	14	15	15
	27	57	12	10	50	16	36	32	16	11

Each dilution was divided and analyzed in triplicate for detectable ATP utilizing the 3M Clean-Trace NG luminometer and the 3M Clean-Trace total ATP water test. Data were expressed in arbitrary relative light units (RLU). To provide an estimate of the number of bacterial cells present in each stock solution, dilutions were prepared in sterile saline, collected by membrane filtration, and cultivated anaerobically on UBA at 28–30°C for 7 days.

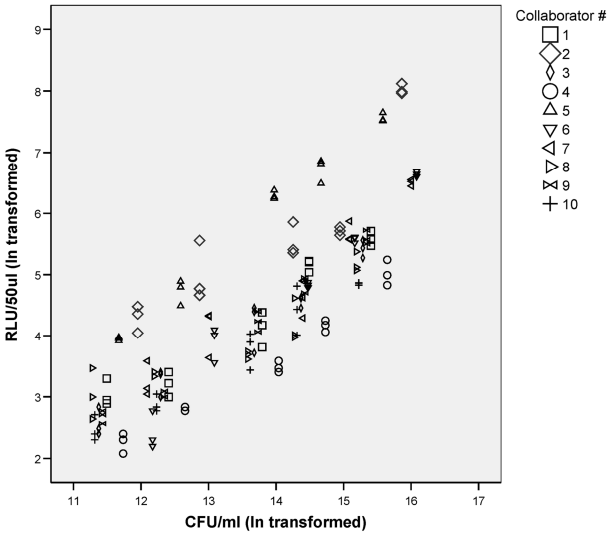
RESULTS AND DISCUSSION

Results for the analysis of water samples containing bacteria using ATP bioluminescence were obtained from 10 collaborators (Table I). The ATP data were used to determine the coefficient of variation (CV) (Table II), and the estimated number of cells (colony forming units [CFU]) was used to produce a linear regression model in conjunction with the measured RLU (Fig. 1).

CV is a measure of relative variability given by the formula (standard deviation/mean). CV is a unitless measure that allows comparison of the relative variability of different data sets with different units of measure. Lower CV values are typically indicative of less variation in a data set. For analysis of ATP in water samples containing bacteria, 48 of 50 data sets exhibited a CV of <10% (with a maximum CV of 13.67%), indicating good repeatability of the method within laboratories. It should be noted that variation increased as the concentration of bacteria decreased, as indicated by the increasing frequency with which a CV value of >10% occurred (Table III).

Linear regression is a statistical method that utilizes the relationship between variables to develop a measure of prediction from one variable to another. The data obtained in this study were used to calculate a predictive model of ATP in water for bacteria, as measured by RLU compared to CFU. The linear equation for the model for bacteria in water was calculated as $\hat{y} = -6.277 + 0.784x_1$, in which x is defined as CFU/mL (ln transformed) and $\hat{y}$ is the calculated value for RLU/50-µL sample size (ln transformed). The R^2 value of the linear regression model was calculated to assess the variability explained in ln(RLU) in relation to ln(CFU) (2). The R^2 value also provides an indication of the strength of a linear relationship (3), where a value of 0 indicates no linear relationship between predictors, while a value of 1 indicates that all of the data points fall on the regression line (3). Analysis of interlaboratory reproducibility using the line calculated by the linear regression model (Fig. 1) indicated that 69.2% (adjusted $R^2 = 0.692$) of the variability in ln(RLU) was explained by ln(CFU). For small-scale testing involving analysis of cultures that may have variable ATP per unit cell (due to physiological differences), it is recommended that a 69.2% explanation of variation in the response is sufficient to call this an acceptable model. Consequently, this suggests that the data were reproducible between laboratories and that all laboratories were able to produce similar results. The range in ln(RLU) values for bacteria that could be viable based on the model of the data generated from all laboratories was given by a 95% confidence level calculated using the equation $\ln RLU = (-6.277 + 0.784x_1) \pm (1.96)(0.75610)$, in which 0.75610 is the variability (standard deviation) of the linear regression model and 1.96 is the z score associated with providing a 95% confidence interval.

As a result of statistical analysis of the experimental data obtained, the method evaluated was deemed to be reproducible between collaborators and repeatable within laboratories. Consequently, detection of ATP bioluminescence was judged to be an acceptable method of determining the presence of bacteria in water samples. It should be noted that, for brevity, collaborative testing was limited to a single *L. brevis* strain and did not involve analysis of the range of contaminants that might be found in brewery water samples. Furthermore, this method is not intended to take the place of traditional routine microbiological tests but to provide a valuable and immediate assessment of hygiene for water samples. The current study, in conjunction with previous data for samples of water containing yeast (1), suggests that ATP measurements may be employed for the analysis of brewery water to determine the presence of microbes or to check general hygiene. It is advised that individual breweries should specify a minimum RLU value



Model Summary[a]

Model	*R*	R^2	Adjusted R^2	SE of Estimate
1	0.833[b]	0.694	0.692	0.75610

[a] Dependent variable: lnRLU.
[b] Predictors: (constant), lnCFU.

ANOVA[a]

Model 1	Sum of Squares	df	Mean Square	*F*	Sigma
Regression	191.826	1	191.826	355.546	0.00[b]
Residual	84.609	148	0.572		
Total	276.435	149			

[a] Dependent variable: lnRLU.
[b] Predictors: (constant), lnCFU.

Coefficients[a]

	Unstandardized Coefficients		Standardized Coefficients			95% Confidence Interval for *B*	
Model 1	*B*	SE	Beta	*t*	Sigma	Lower Bound	Upper Bound
(Constant)	–6.277	0.589		–10.661	0.000	–7.441	–5.114
lnCFU	0.784	0.043	0.833	18.318	0.000	0.700	0.869

[a] Dependent Variable: lnRLU.

Fig. 1. Regression model for the detection of bacteria in water samples.

TABLE II
Determination of Microbial Loading of Prepared Samples

	Colony Forming Units (CFU)									
Dilution	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Calculated stock/mL	98×10^5	16×10^6	87×10^5	12×10^6	12×10^6	19×10^6	18×10^6	79×10^5	92×10^5	82×10^5
10^{-5}	98	155	87	125	117	193	179	79	92	82

TABLE III
Individual Collaborator Precision for the Detection of Bacteria in Water Using Adenosine Triphosphate (ATP)[a]

Dilution	Collaborator	Mean	Median	Min	Max	SD	CV
1:2	1	5.58	5.57	5.47	5.71	0.12	2.14
	2	8.02	7.99	7.96	8.12	0.08	1.04
	3	5.42	5.43	5.27	5.56	0.14	2.66
	4	5.02	4.99	4.83	5.24	0.21	4.15
	5	7.57	7.53	7.52	7.65	0.07	0.96
	6	6.64	6.64	6.60	6.69	0.04	0.65
	7	6.51	6.52	6.45	6.55	0.05	0.83
	8	5.19	5.12	5.07	5.38	0.16	3.17
	9	5.60	5.57	5.50	5.72	0.11	2.03
	10	4.85	4.86	4.83	4.87	0.02	0.43
1:5	1	5.15	5.20	5.04	5.22	0.10	1.97
	2	5.71	5.71	5.64	5.77	0.07	1.16
	3	4.56	4.62	4.45	4.62	0.10	2.10
	4	4.16	4.17	4.06	4.25	0.09	2.28
	5	6.72	6.82	6.49	6.86	0.20	3.00
	6	5.58	5.60	5.52	5.61	0.05	0.92
	7	5.68	5.58	5.57	5.87	0.17	3.00
	8	4.20	4.01	3.97	4.62	0.36	8.63
	9	4.85	4.91	4.70	4.95	0.13	2.73
	10	4.42	4.43	4.01	4.81	0.40	9.12
1:10	1	4.12	4.17	3.81	4.38	0.29	7.07
	2	5.54	5.41	5.36	5.86	0.28	4.98
	3	4.19	4.39	3.71	4.45	0.41	9.83
	4	3.48	3.47	3.40	3.58	0.09	2.65
	5	6.30	6.27	6.24	6.38	0.07	1.18
	6	4.83	4.83	4.79	4.88	0.04	0.91
	7	4.60	4.62	4.29	4.90	0.30	6.61
	8	3.68	3.69	3.61	3.74	0.06	1.74
	9	4.23	4.23	4.06	4.39	0.17	3.95
	10	3.78	3.89	3.43	4.03	0.31	8.20
1:40	1	3.21	3.22	3.00	3.40	0.20	6.34
	2	5.00	4.77	4.66	5.56	0.49	9.76
	3	3.25	3.37	3.00	3.40	0.22	6.91
	4	2.81	2.83	2.77	2.83	0.04	1.24
	5	4.72	4.80	4.49	4.89	0.21	4.45
	6	3.89	4.01	3.56	4.09	0.29	7.45
	7	4.10	4.32	3.64	4.33	0.40	9.68
	8	3.38	3.40	3.33	3.40	0.04	1.18
	9	3.06	3.09	3.00	3.09	0.06	1.80
	10	2.88	2.83	2.77	3.04	0.14	4.95
1:100	1	3.04	2.94	2.89	3.30	0.22	7.23
	2	4.29	4.36	4.04	4.48	0.22	5.22
	3	2.57	2.48	2.40	2.83	0.23	8.96
	4	2.26	2.30	2.08	2.40	0.16	7.23
	5	3.94	3.95	3.91	3.97	0.03	0.75
	6	2.42	2.30	2.20	2.77	0.31	12.64
	7	3.25	3.14	3.04	3.58	0.29	8.87
	8	3.03	3.00	2.64	3.47	0.41	13.67
	9	2.68	2.71	2.56	2.77	0.11	3.96
	10	2.47	2.40	2.30	2.71	0.21	8.58

[a] Valid $N = 3$.

that they deem to be "acceptable" as an indicator of cleanliness prior to employing ATP detection techniques on a routine basis.

LITERATURE CITED

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Miniature Fermentation Method

Subcommittee Members: A. Speers, *Chair*; C. Baugh; D. Cook (EBC); E. Eck; B. Gibson; R. Joy; A. MacLeod; A. Panteloglou; M. Voetz (EBC); S. Walker (EBC); and C. Powell (*ex officio*)

Keywords: Apparent degree of fermentation, In vitro fermentation, Premature yeast flocculation, PYF

CONCLUSIONS

1. Using a commercial malt and a malt that showed premature yeast flocculation (PYF), the change in apparent extract (AE) during fermentation could be modeled by a nonlinear logistic equation using commercial software. The method is suitable to provide estimates of original extract (OE) and AE and to calculate the apparent degree of fermentation (ADF). The ADF within-lab error, or repeatability values, averaged 0.011 for both the control and PYF malts. The ADF between-lab error, or reproducibility values, was 0.030 and 0.016 for the control and PYF malts, respectively. The highest coefficient of variation encountered was approx. 3.5%.
2. The parabolic behavior of the yeast in suspension can be modeled with a variant of a Gaussian curve, termed a "tilted Gaussian" model. Examination of paired data from control and PYF malts indicated there were significant differences ($P < 0.05$) between the curves.

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RECOMMENDATIONS

1. It is proposed that the method be approved for inclusion in the ASBC *Methods of Analysis* for the determination of the ADF of freely fermenting wort and also for the identification of malt causing PYF.
2. Discharge the subcommittee.

This was the second year of the subcommittee's existence. The subcommittee was formed after discussions at the 2009 ASBC Annual Meeting to evaluate a mini-fermentation method (6) that could potentially be used for the identification of malts promoting PYF. While there is a current ASBC method for yeast fermentable extract (Wort-5) that involves a stirred fermentation, Wort-5 (1) is not appropriate for observing yeast flocculation abnormalities caused by yeast or wort defects. During the first year of the subcommittee, the method was shown to be suitable for determining ADF and examining the effects of different yeast strains and fermentation conditions. During the current year, a control (commercial) malt and a malt reported to cause PYF were analyzed using the mini-fermentation method.

PROCEDURE

An industrial lager strain (SMA) was precultivated on YEPD agar and grown aerobically in YEPD broth for 48 hr at 30°C. Yeast cultures were washed in deionized water, and the yeast pellet was resuspended in sterile water and enumerated using a hemacytometer (ASBC Method Yeast-4). Subsequently, the yeast was pitched in

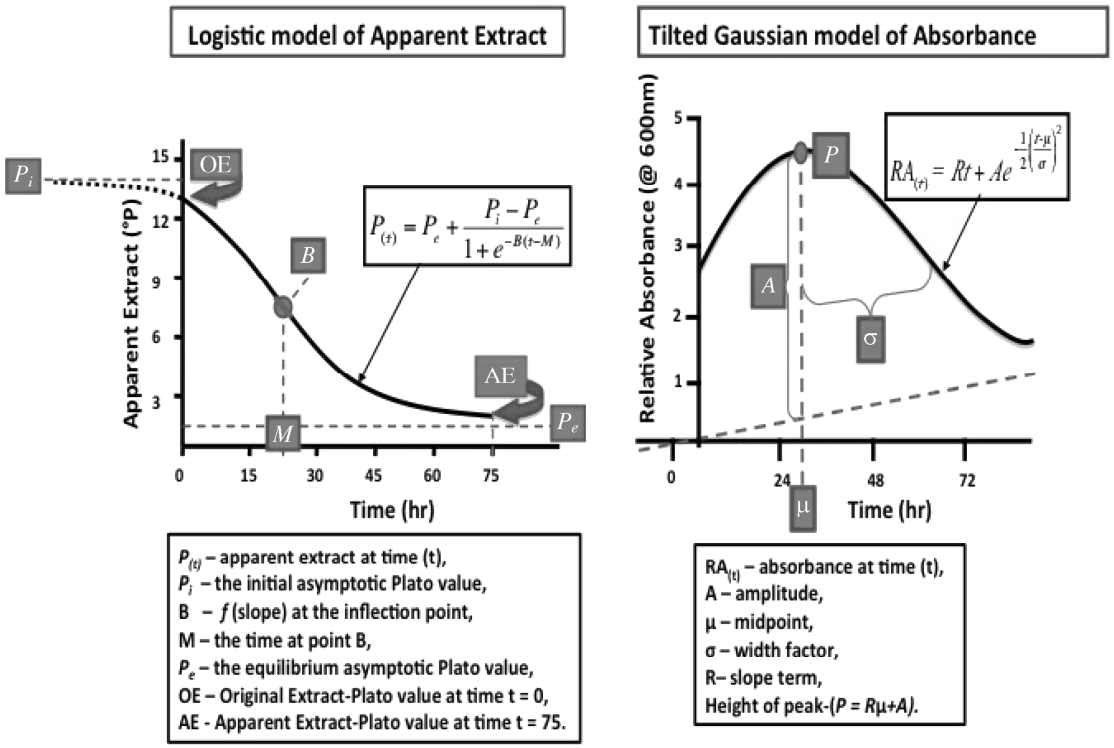

Fig. 1. Fit of tilted Gaussian and logistic models to absorbance and extract data, respectively.

wort prepared from malt mashed according to the standard ASBC/Congress mashing regime (ASBC Method Malt-4) using a mash bath (International Equipment and Control Pty. Ltd. mash bath or equivalent). The mash slurries were stirred by magnetic stir bars or propeller-style mixers. As per the Malt-4 procedure, additional water was added at 55 min. However, the wort was not adjusted to 450 g at the completion of the mash. Instead, wort was filtered to remove particulates (Reeve Angel 802, Whatman, Inc.).

Following filtration, worts were autoclaved and cooled to 4°C for not more than 12 hr. After the cold break had occurred, the worts were removed and centrifuged at 4,400 rpm (3,310 × *g*) for 15 min to remove trub. Removal of trub was important to allow spectrophotometric measurement of yeast in suspension. The worts were diluted to 12.6°P with distilled water and 18 g of lab-grade D-glucose was added to reach an extract level of 16.1°P and a volume of 450 mL. After oxygenation, the wort was pitched with 1.5×10^7 cells/mL. The pitched wort was thoroughly mixed prior to being aseptically distributed in 30 sterile fermentation tubes (15 mL volume each) containing a boiling chip and stoppered with a sponge bung. Tubes were set to ferment in a water bath at 21°C until (de-

TABLE I-A
Relative Absorbance Data for Fermentations Performed Using Control Malt Samples[a]

Time	Collaborator								Time	Collaborator							
(hr)	1	2	3	4	5	6	7	8	(hr)	1	2	3	4	5	6	7	8
0	2.074	0.982	1.025	…	…	…	…	0.730	42	…	…	…	…	…	…	…	1.114
0	2.057	0.991	…	…	…	…	…	0.704	42	…	…	…	…	…	…	…	0.895
0	…	0.978	…	…	…	…	…	0.700	45	14.847	…	2.405	…	…	…	…	…
1	1.641	0.938	1.017	2.560	0.445	0.924	1.172	…	45	7.854	…	2.485	…	…	…	…	…
1	1.785	0.949	1.014	2.558	0.445	0.907	1.178	…	45	…	…	…	…	…	…	…	…
1	…	0.940	…	2.515	0.445	0.918	1.176	…	46	…	8.091	…	4.012	1.877	2.212	2.726	…
4	…	…	1.143	…	…	…	…	0.704	46	…	3.762	…	3.737	2.027	2.185	2.898	…
4	…	…	1.159	…	…	…	…	0.700	46	…	…	…	3.985	1.908	2.294	2.921	…
4	…	…	…	…	…	…	…	0.670	48	…	…	…	…	…	…	…	0.780
5	2.541	…	…	…	…	…	…	…	48	…	…	…	…	…	…	…	0.913
5	2.805	…	…	…	…	…	…	…	48	…	…	…	…	…	…	…	0.224
5	…	…	…	…	…	…	…	…	49	7.854	…	2.649	3.688	…	…	…	…
6	…	1.043	…	2.791	0.363	1.270	1.689	…	49	10.404	…	2.575	3.460	…	…	…	…
6	…	1.201	…	2.891	0.388	1.276	1.693	…	49	…	…	…	3.730	…	…	…	…
6	…	1.158	…	2.917	0.380	1.270	1.695	…	50	…	3.975	…	…	1.793	2.134	2.701	…
20	…	…	…	…	…	…	…	1.197	50	…	3.081	…	…	1.889	2.077	2.782	…
20	…	…	…	…	…	…	…	1.189	50	…	7.095	…	…	1.752	2.115	2.616	…
20	…	…	…	…	…	…	…	1.393	52	…	…	2.183	…	…	…	…	0.950
21	4.223	…	2.413	…	…	…	…	…	52	…	…	2.387	…	…	…	…	0.758
21	16.443	…	2.454	…	…	…	…	…	52	…	…	…	…	…	…	…	0.114
21	…	…	…	…	…	…	…	…	53	11.070	…	…	3.313	…	…	…	…
22	…	2.360	…	…	1.064	2.188	2.802	…	53	6.560	…	…	3.214	…	…	…	…
22	…	2.156	…	…	1.271	2.201	2.800	…	53	…	…	…	3.228	…	…	…	…
22	…	1.489	…	…	1.309	2.209	2.790	…	54	…	3.450	…	…	1.686	2.028	2.672	…
22.5	…	…	…	…	…	…	…	…	54	…	…	…	…	1.873	2.109	2.548	…
22.5	…	…	…	…	…	…	…	…	54	…	…	…	…	1.877	1.975	2.816	…
22.5	…	…	…	…	…	…	…	…	68	…	…	…	…	…	…	…	0.041
23	…	…	…	4.264	…	…	…	…	68	…	…	…	…	…	…	…	0.165
23	…	…	…	4.607	…	…	…	…	68	…	…	…	…	…	…	…	0.197
23	…	…	…	4.472	…	…	…	…	69	1.743	…	1.471	…	…	…	…	…
24	…	…	…	…	…	…	…	1.349	69	2.016	…	1.414	…	…	…	…	…
24	…	…	…	…	…	…	…	1.213	69	…	…	1.527	…	…	…	…	…
24	…	…	…	…	…	…	…	1.343	70	…	2.656	…	…	0.833	0.988	2.254	…
25	17.253	…	2.550	…	…	…	…	…	70	…	2.661	…	…	0.903	0.704	2.175	…
25	12.798	…	2.439	…	…	…	…	…	70	…	…	…	…	0.890	0.989	2.277	…
25	…	…	…	…	…	…	…	…	70.5	…	…	…	3.021	…	…	…	…
26	…	1.972	…	…	1.627	2.263	2.865	…	70.5	…	…	…	2.063	…	…	…	…
26	…	2.203	…	…	1.528	2.257	2.877	…	70.5	…	…	1.610	2.508	…	…	…	…
26	…	2.094	…	…	1.573	2.264	2.886	…	72	…	…	…	…	…	…	…	0.138
27	…	…	…	4.796	…	…	…	…	72	…	…	…	…	…	…	…	0.061
27	…	…	…	4.943	…	…	…	…	73	1.620	…	1.376	…	…	…	…	…
27	…	…	…	4.338	…	…	…	…	73	0.540	…	1.321	…	…	…	…	…
28	…	…	2.548	…	…	…	…	1.312	73	…	…	…	…	…	…	…	…
28	…	…	2.564	…	…	…	…	0.998	74	…	2.598	…	…	0.741	1.035	1.699	…
28	…	…	…	…	…	…	…	1.090	74	…	2.641	…	…	1.031	0.716	1.944	…
29	14.175	…	…	…	…	…	…	…	74	…	…	…	…	0.839	0.684	2.014	…
29	14.985	…	…	…	…	…	…	…	75	…	…	…	1.707	…	…	…	…
29	…	…	…	…	…	…	…	…	75	…	…	…	1.626	…	…	…	…
30	…	1.867	…	…	1.779	2.320	2.909	…	75	…	…	…	2.032	…	…	…	…
30	…	2.351	…	…	1.738	2.295	2.905	…	77	0.640	…	1.227	…	…	…	…	…
30	…	2.303	…	…	1.819	2.294	2.930	…	77	1.560	…	1.318	…	…	…	…	…
36	…	…	…	4.590	…	…	…	…	77	…	…	1.207	…	…	…	…	…
36	…	…	…	4.369	…	…	…	…	78	…	2.417	…	1.564	0.808	0.709	1.930	…
36	…	…	…	4.737	…	…	…	…	78	…	2.768	…	1.957	0.789	0.874	2.014	…
42	…	…	…	…	…	…	…	1.107	78	…	…	…	…	0.765	1.314	…	…

[a] Absorbance values for collaborators were not measured at identical times. Individual values are relative for each collaborator. "…" indicates data were not supplied.

structive) sampling. A total of 30 samples was taken at 10 set time points over 78 hr. For each sample, absorbance readings were taken at 600 nm, the sample was filtered, and the density of the filtrate was measured with a portable density meter.

RESULTS AND DISCUSSION

A total of eight collaborators provided absorbance and fermentation data similar to the curves displayed in Figure 1. The absorbance and extract data for both the PYF and control malts are shown in Tables I-A and I-B and Tables II-A and II-B, respectively. The extract and absorbance trends for the eight collaborators are shown in Figure 2. While most collaborators undertook triplicate testing at the recommended time points, some supplied single or duplicate readings, as shown in Figure 1 and Tables I-A and I-B.

A tilted Gaussian model was used to model the change in absorbance with time:

$$RA_{(t)} = R \cdot t + Ae^{\{-0.5 \cdot [(t-\mu)/\sigma]\}} \quad (1)$$

where $RA_{(t)}$ is absorbance at time (t), R is the slope term, A is the amplitude, μ is the midpoint, and σ is the width factor. This model fit the data of seven of the eight reporting collaborators well. This

TABLE I-B
Relative Absorbance Data for Fermentations Performed Using Premature Yeast Flocculation Malt Samples[a]

Time	Collaborator								Time	Collaborator							
(hr)	1	2	3	4	5	6	7	8	(hr)	1	2	3	4	5	6	7	8
0	2.159	1.007	1.030	…	…	…	…	0.710	42	…	…	…	…	…	…	…	0.666
0	2.151	1.013	…	…	…	…	…	0.700	42	…	…	…	…	…	…	…	0.566
0	…	1.003	…	…	…	…	…	0.710	42	…	…	…	…	…	…	…	0.888
1	1.785	1.001	1.008	2.430	0.393	0.498	1.213	…	45	6.018	…	2.016	…	…	…	…	…
1	1.938	1.004	1.001	2.433	0.393	0.498	1.214	…	45	11.110	…	2.042	…	…	…	…	…
1	…	0.995	…	2.417	0.393	0.943	1.221	…	45	…	…	…	…	…	…	…	…
4	…	…	1.142	…	…	…	…	0.700	46	…	4.115	…	3.905	1.913	1.88	2.834	…
4	…	…	1.160	…	…	…	…	0.720	46	…	2.751	…	3.818	1.820	1.849	2.610	…
4	…	…	…	…	…	…	…	0.724	46	…	…	…	3.747	1.747	1.809	2.540	…
5	2.981	…	…	…	…	…	…	…	48	…	…	…	…	…	…	…	0.313
5	3.091	…	…	…	…	…	…	…	48	…	…	…	…	…	…	…	0.339
5	…	…	…	…	…	…	…	…	48	…	…	…	…	…	…	…	0.217
6	…	1.202	…	2.673	0.366	1.292	1.726	…	49	4.998	…	2.095	3.488	…	…	…	…
6	…	1.212	…	2.794	0.403	1.308	1.727	…	49	4.080	…	2.130	3.897	…	…	…	…
6	…	1.290	…	2.736	0.405	1.241	1.733	…	49	…	…	…	3.635	…	…	…	…
20	…	…	…	…	…	…	…	1.413	50	…	6.740	…	…	1.569	1.421	2.445	…
20	…	…	…	…	…	…	…	1.572	50	…	3.712	…	…	1.747	1.656	2.479	…
20	…	…	…	…	…	…	…	1.227	50	…	3.103	…	…	1.555	1.552	2.382	…
21	10.287	…	2.609	…	…	…	…	…	52	…	…	2.132	…	…	…	…	0.206
21	17.496	…	2.475	…	…	…	…	…	52	…	…	2.063	…	…	…	…	0.102
21	…	…	…	…	…	…	…	…	52	…	…	…	…	…	…	…	0.390
22	…	2.186	…	…	1.480	2.268	2.891	…	53	2.562	…	…	3.354	…	…	…	…
22	…	1.800	…	…	1.194	2.258	2.887	…	53	2.394	…	…	3.050	…	…	…	…
22	…	1.528	…	…	1.356	2.264	2.903	…	53	…	…	…	3.177	…	…	…	…
22.5	…	…	…	…	…	…	…	…	54	…	3.156	…	…	1.795	1.318	2.328	…
22.5	…	…	…	…	…	…	…	…	54	…	…	…	…	1.369	1.318	2.381	…
22.5	…	…	…	…	…	…	…	…	54	…	…	…	…	1.396	1.395	2.250	…
23	…	…	…	4.524	…	…	…	…	66	…	…	…	…	…	…	…	0.124
23	…	…	…	4.845	…	…	…	…	66	…	…	…	…	…	…	…	0.172
23	…	…	…	5.036	…	…	…	…	66	…	…	…	…	…	…	…	…
24	…	…	…	…	…	…	…	1.645	69	0.583	…	1.511	…	…	…	…	…
24	…	…	…	…	…	…	…	1.603	69	0.737	…	1.486	…	…	…	…	…
24	…	…	…	…	…	…	…	1.428	69	…	…	1.454	…	…	…	…	…
25	6.929	…	2.605	…	…	…	…	…	70	…	3.626	…	…	0.716	0.735	1.874	0.059
25	13.284	…	2.512	…	…	…	…	…	70	…	2.557	…	…	0.793	0.727	1.830	0.082
25	…	…	…	…	…	…	…	…	70	…	…	…	…	0.994	0.836	…	…
26	…	2.345	…	…	1.690	2.318	2.930	…	70.5	…	…	…	2.228	…	…	…	…
26	…	2.145	…	…	1.660	2.331	2.920	…	70.5	…	…	…	1.782	…	…	…	…
26	…	1.641	…	…	1.677	2.322	2.919	…	70.5	…	…	…	1.233	…	…	…	…
27	…	…	…	4.591	…	…	…	…	73	0.720	…	1.275	…	…	…	…	…
27	…	…	…	4.737	…	…	…	…	73	0.648	…	1.422	…	…	…	…	…
27	…	…	…	4.349	…	…	…	…	73	…	…	1.272	…	…	…	…	…
28	…	…	2.563	…	…	…	…	1.316	74	…	…	…	…	0.730	0.566	1.525	…
28	…	…	2.545	…	…	…	…	0.909	74	…	…	…	…	0.774	0.646	1.429	…
28	…	…	…	…	…	…	…	0.632	74	…	…	…	…	0.498	0.544	…	…
29	13.851	…	…	…	…	…	…	…	75	…	3.038	…	1.605	…	…	…	…
29	12.960	…	…	…	…	…	…	…	75	…	4.603	…	1.375	…	…	…	…
29	…	…	…	…	…	…	…	…	75	…	…	…	0.973	…	…	…	…
30	…	2.199	…	…	1.817	2.346	2.940	…	77	0.960	…	1.302	…	…	…	…	…
30	…	2.331	…	…	1.606	2.357	2.918	…	77	1.400	…	1.146	…	…	…	…	…
30	…	2.142	…	…	1.808	2.347	2.934	…	77	…	…	1.311	…	…	…	…	…
36	…	…	…	4.055	…	…	…	…	78	…	…	…	1.949	0.515	0.661	1.512	…
36	…	…	…	4.262	…	…	…	…	78	…	…	…	1.305	0.575	0.508	1.427	…
36	…	…	…	3.994	…	…	…	…	78	…	…	…	…	0.640	0.569	…	…

[a] Absorbance values for collaborators were not measured at identical times. Individual values are relative for each collaborator. "…" indicates data were not supplied.

expression (3) has been used to replace the nine-parameter model described by Lake et al (5) and allows a model to be fit to substantially less data. A common curve fitting software package, Prism 5.0c (Graph Pad Software), was used to fit the absorbance data.

As shown in Table III, the model fit the absorbance data well in all cases, except for collaborator 2, which reported problems with their procedure. Collaborator 3 reported that they used SMA yeast obtained from a yeast supply house instead of growing the yeast in-house. Collaborator 5, apparently, did not fortify the wort with 4% (wt/wt) glucose. However, because the results from all collaborators, with the exception of collaborator 2, showed general agreement, only data from collaborator 2 were discarded.

Using the Prism 5.0c software, it was possible to compare nonlinear data sets with an ANOVA-type procedure described on the Graph Pad website (http://www.graphpad.com). Table III shows the results of the ANOVA procedure. All collaborators, with the exception of collaborator 2, showed that the control and PYF malts were significantly different ($P < 0.05$) based on an F test using the Prism 5.0c software. A paired Student's t test of the width factors (σ), as well as the midpoint (μ) values of the curves, of fermentation data from collaborators 1 and 3–8 indicated that both parameters were significantly different ($P < 0.05$) from the control. Visual examination of the fermentation curves also indicated that the PYF malt did in fact cause PYF.

TABLE II-A
Extract Data for Fermentations Performed Using Control Malt Samples[a]

Time	Collaborator								Time	Collaborator							
(hr)	1	2	3	4	5	6	7	8	(hr)	1	2	3	4	5	6	7	8
0	14.73	13.16	16.03	…	…	…	…	15.47	42	…	…	…	…	…	…	…	3.37
0	14.73	13.18	…	…	…	…	…	15.47	42	…	…	…	…	…	…	…	3.12
0	…	13.19	…	…	…	…	…	15.47	42	…	…	…	…	…	…	…	3.52
1	14.69	13.70	15.84	14.50	11.78	15.00	15.30	…	45	3.88	…	3.55	…	…	…	…	…
1	14.68	13.68	15.90	14.50	11.78	15.00	15.40	…	45	3.32	…	3.86	…	…	…	…	…
1	…	13.70	…	14.50	11.78	15.00	15.40	…	45	…	…	…	…	…	…	…	…
4	…	…	15.33	…	…	…	…	14.75	46	…	1.66	…	3.20	3.05	2.80	2.90	…
4	…	…	15.44	…	…	…	…	14.84	46	…	0.31	…	3.20	3.08	2.50	3.20	…
4	…	…	…	…	…	…	…	14.84	46	…	…	…	3.00	3.11	3.40	3.20	…
5	14.14	…	…	…	…	…	…	…	48	…	…	…	…	…	…	…	3.10
5	14.15	…	…	…	…	…	…	…	48	…	…	…	…	…	…	…	3.15
5	…	…	…	…	…	…	…	…	48	…	…	…	…	…	…	…	5.41
6	…	13.22	…	14.20	11.66	14.40	14.60	…	49	3.12	…	3.00	2.90	…	…	…	…
6	…	13.20	…	14.20	11.69	14.30	14.60	…	49	5.04	…	2.82	3.20	…	…	…	…
6	…	13.12	…	14.00	11.69	14.30	14.60	…	49	…	…	…	3.00	…	…	…	…
20	…	…	…	…	…	…	…	8.92	50	…	1.67	…	…	2.50	2.30	2.70	…
20	…	…	…	…	…	…	…	8.71	50	…	0.85	…	…	2.62	2.20	2.60	…
20	…	…	…	…	…	…	…	9.03	50	…	…	…	…	2.60	2.40	2.40	…
21	8.44	…	8.54	…	…	…	…	…	52	…	…	2.84	…	…	…	…	2.31
21	11.63	…	8.67	…	…	…	…	…	52	…	…	3.12	…	…	…	…	2.72
21	…	…	…	…	…	…	…	…	52	…	…	…	…	…	…	…	6.18
22	…	9.21	…	…	9.38	7.80	7.70	…	53	2.84	…	…	2.80	…	…	…	…
22	…	7.19	…	…	9.59	7.80	7.70	…	53	2.4	…	…	2.80	…	…	…	…
22	…	7.51	…	…	9.33	7.80	7.80	…	53	…	…	…	2.90	…	…	…	…
22.5	…	…	…	…	…	…	…	…	54	…	1.02	…	…	1.99	2.10	…	…
22.5	…	…	…	…	…	…	…	…	54	…	0.41	…	…	2.11	2.20	…	…
22.5	…	…	…	…	…	…	…	…	54	…	…	…	…	2.06	2.20	…	…
23	…	…	…	7.40	…	…	…	…	66	…	…	…	…	…	…	2.50	4.46
23	…	…	…	7.20	…	…	…	…	66	…	…	…	…	…	…	2.40	1.95
23	…	…	…	7.70	…	…	…	…	66	…	…	…	…	…	…	2.60	1.62
24	…	…	…	…	…	…	…	7.63	69	2.01	…	1.95	…	…	…	…	…
24	…	…	…	…	…	…	…	7.44	69	2.85	…	1.94	…	…	…	…	…
24	…	…	…	…	…	…	…	8.17	69	…	…	2.03	…	…	…	…	…
25	7.77	…	7.16	…	…	…	…	…	70	…	1.06	…	…	…	1.60	2.10	3.75
25	7.08	…	7.44	…	…	…	…	…	70	…	0.36	…	…	…	1.80	2.10	3.07
25	…	…	…	…	…	…	…	…	70	…	…	…	…	…	1.70	…	…
26	…	8.62	…	…	7.99	6.30	6.50	…	70.5	…	…	…	2.50	…	…	…	…
26	…	7.66	…	…	7.79	6.40	6.50	…	70.5	…	…	…	2.60	…	…	…	…
26	…	8.36	…	…	7.74	6.30	6.50	…	70.5	…	…	…	2.50	…	…	…	…
27	…	…	…	5.90	…	…	…	…	73	1.83	…	1.96	…	…	…	…	…
27	…	…	…	5.90	…	…	…	…	73	1.84	…	1.96	…	…	…	…	…
27	…	…	…	6.00	…	…	…	…	73	…	…	2.12	…	…	…	…	…
28	…	…	6.41	…	…	…	…	6.65	74	…	0.43	…	…	1.58	1.70	2.00	…
28	…	…	6.49	…	…	…	…	6.40	74	…	1.04	…	…	1.50	1.70	2.10	…
28	…	…	…	…	…	…	…	6.77	74	…	…	…	…	1.55	1.70	2.10	…
29	6.06	…	…	…	…	…	…	…	75	…	…	…	2.60	…	…	…	…
29	6.09	…	…	…	…	…	…	…	75	…	…	…	2.60	…	…	…	…
29	…	…	…	…	…	…	…	…	75	…	…	…	2.50	…	…	…	…
30	…	6.00	…	…	6.76	5.40	5.60	…	77	1.81	…	1.97	…	…	…	…	…
30	…	6.51	…	…	6.58	5.20	5.40	…	77	2.93	…	1.98	…	…	…	…	…
30	…	5.56	…	…	6.68	5.20	5.60	…	77	…	…	1.97	…	…	…	…	…
36	…	…	…	4.10	…	…	…	…	78	…	0.13	…	2.40	1.53	1.70	2.10	…
36	…	…	…	4.00	…	…	…	…	78	…	0.12	…	2.40	1.48	1.70	2.10	…
36	…	…	…	5.20	…	…	…	…	78	…	…	…	…	1.50	1.90	…	…

[a] Extract values for collaborators were not measured at identical times. "…" indicates data were not supplied.

As reported in the first year (2), the extract data could be successfully fit using a nonlinear regression routine (Prism 5.0c) to obtain the four parameters of the logistic equation:

$$P_t = (P_i - P_e)/\{1 + \exp[-B \cdot (t - M)]\} + P_e \quad (2)$$

where P_t, P_i, and P_e are the extract levels at time t and upper and lower limits the function asymptotically approaches (expressed in degrees Plato), respectively; B is the value proportional to the slope at the time of inflection (M). From these values, OE and AE were calculated as shown in Figure 1. ADF was calculated as shown in equation 3:

$$\text{ADF} = (\text{OE} - \text{AE})/\text{OE} \quad (3)$$

To compare the within- and between-lab errors of the fermentations, the standard error of ADF was calculated and compared for each collaborator and yeast strain. To estimate the individual ADF within-lab errors, a calculation of the propagation of error (4,7) was undertaken as described previously (2).

The individual ADF values, as well as their average and standard errors (between-lab errors) and individual ADF errors (ADF within error), are shown in Table IV. Only the data set for collaborator 2 gave unrealistic estimates of ADF values and, therefore, was eliminated from further analysis.

TABLE II-B
Extract Data for Fermentations Performed Using Premature Yeast Flocculation Malt Samples[a]

Time	Collaborator								Time	Collaborator							
(hr)	1	2	3	4	5	6	7	8	(hr)	1	2	3	4	5	6	7	8
0	14.62	13.64	16.14	…	…	…	…	15.47	42	…	…	…	…	…	…	…	1.88
0	14.63	13.65	…	…	…	…	…	15.47	42	…	…	…	…	…	…	…	3.07
0	…	13.71	…	…	…	…	…	15.47	42	…	…	…	…	…	…	…	2.82
1	14.59	13.95	12.79	14.60	11.90	15.10	15.30	…	45	3.13	…	4.12	…	…	…	…	…
1	14.59	14.01	16.09	14.70	11.90	15.10	15.30	…	45	2.51	…	3.74	…	…	…	…	…
1	…	13.12	…	14.60	11.90	15.10	15.30	…	45	…	…	…	…	…	…	…	…
4	…	…	15.62	…	…	…	…	14.46	46	…	1.66	…	2.70	2.17	2.30	2.70	…
4	…	…	15.64	…	…	…	…	14.39	46	…	0.73	…	2.70	1.81	2.40	2.70	…
4	…	…	…	…	…	…	…	14.39	46	…	…	…	2.50	2.62	2.40	2.70	…
5	14.03	…	…	…	…	…	…	…	48	…	…	…	…	…	…	…	3.6
5	14.00	…	…	…	…	…	…	…	48	…	…	…	…	…	…	…	3.3
5	…	…	…	…	…	…	…	…	48	…	…	…	…	…	…	…	2.00
6	…	14.04	…	13.80	11.81	14.40	14.50	…	49	2.36	…	3.15	2.40	…	…	…	…
6	…	13.00	…	14.20	11.81	14.50	14.60	…	49	2.43	…	3.59	2.30	…	…	…	…
6	…	12.52	…	14.20	11.81	14.40	14.50	…	49	…	…	…	2.30	…	…	…	…
20	…	…	…	…	…	…	…	7.49	50	…	0.36	…	…	1.96	2.20	2.70	…
20	…	…	…	…	…	…	…	6.94	50	…	0.54	…	…	2.11	2.30	2.30	…
20	…	…	…	…	…	…	…	8.22	50	…	…	…	…	1.81	2.30	2.50	…
21	7.57	…	8.31	…	…	…	…	…	52	…	…	3.13	…	…	…	…	2.13
21	8.93	…	8.19	…	…	…	…	…	52	…	…	3.25	…	…	…	…	3.63
21	…	…	…	…	…	…	…	…	52	…	…	…	…	…	…	…	2.97
22	…	9.34	…	…	9.09	7.10	6.90	…	53	1.22	…	…	2.10	…	…	…	…
22	…	8.70	…	…	9.57	7.20	6.90	…	53	2.06	…	…	2.30	…	…	…	…
22	…	7.67	…	…	9.30	7.20	7.00	…	53	…	…	…	2.30	…	…	…	…
22.5	…	…	…	…	…	…	…	…	54	…	0.37	…	…	1.91	2.10	2.50	…
22.5	…	…	…	…	…	…	…	…	54	…	0.37	…	…	1.91	2.10	2.30	…
22.5	…	…	…	…	…	…	…	…	54	…	…	…	…	1.73	2.10	2.20	…
23	…	…	…	7.40	…	…	…	…	66	…	…	…	…	…	…	…	2.28
23	…	…	…	4.60	…	…	…	…	66	…	…	…	…	…	…	…	2.28
23	…	…	…	4.10	…	…	…	…	66	…	…	…	…	…	…	…	…
24	…	…	…	…	…	…	…	5.28	69	1.93	…	2.71	…	…	…	…	…
24	…	…	…	…	…	…	…	5.93	69	1.86	…	2.78	…	…	…	…	…
24	…	…	…	…	…	…	…	4.78	69	…	…	2.68	…	…	…	…	…
25	8.62	…	7.11	…	…	…	…	…	70	…	0.20	…	…	1.58	2.10	2.20	3.63
25	6.23	…	6.66	…	…	…	…	…	70	…	0.22	…	…	1.55	2.20	2.20	2.82
25	…	…	…	…	…	…	…	…	70	…	…	…	…	1.63	2.10	2.20	…
26	…	7.98	…	…	7.60	5.50	5.70	…	70.5	…	…	…	2.10	…	…	…	…
26	…	6.35	…	…	7.62	5.50	5.60	…	70.5	…	…	…	2.10	…	…	…	…
26	…	6.17	…	…	7.47	5.60	5.70	…	70.5	…	…	…	2.10	…	…	…	…
27	…	…	…	5.90	…	…	…	…	73	2.51	…	2.61	…	…	…	…	…
27	…	…	…	5.90	…	…	…	…	73	1.87	…	2.33	…	…	…	…	…
27	…	…	…	5.80	…	…	…	…	73	…	…	2.51	…	…	…	…	…
28	…	…	6.01	…	…	…	…	6.33	74	…	…	…	…	1.60	2.20	2.20	…
28	…	…	6.06	…	…	…	…	5.88	74	…	…	…	…	1.65	2.10	2.20	…
28	…	…	…	…	…	…	…	4.91	74	…	…	…	…	1.58	2.10	…	…
29	4.84	…	…	…	…	…	…	…	75	…	0.17	…	2.10	…	…	…	…
29	4.83	…	…	…	…	…	…	…	75	…	0.20	…	2.20	…	…	…	…
29	…	…	…	…	…	…	…	…	75	…	…	…	2.20	…	…	…	…
30	…	4.87	…	…	6.21	4.40	5.00	…	77	4.00	…	2.34	…	…	…	…	…
30	…	4.59	…	…	6.78	4.40	4.90	…	77	1.84	…	2.46	…	…	…	…	…
30	…	4.87	…	…	6.16	4.40	4.90	…	77	…	…	2.61	…	…	…	…	…
36	…	…	…	3.60	…	…	…	…	78	…	0.17	…	2.20	1.50	2.00	2.20	…
36	…	…	…	3.80	…	…	…	…	78	…	0.14	…	2.20	1.53	2.00	2.20	…
36	…	…	…	3.50	…	…	…	…	78	…	…	…	…	1.55	2.10	…	…

[a] Extract values for collaborators were not measured at identical times. "…" indicates data were not supplied.

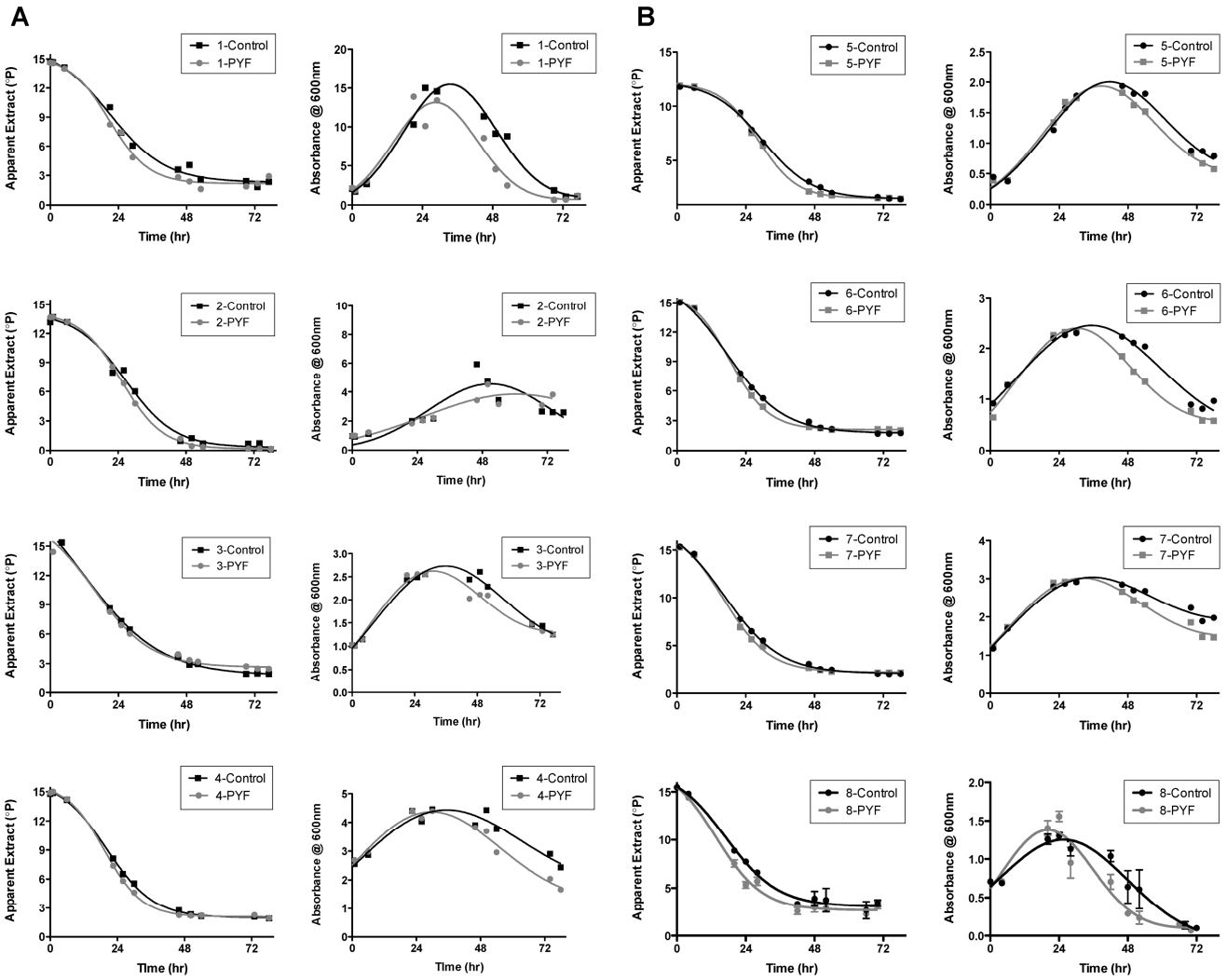

Fig. 2. Extract and absorbance trends for collaborators 1–4 (**A**) and 5–8 (**B**) using the mini-fermentation method.

TABLE III
Comparison of Nonlinear Fits of Absorbance Data for Fermentations of Control (Con.) and Premature Yeast Flocculation (PYF) Malts Using the Tilted Gaussian Model for Individual Collaborative Data

	Collaborator and Malt															
Code[a]	1-PYF	1-Con.	2-PYF	2-Con.	3-PYF	3-Con.	4-PYF	4-Con.	5-PYF	5-Con.	6-PYF	6-Con.	7-PYF	7-Con.	8-PYF	8-Con.
A (°P)	12.900	15.170	7.860	8.542	2.182	2.385	4.326	4.167	1.743	1.791	2.209	2.325	2.532	2.380	1.355	1.276
M (hr)	28.380	33.360	67.840	60.690	27.720	33.190	27.380	26.670	37.600	40.330	29.050	34.370	28.620	30.020	19.87	25.84
σ (hr)	14.450	15.790	33.650	28.410	21.550	24.560	25.140	25.750	19.430	20.400	19.630	24.780	23.550	25.370	15.91	22.07
R (°P/hr)	0.009	0.010	–0.059	–0.066	0.015	0.010	0.011	0.017	0.005	0.005	0.006	0.004	0.016	0.020	0.001	–0.001
df	20.000	20.000	24.000	26.000	22.000	22.000	25.000	25.000	29.000	29.000	29.000	29.000	26.000	28.000	24	25
r^2	0.842	0.823	0.709	0.638	0.961	0.974	0.946	0.948	0.959	0.965	0.976	0.946	0.978	0.967	0.865	0.821
Abs. SS	94.340	137.300	14.280	28.720	0.326	0.259	2.298	1.675	0.416	0.365	0.364	0.702	0.254	0.340	0.930	0.935
P[b]	<0.05		>0.5		<0.001		<0.05		<0.001		<0.001		<0.001		<0.01	
F[c]	2.956 (4, 40)		0.8050 (4, 50)		7.371 (4, 44)		2.894 (4, 50)		5.476 (4, 58)		21.99 (4, 58)		23.06 (4, 54)		4.289 (4, 49)	

[a] *A* = amplitude; *M* = time of inflection; σ = width factor; *R* = slope term; df = degree of freedom; r^2 = correlation coefficient; and Abs. SS = absolute sum of squares.
[b] Probability values <0.05, <0.01, and <0.001 indicate a significant difference between the change in absorbance of the paired control and PYF malts.
[c] The reported *F* value is followed by the degrees of freedom of the nominator and denominator.

Examination of Table IV indicates the within-lab error averaged 0.011 for both the control and PYF malts. The between-lab error, or standard deviation, of the ADF values was 0.030 and 0.016 for the control and PYF malts, respectively. If one considers an average ADF value of 0.85 and the worst errors encountered (approx. 0.03), the highest coefficient of variation noted was approx. 3.5%.

LITERATURE CITED

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TABLE IV
Nonlinear Analysis and Determination of Original Extract (OE), Apparent Extract (AE), and Apparent Degree of Fermentation (ADF) for Fermentations of Control and Premature Yeast Flocculation (PYF) Malts for Individual Collaborative Data

Malt	Collaborator							
Parameter[a]	1	2	3	4	5	6	7	8
Control								
Best-fit value								
P_e (°P)	2.319	0.308	1.812	2.599	1.530	1.760	2.121	3.062
P_i (°P)	16.380	14.160	22.500	16.390	12.270	17.750	18.390	17.510
B (hr^{-1})	–0.101	–0.114	–0.074	–0.119	–0.115	–0.103	–0.103	–0.109
M (hr)	20.890	26.410	11.570	18.100	29.700	17.450	16.420	16.960
df	20	26	22	28	29	29	27	25
r^2	0.977	0.985	0.999	0.996	0.999	0.998	0.998	0.965
Abs. SS	13.290	12.340	0.999	2.589	0.772	1.626	1.290	2.113
OE (°P)	14.862	13.506	16.341	14.952	11.932	15.463	15.841	15.550
AE (°P)	2.378	0.362	1.998	2.615	1.587	1.803	2.161	3.087
ADF	0.840	0.973	0.878	0.825	0.867	0.883	0.864	0.801
ADF within-lab error	0.029	0.021	0.013	0.010	0.005	0.007	0.007	0.007
Avg. ADF[b]	0.851							
Between-lab error[b]	0.030							
PYF								
Best-fit value								
P_e (°P)	2.177	0.1592	2.641	2.144	1.565	2.104	2.251	2.665
P_i (°P)	15.28	14.16	18.52	15.81	12.11	16.27	17.53	17.96
B (hr^{-1})	–0.1436	–0.1359	–0.0999	–0.1335	0.1501	–0.1467	–0.1248	–0.1224
M (hr)	20.69	25.07	15.37	19.62	28.45	18.26	16.21	13.57
df	20	26	22	28	29	29	27	24
r^2	0.9827	0.9935	0.9814	0.9991	0.998	0.9995	0.9979	0.982
Abs. SS	10.55	5.921	11.02	0.628	1.103	0.3495	1.438	1.144
OE (°P)	14.641	13.711	15.706	14.882	11.965	15.360	15.745	15.518
AE (°P)	2.182	0.175	2.682	2.152	1.575	2.107	2.261	2.673
ADF	0.851	0.987	0.829	0.855	0.868	0.863	0.856	0.828
ADF within-lab error	0.019	0.013	0.032	0.004	0.005	0.002	0.007	0.007
Avg. ADF[b]	0.850							
Between-lab error[b]	0.0158							

[a] P_e = lower limit the function asymptotically approaches; P_i = upper limit the function asymptotically approaches; B = value proportional to the slope at the time of inflection (M); df = degree of freedom; r^2 = correlation coefficient; and Abs. SS = absolute sum of squares.
[b] Collaborator 2 was not included in this calculation.

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Determination of Iso-α-acids in Beer and Wort by High-Performance Liquid Chromatography

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Keywords: Hop acids, HPLC, IAA

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the determination of iso-α-acids in wort without the use of solid-phase extraction (SPE) ranged from 1.5 to 11.1% and 9.7 to 16.6%, respectively, and were judged acceptable.
2. Repeatability and reproducibility coefficients of variation for the determination of iso-α-acids in beer without the use of SPE ranged from 3.15 to 3.20% and 6.2 to 11.6%, respectively, and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends the modified high-performance liquid chromatography (HPLC) method used for determination of iso-α-acids in beer and wort be included in the ASBC *Methods of Analysis.*
2. Discharge the subcommittee.

This is the subcommittee's first year of existence. The subcommittee was formed on the recommendation of the Subcommittee on Coordination of New and Alternate Methods (2). This method is designed to test iso-α-acids (IAA) in beer and wort to determine whether adequate chromatography can be achieved without the use of SPE, as is used in ASBC Method Beer-23C (1). This method was based on EBC Method 7.9 (3). The primary modification made to EBC Method 7.9 was the incorporation of beer and wort in place of hop products.

PROCEDURE

Six sample pairs were sent to each collaborator: three wort sample pairs and three beer sample pairs. The wort and beer pairs covered a range of IAA levels.

Collaborators were requested to follow the modified EBC Method 7.9. The procedure utilized a C18 column and a methanol-water-phosphoric acid mobile phase. Results were evaluated using the Youden unit block design (2).

RESULTS AND DISCUSSION

Results from nine collaborators were received for the wort sample pairs (A1/A2, B1/B2, and C1/C2) and beer sample pairs (D1/D2, E1/E2, and F1/F2). The results are summarized in Tables I and II, respectively. Outliers were identified using Dixon's ratio test, as described in the Youden unit block procedure (2).

In Table I, data from two identified outliers were excluded from the test results for the wort sample pairs. In Table II, data from a single identified outlier were excluded from the test results for the beer sample pairs. One outlier for both the wort and beer sample pairs can be attributed to inexperience with HPLC analysis.

A statistical summary of both the wort and beer IAA data is presented in Table III. Repeatability and reproducibility coefficients of variation for wort ranged from 1.5 to 11.1% and 9.7 to 16.6%, respectively, and were judged acceptable. Repeatability and reproducibility coefficients of variation for beer ranged from 3.15 to 3.20% and 6.2 to 11.6%, respectively, and were judged acceptable.

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TABLE I
Iso-α-acids in Wort (ppm [mg/L])

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	A1	A2	B1	B2	C1	C2
1	6.26	7.96	31.84	35.18	43.60	44.76
2	7.63	11.48	30.83	35.07	64.43	65.30
3	7.17	9.87	33.60	35.80	57.50	57.15
4	6.11	8.02	31.61	34.67	49.13	49.64
5	6.33	6.33	34.69	35.84	57.61	55.46
6	7.30	10.6	27.40	26.30	47.00	47.30
7	6.97	9.05	28.65	30.61	49.33	48.75
8	2.30[a]	2.90[a]	9.30[a]	9.80[a]	9.80[a]	13.80[a]
9	2.60[a]	4.40[a]	15.70[a]	17.40[a]	27.70[a]	27.70[a]
Mean[b]	6.824	9.044	31.231	33.352	52.657	52.622
Grand mean[b]	7.934		32.292		52.640	

[a] Outlier at $P \leq 0.05$ based on totals and differences.
[b] Calculated excluding outliers.

TABLE II
Iso-α-acids in Beer (mg/L)

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	D1	D2	E1	E2	F1	F2
1	23.32	21.04	26.20	29.64	37.04	35.64
2	24.45	22.54	28.28	30.33	44.98	47.42
3	24.80	23.40	30.20	30.30	46.30	47.30
4	21.96	20.94	28.25	28.10	41.80	43.84
5	16.68	17.35	27.10	27.48	38.22	40.75
6	18.80	18.20	24.50	25.40	43.20	43.00
7	21.68	20.73	25.54	27.76	44.80	42.27
8	8.30[a]	7.80[a]	10.10[a]	10.30[a]	12.20[a]	12.70[a]
9	23.60	21.60	27.20	28.00	44.20	46.50
Mean[b]	21.911	20.725	27.159	28.376	42.568	43.340
Grand mean[b]	21.318		27.768		42.954	

[a] Outlier at $P \leq 0.05$ based on totals and differences.
[b] Calculated excluding outliers.

TABLE III
Statistical Summary of Results[a]

Medium Sample Pair	No. of Labs	Grand Mean	Repeatability			Reproducibility		
			S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Wort								
A1/A2	7	7.934	0.88	11.14	2.47	1.31	16.56	3.68
B1/B2	7	32.292	1.23	3.81	3.45	3.12	9.67	8.74
C1/C2	7	52.640	0.79	1.50	2.22	7.23	13.74	20.25
Beer								
D1/D2	8	21.318	0.67	3.15	1.88	2.48	11.62	6.93
E1/E2	8	27.768	0.88	3.15	2.45	1.73	6.21	4.83
F1/F2	8	42.954	1.37	3.20	3.85	3.66	8.52	10.25

[a] All calculations were made based on Tables I and II.

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Alternative Method for ASBC Malt-2B (Assortment)

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Keywords: Eureka-Niagara, Sortimat

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for assortment using the Pfeuffer Sortimat sample grader with imperial screens ranged from 1.31 to 65.17% and 1.70 to 65.17%, respectively, and were judged acceptable.
2. Repeatability and reproducibility coefficients of variation for assortment using the Pfeuffer Sortimat sample grader with metric screens ranged from 0.54 to 26.65% and 1.72 to 55.26%, respectively, and were judged acceptable.
3. Based on the *t* test, assuming unequal variances for malt assortment, the S. Howe Co., Inc. Eureka-Niagara and Pfeuffer Sortimat sample graders with imperial screens were not significantly different at the 95% confidence level.
4. Based on the *t* test, assuming unequal variances for malt assortment, the Pfeuffer Sortimat sample grader with different metric screens was not significantly different at 2.8 and 2.5 mm but was significantly different for the 2.2-mm and sample through 2.2-mm screens at the 95% confidence level.

RECOMMENDATIONS

1. The subcommittee recommends changing the screen specifications for determining assortment of malt kernel sizes to $^{7}/_{64}$ in. (2.78 mm), $^{6}/_{64}$ in. (2.38 mm), and $^{5}/_{64}$ in. (1.98 mm), which are accurate to ±0.005 in. (±0.05 mm).
2. The subcommittee recommends that the alternative method for assortment be included in the ASBC *Methods of Analysis.*
3. Discharge the subcommittee.

This was the second year of the subcommittee's evaluation of assortment using the Pfeuffer Sortimat sample grader. The subcommittee was formed on the recommendation of the Subcommittee on *Methods of Analysis* Malt Review (2). In the first year, members of the subcommittee were asked to evaluate the screen slot widths to determine the recommendations for screen tolerances. Because the method for assortment was produced in 1956, it was important to look at tolerances to understand the limitations of the machine. The last time screen tolerances were reviewed was in 1955 and 1956 (3,4), and no changes were made at that time. Stamping accuracy today does not meet the rigid tolerances set by the original subcommittees. In its first year, the method produced unacceptable repeatability and reproducibility coefficients of variation for all screens (5). The Pfeuffer Sortimat sample grader is becoming standard equipment for assortment in the malting industry and is slowly replacing the S. Howe Co., Inc. Eureka-Niagara sample grader.

PROCEDURE

Collaborators were provided with four sample pairs (A/B through G/H) that were similar but distinct. Samples A and B were six-row Lacey malts from different production dates. Samples C and D were two-row Conlon malts from different production dates. Samples E and F were two-row customer malt shipments blended on different dates. Samples G and H were six-row customer malt shipments

TABLE I
Assortment (%) for a $^{7}/_{64}$-in. Screen for a Eureka-Niagara Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	63.0	61.5	92.9	92.1	72.4	73.9	64.9	62.4
2	65.6	62.0	95.4	94.2	73.2	77.0	64.9	63.6
3	64.0	61.1	95.6	95.2	73.9	73.6	65.1	66.3
4	64.9	61.1	93.0	90.6	73.7	72.0	62.4	61.4
5	61.9	59.7	92.6	93.1	70.4	70.3	61.7	61.7
6	65.5	57.2	94.2	93.5	75.1	73.8	65.0	66.1
7	69.3	69.0	94.9	94.2	74.2	77.5	68.8	68.8
Mean	64.89	61.66	94.09	93.27	73.27	74.01	64.69	64.33
Grand mean	63.27		93.68		73.64		64.51	

TABLE II
Assortment (%) for a $^{6}/_{64}$-in. Screen for a Eureka-Niagara Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	30.8	31.3	5.9	6.3	22.7	21.5	28.7	31.1
3	28.6	30.3	2.9	3.9	21.3	18.4	28.3	29.1
3	28.9	31.8	4.1	2.0	20.9	20.7	27.7	2.7
4	31.9	34.2	6.2	8.2	22.9	24.6	33.5	34.0
5	34.0	35.6	6.4	6.4	26.2	26.0	33.3	33.3
6	28.0	32.4	4.5	4.7	19.6	20.8	28.1	26.4
7	25.2	25.6	4.3	4.3	19.5	17.6	25.1	24.8
Mean	29.63	31.60	4.90	5.11	21.87	21.37	29.24	29.49
Grand mean	30.61		5.01		21.62		29.36	

TABLE III
Assortment (%) for a $^{5}/_{64}$-in. Screen for a Eureka-Niagara Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	5.6	6.2	1.0	1.4	4.5	3.8	5.9	5.7
2	5.0	6.8	1.5	1.6	4.8	4.3	6.2	6.3
3	6.1	6.2	1.2	1.2	4.7	5.0	6.7	5.5
4	2.5	4.1	0.6	0.9	2.8	2.7	3.5	3.9
5	3.5	3.7	0.8	0.3	2.5	3.2	4.5	4.0
6	5.9	9.5	1.0	1.6	4.9	4.9	6.6	7.2
7	4.9	4.8	0.7	1.3	5.1	4.3	5.8	5.8
Mean	4.79	5.90	0.97	1.19	4.19	4.03	5.60	5.49
Grand mean	5.34		1.08		4.11		5.54	

TABLE IV
Assortment (%) for Sample Through a $^{5}/_{64}$-in. Screen for a Eureka-Niagara Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	0.5	0.9	0.2	0.2	0.4	0.6	0.5	0.8
2	0.8	0.9	0.2	0.3	0.7	0.3	0.6	1.0
3	1.0	0.9	0.2	0.2	0.5	0.7	0.5	0.5
4	0.7	0.7	0.2	0.4	0.7	0.7	0.5	0.7
5	0.6	1.0	0.2	0.2	0.8	0.5	0.5	0.9
6	0.6	0.9	0.3	0.2	0.4	0.5	0.3	0.3
7	0.6	0.7	0.2	0.2	0.1	0.7	0.4	0.6
Mean	0.69	0.86	0.21	0.24	0.51	0.57	0.47	0.69
Grand mean	0.77		0.23		0.54		0.58	

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blended on different dates. Collaborators were asked to use either the Eureka-Niagara sample grader with imperial screens of 7/64, 6/64, and 5/64 in. or the Sortimat sample grader with either imperial screens of 7/64, 6/64, and 5/64 in. or metric screens of 2.8, 2.5, and 2.2 mm. If the collaborators had the capability to run samples on multiple graders they were asked to do so. In the first year of study it was determined that the ±0.0005 in. tolerance set for each screen was beyond the capability of the manufacturer's 0.003 in. stamping accuracy. It was proposed that the tolerance be set at ±0.005 in. and ±0.05 mm. Results were evaluated using the Youden unit block design (1) and the *t* test, assuming unequal variances (Minitab).

TABLE V
Assortment (%) for a 7/64-in. Screen for a Sortimat Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	65.3	62.5	93.9	93.0	75.8	76.8	68.3	66.0
2	71.5	70.7	91.4	93.6	79.9	81.7	72.5	73.0
3	74.8	69.7	92.3	93.6	80.1	82.6	70.9	73.2
4	67.5	63.4	95.0	89.7	75.4	73.9	64.9	66.9
5	65.4	65.0	94.5	92.1	76.8	76.6	68.4	66.1
6	68.8	68.1	94.1	94.8	77.7	77.9	68.3	69.8
Mean	68.88	66.57	93.53	92.80	77.62	78.25	68.88	69.17
Grand mean	67.73		93.17		77.93		69.03	

TABLE VI
Assortment (%) for a 6/64-in. Screen for a Sortimat Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	28.9	29.9	5.0	5.6	19.3	18.8	25.9	27.7
2	24.1	25.2	7.6	5.6	16.9	15.4	23.2	23.1
3	21.5	25.3	6.8	5.4	17.4	14.9	24.4	23.2
4	27.7	29.2	3.8	8.8	19.6	20.9	28.4	27.5
5	29.7	29.3	4.6	6.0	19.1	18.9	26.7	28.2
6	26.4	27.5	4.9	4.3	18.9	18.1	27.2	25.8
Mean	26.38	27.73	5.45	5.95	18.53	17.83	25.97	25.92
Grand mean	27.06		5.70		18.18		25.94	

TABLE VII
Assortment (%) for a 5/64-in. Screen for a Sortimat Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	5.3	6.6	0.8	1.2	4.5	3.8	5.3	5.7
2	4.0	3.5	0.8	0.7	2.7	2.5	3.8	3.5
3	3.0	4.5	0.8	0.9	2.2	2.3	4.3	3.2
4	4.8	6.3	1.1	1.2	4.2	4.8	6.1	5.2
5	4.4	4.9	0.7	1.5	3.7	3.6	4.6	5.1
6	4.3	3.7	0.9	0.7	3.1	3.3	4.3	4.0
Mean	4.30	4.92	0.85	1.03	3.40	3.38	4.73	4.45
Grand mean	4.61		0.94		3.39		4.59	

TABLE VIII
Assortment (%) for Sample Through a 5/64-in. Screen for a Sortimat Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	0.5	1.0	0.2	0.2	0.4	0.6	0.5	0.6
2	0.4	0.6	0.2	0.1	0.5	0.4	0.5	0.4
3	0.4	0.6	0.6	0.2	0.4	0.4	0.4	0.3
4	0.4	0.9	0.1	0.2	0.7	0.5	0.7	0.5
5	0.5	0.8	0.2	0.4	0.4	0.8	0.3	0.3
6	0.7	0.7	0.2	0.1	0.4	0.8	0.4	0.5
Mean	0.48	0.77	0.25	0.20	0.47	0.58	0.53	0.45
Grand mean	0.63		0.23		0.53		0.49	

RESULTS AND DISCUSSION

Results from 12 collaborators were received for the four sample pairs. Seven collaborators used the Eureka-Niagara sample grader,

TABLE IX
Assortment (%) for a 2.8-mm Screen for a Sortimat Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	69.5	64.1	94.9	94.2	76.8	76.1	67.9	69.8
2[a]	…	…	…	…	…	…	…	…
3	67.0	65.4	94.3	93.6	77.0	77.1	66.2	65.7
4	65.3	57.3	92.8	92.1	75.2	74.0	64.8	65.7
5	63.4	62.5	93.6	91.5	73.6	74.8	66.3	64.5
6	68.3	67.5	93.8	92.1	73.5	76.3	66.3	67.0
7	61.1	58.7	91.1	89.0	70.1	70.8	58.3[a]	58.1[a]
Mean	65.76	62.58	93.42	92.08	74.37	74.86	64.96	65.13
Grand mean	64.17		92.75		74.61		65.05	

[a] Outlier at $P \leq 0.05$ based on total and/or differences.

TABLE X
Assortment (%) for a 2.5-mm Screen for a Sortimat Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	23.5	26.1	3.3	4.1	17.1	17.4	23.6	23.1
2[a]	…	…	…	…	…	…	…	…
3	27.8	29.2	4.5	5.5	19.6	19.7	28.1	28.7
4	25.6	29.5	5.5	5.8	18.2	19.0	26.0	24.9
5	26.2	25.9	4.5	5.6	18.1	17.2	24.0	24.6
6	23.4	24.1	5.0	5.1	18.4	17.6	24.3	23.7
7	25.6	28.1	6.2	6.9	19.4	19.1	27.2	27.0
Mean	25.35	27.14	4.83	5.50	18.47	18.34	25.54	25.33
Grand mean	26.25		5.16		18.40		25.43	

[a] Outlier at $P \leq 0.05$ based on total and/or differences.

TABLE XI
Assortment (%) for a 2.2-mm Screen for a Sortimat Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	6.1	8.0	1.5	1.3	5.1	5.5	7.1	6.1
2[a]	…	…	…	…	…	…	…	…
3	4.4	4.7	1.0	0.9	3.0	3.1	5.2	5.1
4	7.4	10.6	1.2	1.7	5.2	5.5	7.7	8.1
5	8.8	9.3	1.5	2.2	6.8	6.7	8.3	9.1
6	6.9	7.1	1.0	1.6	6.3	4.9	8.2	7.9
7	11.5	11.3	2.2	3.3	8.7	8.3	12.2	12.7
Mean	7.52	8.50	1.40	1.83	5.85	5.67	8.11	8.16
Grand mean	8.01		1.62		5.76		8.13	

[a] Outlier at $P \leq 0.05$ based on total and/or differences.

TABLE XII
Assortment (%) for Sample Through a 2.2-mm Screen for a Sortimat Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	0.9	1.7	0.3	0.3	1.0	1.0	1.5	1.1
2[a]	…	…	…	…	…	…	…	…
3	0.8	0.7	0.2	0.1	0.4	0.1	0.5	0.5
4	1.7	2.6	0.5	0.4	1.4	1.5	1.5	1.3
5	1.6	2.3	0.4	0.7	1.5	1.3	1.4	1.8
6	1.5	1.4	0.3	0.4	1.8	1.2	1.3	1.5
7	2.3	2.0	0.7	0.9	1.8	1.5	2.5	2.3
Mean	1.47	1.79	0.40	0.47	1.32	1.11	1.44	1.42
Grand mean	1.63		0.43		1.21		1.43	

[a] Outlier at $P \leq 0.05$ based on total and/or differences.

and six used the Sortimat sample grader with imperial screens. Seven collaborators used the Sortimat sample grader with metric screens. Results from one collaborator were excluded prior to statistical analysis because of known deviations from the prescribed experimental protocol. Each screen from each piece of equipment was treated as a separate test condition. Results from the Eureka-Niagara sample grader are presented in Tables I–IV. Results from the Sortimat sample grader with imperial screens are presented in Tables V–VIII. Results from the Sortimat sample grader with metric screens are presented in Tables IX–XII. Outliers were determined using Dixon's ratio test (1).

Statistical summaries of the assortment data for the Eureka-Niagara sample grader, Sortimat sample grader with imperial screens, and Sortimat sample grader with metric screens are presented in Tables XIII–XV, respectively. Repeatability and reproducibility coefficients of variation for assortment using a Eureka-Niagara sample grader with imperial screens ranged from 0.66 to 43.76% and 1.50 to 36.95%, respectively, and were judged acceptable. Repeatability and reproducibility coefficients of variation for assortment using the Sortimat sample grader with imperial screens ranged from 1.31 to 65.17% and 1.70 to 65.17%, respectively, and were judged acceptable. Each screen was looked at individually to determine repeatability and reproducibility coefficients of variation:

- Repeatability and reproducibility coefficients of variation for the Sortimat sample grader with $^{7}/_{64}$-in. screen ranged from 1.31 to 2.14% and 1.70 to 5.26%, respectively, and were judged acceptable. Repeatability and reproducibility coefficients of variation for assortment using the Sortimat sample grader with 2.8-mm screen ranged from 0.54 to 3.20% and 1.72 to 5.62%, respectively, and were judged acceptable. In comparison, the repeatability and reproducibility coefficients of variation for the Eureka-Niagara sample grader ranged from 0.66 to 2.85% and 1.50 to 4.83%, respectively.
- Repeatability and reproducibility coefficients of variation for the Sortimat sample grader with $^{6}/_{64}$-in. screen ranged from 3.57 to 31.45% and 8.12 to 25.90%, respectively, and were judged acceptable. Repeatability and reproducibility coefficients of variation for assortment using the Sortimat sample grader with 2.5-mm screen ranged from 1.91 to 5.47% and 5.37 to 18.49%, respectively, and were judged acceptable. In comparison, the repeatability and reproducibility coefficients of variation for the Eureka-Niagara sample grader ranged from 2.99 to 17.57% and 9.92 to 33.96%, respectively.
- Repeatability and reproducibility coefficients of variation for the Sortimat sample grader with $^{5}/_{64}$-in. screen ranged from 9.08 to 27.45% and 20.28 to 26.62%, respectively, and were judged acceptable. Repeatability and reproducibility coefficients of variation for assortment using the Sortimat sample grader with 2.2-mm screen ranged from 5.69 to 21.72% and 30.37 to 41.65%, respectively, and were judged acceptable. In comparison, the repeatability and reproducibility coefficients

TABLE XIV
Statistical Summary of Results for Imperial Sortimat Screens[a]

Screen Sample Pair	No. of Labs	Grand Mean	Repeatability			Reproducibility		
			S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
$^{7}/_{64}$ in.								
A/B	6	67.73	1.4	2.08	3.94	3.56	5.3	9.98
C/D	6	93.17	2.0	2.11	5.49	1.58	1.7	4.43
E/F	6	77.93	1.0	1.31	2.86	2.74	3.5	7.67
G/H	6	69.03	1.5	2.14	4.14	3.00	4.3	8.39
$^{6}/_{64}$ in.								
A/B	6	27.06	1.0	3.57	2.71	2.64	9.8	7.40
C/D	6	5.70	1.8	31.45	5.02	1.48	25.9	4.13
E/F	6	18.18	0.9	4.97	2.53	1.80	9.9	5.03
G/H	6	25.94	1.0	3.80	2.76	2.11	8.1	5.90
$^{5}/_{64}$ in.								
A/B	6	4.61	0.7	14.98	1.93	1.07	23.2	3.00
C/D	6	0.94	0.3	27.45	0.72	0.25	26.2	0.69
E/F	6	3.39	0.3	9.08	0.86	0.90	26.6	2.53
G/H	6	4.59	0.5	10.05	1.29	0.93	20.3	2.61
Through $^{5}/_{64}$ in.								
A/B	6	0.63	0.1	21.96	0.38	0.14	22.7	0.40
C/D	6	0.23	0.2	65.17	0.41	0.15	65.2	0.41
E/F	6	0.53	0.2	34.50	0.51	0.16	29.6	0.44
G/H	6	0.45	0.1	19.03	0.24	0.13	28.7	0.36

[a] Calculations based on Tables V–VIII.

TABLE XIII
Statistical Summary of Results for a Eureka-Niagara Sample Grader[a]

Screen Sample Pair	No. of Labs	Grand Mean	Repeatability			Reproducibility		
			S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
$^{7}/_{64}$ in.								
A/B	7	63.27	1.80	2.9	5.04	3.06	4.8	8.55
C/D	7	93.68	0.62	0.7	1.73	1.40	1.5	3.92
E/F	7	73.64	1.54	2.1	4.31	2.10	2.9	5.88
G/H	7	64.51	0.94	1.5	2.64	2.55	4.0	7.15
$^{6}/_{64}$ in.								
A/B	7	30.61	0.99	3.2	2.77	3.04	9.9	8.51
C/D	7	5.01	0.88	17.6	2.46	1.70	4.0	4.76
E/F	7	21.62	1.16	5.4	3.25	2.71	12.5	7.59
G/H	7	39.36	0.88	3.0	2.45	3.28	11.2	9.18
$^{5}/_{64}$ in.								
A/B	7	5.34	0.94	17.5	2.62	1.68	31.4	4.70
C/D	7	1.08	0.28	25.5	0.77	0.39	36.3	1.10
E/F	7	4.11	0.39	9.4	1.08	0.97	23.5	2.71
G/H	7	5.54	0.43	7.7	1.19	1.18	21.3	3.31
Through $^{5}/_{64}$ in.								
A/B	7	0.77	0.14	18.1	0.39	0.14	18.6	0.40
C/D	7	0.23	0.07	29.4	0.19	0.06	27.0	0.17
E/F	7	0.54	0.24	43.8	0.67	0.20	37.0	0.56
G/H	7	0.58	0.12	20.5	0.33	0.18	31.7	0.51

[a] Calculations based on Tables I–IV.

TABLE XV
Statistical Summary of Results for a Metric Sortimat Sample Grader[a]

Screen Sample Pair	No. of Labs	Grand Mean	Repeatability			Reproducibility		
			S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
2.8 mm								
A/B	6	64.17	2.06	3.2	5.76	3.56	5.5	9.96
C/D	6	92.75	0.50	0.5	1.41	1.60	1.7	4.48
E/F	6	74.61	1.02	1.4	2.85	2.43	3.3	6.80
G/H	6	65.05	0.91	1.4	2.55	3.66	5.6	10.24
2.5 mm								
A/B	6	26.25	1.07	4.1	2.99	1.92	7.3	5.38
C/D	6	5.16	0.28	5.5	0.79	0.96	18.5	2.67
E/F	6	18.40	0.46	2.5	1.30	0.99	5.4	2.77
G/H	6	25.43	0.49	1.9	1.36	1.99	7.8	5.58
2.2 mm								
A/B	6	8.01	0.92	11.5	2.58	2.43	30.4	6.81
C/D	6	1.62	0.35	21.7	0.98	0.67	41.7	1.89
E/F	6	5.76	0.47	8.1	1.31	1.83	31.8	5.13
G/H	6	8.13	0.43	5.7	1.30	2.48	30.5	6.95
Through 2.2 mm								
A/B	6	1.63	0.38	23.2	1.06	0.62	38.1	1.73
C/D	6	0.43	0.12	26.7	0.32	0.24	55.3	0.67
E/F	6	1.21	0.17	14.1	0.48	0.53	44.0	1.49
G/H	6	1.43	0.22	15.3	0.62	0.63	43.9	1.76

[a] Calculations based on Tables IX–XII.

TABLE XVI
Comparison of 7/64-in. and 2.8-mm Screens for Eureka-Niagara and Sortimat Graders with Metric Screens and a Sortimat Grader with Imperial Screens for the Determination of Assortment in Malt Using the *t* Test, Assuming Unequal Variances[a]

Statistical Parameter	Imperial Screens	Metric Screens
Number of sample pairs (*N*)	48	48
Mean of differences (*D*)	–3.7	–0.92
Standard error of differences (SD)	3.43	3.59
Calculated *t*	–1.47[b]	–0.23[b]
$t_{0.05}$	1.98	1.98

[a] Calculations based on Youden unit block design analysis (1).
[b] Not significant at the 95% confidence level.

TABLE XVII
Comparison of 6/64-in. and 2.5-mm Screens for Eureka-Niagara and Sortimat Graders with Metric Screens and a Sortimat Grader with Imperial Screens for the Determination of Assortment in Malt Using the *t* Test, Assuming Unequal Variances[a]

Statistical Parameter	Imperial Screens	Metric Screens
Number of sample pairs (*N*)	48	48
Mean of differences (*D*)	2.99	3.39
Standard error of differences (SD)	2.84	3.85
Calculated *t*	1.27[b]	1.50[b]
$t_{0.05}$	1.98	1.98

[a] Calculations based on Youden unit block design analysis (1).
[b] Not significant at the 95% confidence level.

TABLE XVIII
Comparison of 5/64-in. and 2.2-mm Screens for Eureka-Niagara and Sortimat Graders with Metric Screens and a Sortimat Grader with Imperial Screens for the Determination of Assortment in Malt Using the *t* Test, Assuming Unequal Variances[a]

Statistical Parameter	Imperial Screens	Metric Screens
Number of sample pairs (*N*)	48	48
Mean of differences (*D*)	0.62	1.88
Standard error of differences (SD)	1.75	2.22
Calculated *t*	1.69[b]	–3.40[c]
$t_{0.05}$	1.98	1.99

[a] Calculations based on Youden unit block design analysis (1).
[b] Not significant at the 95% confidence level.
[c] Significant at the 95% confidence level.

TABLE XIX
Comparison of Sample Through 5/64-in. and 2.2-mm Screens for Eureka-Niagara and Sortimat Graders with Metric Screens and a Sortimat Grader with Imperial Screens for the Determination of Assortment in Malt Using the *t* Test, Assuming Unequal Variances[a]

Statistical Parameter	Imperial Screens	Metric Screens
Number of sample pairs (*N*)	48	48
Mean of differences (*D*)	0.09	–0.61
Standard error of differences (SD)	0.22	0.62
Calculated *t*	–6.00[b]	1.61[b]
$t_{0.05}$	2.00	1.98

[a] Calculations based on Youden unit block design analysis (1).
[b] Significant at the 95% confidence level.

of variation for the Eureka-Niagara sample grader ranged from 7.67 to 25.51% and 21.34 to 36.33%, respectively.

- Repeatability and reproducibility coefficients of variation for the Sortimat sample grader with sample through a 5/64-in. screen ranged from 19.03 to 65.17% and 22.72 to 65.17%, respectively, and were judged acceptable. Repeatability and reproducibility coefficients of variation for assortment using the Sortimat sample grader with sample through a 2.2-mm screen ranged from 14.14 to 26.65% and 38.05 to 55.26%, respectively, and were judged acceptable. In comparison, the repeatability and reproducibility coefficients of variation for the Eureka-Niagara sample grader ranged from 18.11 to 43.76% and 18.55 to 36.95%, respectively.

Only two samples were used to establish the reproducibility coefficients of variation in the 1955 and 1956 studies. This led to difficulty in comparing them to this study. When comparing the results from this subcommittee with those of the previous subcommittees (3,4), repeatability coefficients of variation were not established. The samples used in the previous studies were also not consistent with what is used today in terms of sizing. The coefficient of variation for the 7/64-in. screen ranged from 11.3 to 48.2%, which is much higher than what was observed in the current study. The 6/64-in. screen ranged from 8.3 to 9.8%, the 5/64-in. screen ranged from 19.2 to 20.3%, and the sample through a 5/64-in. screen ranged from 23.0 to 60.0%.

One difficulty with the Sortimat sample grader is the 7/64-in. (or 2.8-mm) receptor cup size. It was difficult to measure the 7/64-in. screen contents accurately for very large values without the use of a double cup. A double cup is an additional chamber inserted in the 7/64-in cup to catch additional grain. If a double cup is not used, loss of malt occurs when the tray is pulled out, leading to variation in results.

The results of the *t* test, assuming unequal variances, comparing the Eureka-Niagara and Sortimat sample graders with imperial and metric screens are presented in Tables XVI–XIX. The reproducibility precision *F* test showed no significant difference between the Eureka-Niagara and Sortimat sample graders with imperial screens or the Eureka-Niagara and Sortimat sample graders with metric screens at the 95% confidence level.

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International Bitterness Unit Analysis in Wort by Segmented Flow Analyzer (International Method)

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Keywords: Automated IBU, BU, Iso-α-acids, SFA

CONCLUSIONS

1. Repeatability coefficients of variation for the determination of international bitterness units (IBU) in wort by segmented flow analyzer ranged from 1.9 to 5.4% and were judged acceptable.
2. Reproducibility coefficients of variation for the determination of IBU in wort by segmented flow analyzer ranged from 5.7 to 7.4% and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends that the method for international bitterness unit analysis in wort by segmented flow analyzer be adopted for inclusion in the ASBC *Methods of Analysis.*
2. Discharge the subcommittee.

This is the first year of the subcommittee's existence. Based on membership polling, the ASBC Technical Committee recommended formation of this subcommittee to evaluate IBU analysis in wort by segmented flow analyzer to update the ASBC *Methods of Analysis* (1). Segmented flow analysis (SFA) is currently used to measure beer bitterness, as described in ASBC Method Beer-23D (1).

PROCEDURE

Collaborators received three wort sample pairs for IBU analysis using the SFA method. Wort samples covered a wide range of bitterness levels. Sample pairs were similar but distinct. Results were evaluated using the Youden unit block design (1).

Collaborators were asked to follow the segmented flow analyzer manufacturer's recommended procedures for instrument operation. The three sample pairs were analyzed for bitterness, and results were reported for each sample along with all pertinent calibration information and notes. A beer with an assigned IBU value of 32.3 was analyzed using ASBC Method Beer-23A (1), diluted, and used to create the calibration curve to which the three wort sample pair results were compared.

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RESULTS AND DISCUSSION

IBU results from 16 collaborators were received for the three sample pairs and are presented in Table I. Outliers were determined using Dixon's ratio test (1). IBU results not used in the statistical calculations were removed due to sample dilution error, possible contamination, or storage issues.

A statistical summary of the analysis of IBU in wort by segmented flow analyzer is shown in Table II. Repeatability and reproducibility coefficients of variation for the determination of IBU in wort by segmented flow analyzer ranged from 1.9 to 5.4% and 5.7 to 7.4%, respectively, and were judged acceptable.

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*. Beer-23A Beer bitterness: Bitterness units (International Method), -23D Beer bitterness: Bitterness units by automated flow analysis; Statistical Analysis-4 Youden unit block collaborative testing procedure, -5 Comparison of test methods. The Society, St. Paul, MN, 2009.

TABLE I
International Bitterness Unit Analysis in Wort by Segmented Flow Analyzer

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	A1	A2	B1	B2	C1	C2
1	23.7	27.2	54.6	56.7	87.6	95.2
2	24.2	26.6	52.0	54.1	81.1	80.5
3	20.9	27.5	56.3	56.1	96.7	96.9
4	24.3	25.3	44.6[a]	54.1[a]	69.9[a]	80.4[a]
5	23.9	28.1	58.9	61.9	97.2	100.6
6	24.3	28.0	60.9	62.9	102.0	102.6
7	24.2	25.9	51.3	55.7	91.4	90.5
8	24.0	25.5	51.0	53.7	79.6	79.3
9	24.0	24.0	53.1	51.6	85.5	85.8
10	21.4	23.7	51.3	52.6	84.6	87.2
11	23.7	24.5	53.3	53.7	88.5	88.1
12	21.7	21.8	53.7	55.7	84.6	88.1
13	23.1	24.5	47.4[a]	48.6[a]	67.8[a]	65.7[a]
14	25.8	25.2	51.5	53.8	86.6	89.2
15	23.5	24.9	52.5	55.8	86.5	88.5
16	17.5[b]	17.7[b]	54.2	58.8	84.3	88.1
Mean[c]	23.49	25.50	53.90	55.94	88.32	90.04
Grand mean[c]	24.50		54.92		89.18	

[a] Result removed due to dilution error.
[b] Result removed due to contamination or storage error.
[c] Calculated excluding outliers.

TABLE II
Statistical Summary of Results[a]

Sample Pair	No. of Labs	Grand Mean	Repeatability			Reproducibility		
			S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
A1/A2	15	24.50	1.32	5.39	3.70	1.53	6.26	4.29
B1/B2	14	54.92	1.17	2.13	3.27	3.13	5.69	8.75
C1/C2	14	89.18	1.67	1.87	4.66	6.57	7.36	18.38

[a] Calculations based on Table I.

Determination of International Bitterness Units in Wort Using the Spectrophotometric Method

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Keywords: BU, IBU

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the determination of international bitterness unit (IBU) levels in wort using the spectrophotometric method ranged from 1.5 to 4.5% and 4.3 to 10.0%, respectively, and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends that the method for International Bitterness Unit Analysis in Wort by Spectrophotometer be adopted for inclusion in the ASBC *Methods of Analysis*.
2. Discharge the subcommittee.

This was the first year of the subcommittee's evaluation of the spectrophotometric method for analyzing IBU levels in wort. The existing European Brewing Congress (EBC) Method 8.8 Bitterness of wort (2) was used as the reference method.

PROCEDURE

Collaborators received three wort sample pairs for IBU analysis using the spectrophotometric method. Wort samples covered a wide range of bitterness levels. Sample pairs were similar but distinct. Results were evaluated using the Youden unit block design (1).

The samples consisted of two low hopped worts (A/B), two mid-range hopped worts (B/C), and two high hopped worts (E/F). Participants were asked to follow the method as closely as possible and document any major adjustments or modifications.

RESULTS AND DISCUSSION

Results for analysis of IBU in wort using the spectrophotometric method were received from 11 collaborators for sample pairs A/B, C/D, and E/F (Table I). Outliers were identified using Dixon's ratio test, as described in the Youden unit block procedure (1). Upon investigation of the two outliers in the E/F pair set, it was determined that the collaborators did not follow the recommended procedure with regard to the timing of the iso-octane spectrophotometric reading and the vigor of agitation on the shaker table used.

Test results for the two outliers identified in pair set E/F pairs were excluded from data analyses in Tables I and II. A statistical summary of the wort IBU spectrophotometric data is presented in Table II. Repeatability and reproducibility coefficients of variation for the determination of IBU levels in wort using the spectrophotometric method ranged from 1.5 to 4.6% and 4.2 to 10.0%, respectively, and were judged acceptable.

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*. Beer-23A Beer bitterness: Bitterness units (International Method), -23D Beer bitterness: Bitterness units by automated flow analysis; Statistical Analysis-4 Youden unit block collaborative testing procedure, -5 Comparison of test methods. The Society, St. Paul, MN, 2009.
2. European Brewing Congress. *Analytica-EBC*. Section 8 Wort, Method 8.8 Bitterness of wort. Fachverlag Hans Carl, Nürnberg, Germany, 1998.

TABLE I
Analysis of International Bitterness Units in Wort Using the Spectrophotometric Method

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F
1	21.0	24.0	49.5	53.0	79.5	83.0
2	27.0	27.5	59.5	63.5	95.0[a]	101.0[a]
3	23.0	26.0	52.5	54.5	81.0	87.0
4	21.5	21.5	41.0	45.5	72.0	82.0
5	27.0	26.5	53.0	57.0	85.0	89.0
6	26.5	25.5	52.0	57.5	87.5	90.0
7	23.5	24.0	51.0	55.0	83.5	86.5
8	23.5	25.5	52.0	54.5	82.5	85.0
9	24.5	25.5	51.5	54.0	83.0	88.0
10	24.0	25.5	51.0	54.0	82.0	86.5
11	22.0	26.0	42.0	44.0	73.5[a]	67.5[a]
Mean[b]	23.96	25.23	50.46	53.86	81.78	86.33
Grand mean[b]	24.59		52.16		84.06	

[a] Outlier at $P \leq 0.05$ based on totals and differences.
[b] Calculated excluding outliers.

TABLE II
Statistical Summary of Results[a]

Sample Pair	No. of Labs	Grand Mean	Repeatability			Reproducibility		
			S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
A/B	11	24.59	1.12	4.56	3.14	1.88	7.63	5.25
C/D	11	52.16	0.79	1.51	14.64	5.23	10.02	14.64
E/F	9	84.06	1.66	1.98	4.66	3.58	4.26	10.02

[a] Calculations based on Table I.

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Determination of β-Glucan in Wort by Segmented Flow Analysis

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Keywords: Calcofluor, Fluorescence, SFA

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for determination of β-glucan in wort by segmented flow analysis (SFA) ranged from 3.3 to 6.6% and 15.5 to 21.5%, respectively, and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends that the method for the determination of β-glucan in wort by SFA be included in the ASBC *Methods of Analysis* (1).
2. Discharge the subcommittee.

This is the subcommittee's first year of existence. The subcommittee was formed on the recommendation of the subcommittee for *Methods of Analysis* Wort Review (8). In recent years, use of SFA has become increasingly common for the determination of β-glucan in wort. However, check service results continue to show poor reproducibility between laboratories, possibly due to deviations from the official method with respect to Calcofluor concentration, buffer type, and pH.

PROCEDURE

A total of eight malt samples representing four sample pairs (similar but distinct) with a range of β-glucan levels were sent to each collaborator. For each sample, each collaborator prepared a Congress wort according ASBC Method Malt-4 and measured β-glucan using segmented flow instrumentation. Collaborators were asked to complete a survey detailing the exact conditions of their protocol if it deviated from the prescribed method. Results were evaluated using the Youden unit block design (1).

RESULTS AND DISCUSSION

Fifteen collaborators submitted results for SFA for all four sample pairs: A/B, C/D, E/F, and G/H. Participating laboratories were instructed to note any deviations from the prescribed method. Considerable variation was found in the application of the official method (Wort-18) augmented to perform SFA. Details of the methods used by the individual laboratories are provided in Table I. Results for determination of β-glucan in wort by SFA are presented in Table II.

A statistical summary of the data for SFA is presented in Table III. Repeatability and reproducibility coefficients of variation for determination of β-glucan in wort ranged from 3.3 to 6.6% and 15.5 to 21.5%, respectively. Previous collaborative studies on flow injection methods for β-glucan determination showed slightly higher coefficients of variation for reproducibility ranging from 17 to 27% (7).

Seven of the fifteen collaborators followed the method, using the same buffer solution for preparation of the Calcofluor reagent (Tri, pH 8.0); therefore, the statistics for these laboratories were examined separately. The reproducibility coefficients of variation for the seven laboratories using a common method were very similar to those for all fifteen laboratories (data not shown). This indicates that using common buffer did not improve the precision of the method and that there are other more significant sources of error contributing to variation between laboratories.

Since its adoption into the ASBC *Methods of Analysis*, the flow injection by fluorescence method for determination of β-glucan in wort has undergone seven collaborative studies (2–7). Each has attempted to achieve an improvement in reproducibility by standardizing either the method conditions or calibration procedures. Despite these attempts, the between-laboratory variation has remained high, perhaps demonstrating there are inherent limitations of the method.

TABLE I
Method Conditions Used by Participating Laboratories

Collaborators	Buffer	Calcofluor Concentration (ppm)	pH
7, 8	Glycine	35	9
1, 9	Glycine	40	9
4	Glycine	35	10
2, 3, 6, 11, 12, 14, 15	Tris	35	8
5	Tris	35	9
10, 13	Tris	35	10

TABLE II
β-Glucan in Wort (ppm) Determined by Segmented Flow Analysis

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	391	404	126	102	137	133	77	76
2	365	360	123	124	141	140	80	76
3	357	383	139	133	160	147	91	92
4	348	325	127	122	144	135	70	66
5	343	357	110	104	127	115	70	67
6	311	293	99	115	115	111	70	63
7	527	515	118	106	130	116	45	41
8	381	381	109	102	136	133	77	73
9	458	444	147	135	161	160	102	100
10	266	266	81	93	102	97	47	45
11	256	236	87	90	96	82	55	59
12	370	364	124	114	130	123	66	72
13	324	324	102	108	120	108	68	71
14	266	264	97	97	109	130	72	72
15	479	437	147	130	150	150	83	83
Mean	362.8	356.9	115.7	111.7	130.5	125.3	71.5	70.4
Grand mean	359.8		113.7		127.9		71.0	

TABLE III
Statistical Summary of Results[a]

Sample Pair	No. of Labs	Grand Mean	Repeatability			Reproducibility		
			S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
A/B	15	362.8	11.9	3.3	33.2	77.0	21.4	215.5
C/D	15	113.7	7.6	6.6	21.1	17.6	15.5	49.4
E/F	15	127.9	6.2	4.8	17.4	20.3	15.8	56.7
G/H	15	71.0	2.5	3.5	7.0	15.2	21.5	42.7

[a] All calculations were made based on Table I.

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It is important to note that the current ASBC and EBC standard methods for determination of β-glucan in wort by fluorescence differ with respect to their method conditions. Previous ruggedness testing (3) demonstrated that many factors, including buffer, excitation and emission wavelength, Calcofluor concentration, and glucan standard all have a significant effect on the measured result and must be considered when comparing analytical results from different labs. These conditions affect the range of β-glucan molecular weights effectively complexed by Calcofluor. However, few studies have attempted to determine the conditions that produce an analytical result that provides the most useful information to the brewer. This is perhaps an area for future research.

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Sensory Science Report

Committee Members: A. Fritsch, *Chair*; A. Benson; G. Conley; C. Haddock; J. Helber; T. Horner; I. McLaughlin; R. Mosher; L. Salazar; S. Thompson; W. Ye; and K. Zigich

FLAVOR WHEEL/FLAVOR TERMINOLOGY

The subcommittee has begun to discuss revamping the flavor wheel. The current approach is to provide a general flavor wheel that caters to novice tasters and can be used as a promotional item for ASBC. However, we have the objective of creating opportunities for more experienced tasters. This includes focused flavor wheels and a possible electronic search engine that will utilize keywords to indicate potential flavor profiles.

The next step is to meet for a one-day focused ideation session to determine the direction for the wheel/terminology. After this is accomplished, we will work to create a system for novice tasters, expert tasters, and sensory professionals.

DISCUSSION FORUM

During our meetings, we have begun to discuss and address issues that are particular to sensory and brewing. This has brought new issues to the forefront for possible contributions from ASBC, including

- Decision trees for sensory evaluation
- A shelf-life protocol

Anyone interested in joining this subcommittee should contact Annette Fritsch at annette.fritsch@bostonbeer.com or +1.617.368.5092.

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Determination of Alpha-Amylase in Malt by Segmented Flow Analysis Using Potassium Ferricyanide

Subcommittee Members: A. Budde, *Chair;* J. Barr; T. Chicos; K. Churchill; K. French; R. Joy; A. MacLeod; J. McCann; T. Mead-Shirley; J. Menert; M. Schmitt (EBC); A. Stern; and R. Jennings (*ex officio*)

Keywords: SFA

CONCLUSIONS

1. Repeatability coefficients of variation for the determination of α-amylase by segmented flow analysis (SFA) using potassium ferricyanide ranged from 1.5 to 4.6% and were judged acceptable.
2. Reproducibility coefficients of variation for the determination of α-amylase by SFA using potassium ferricyanide ranged from 46.1 to 51.2% and were judged unacceptable.

RECOMMENDATIONS

1. The subcommittee recommends that collaborative testing be repeated.
2. Send samples representing a wide range of enzyme concentrations to be determined using glucose as the calibration standard. A sample having a known amount of α-amylase should be used as an internal standard.
3. Engage collaborators using ASBC Method Malt-7C (1) for the reference method.

This was the second year of the subcommittee's evaluation of an alternative method for determining α-amylase activities in malt by SFA using potassium ferricyanide for detection. The subcommittee was formed based on the recommendation from the Subcommittee on *Methods of Analysis* Malt Review (5). Some members had expressed concern that the current approved method (Malt-7C) for automated flow analysis (1), which uses β-limit dextrin and iodine for detection, might not have the dynamic range to accurately cover a broader spectrum of malts with very high or very low levels of α-amylase activity.

In the first year of study, determination of α-amylase by SFA using potassium ferricyanide produced acceptable repeatability coefficients of variation and unacceptable reproducibility coefficients of variation compared with ASBC Methods Malt-7A and -7B using glucose as the standard (1,4). The subcommittee recommended that the collaborative testing be repeated with a focus on Malt-7C as a comparison using the Megazyme malt amylase standard E-MAST (1).

Previous attempts to establish α-amylase activity by SFA experienced high reproducibility coefficients of variation (3). Since most analytical labs were performing SFA and the number of labs routinely using the standard reference method was decreasing, an effort was made to include only collaborators who use the most prevalent methodology (SFA using iodine for detection). Satisfactory results from this collaborative study resulted in approval of ASBC Method Malt-7C as a method for determination of α-amylase activity (2). SFA using potassium ferricyanide to determine α-amylase activity may allow for more accurate assessment of very low or very high activity compared with Malt-7C, while yielding similar results in the 40–60 dextrinizing units (DU) range.

PROCEDURE

A total of eight malt samples representing commercial varieties and experimental lines malted on separate days were ground on a Buhler DFLU disc mill according to ASBC Method Malt-4 (1) for fine grind, vacuum packed, and sent to each collaborator. The samples were similar but distinct. The eight malt samples consisted of four sample pairs (A/B through G/H) that spanned a wide range of α-amylase activities. The method was calibrated using the Megazyme malt amylase standard E-MAST as described in Malt-7C. Results were evaluated using the Youden unit block design (1).

TABLE I
α-Amylase Activity at 20° DU (as is) Determined by Segmented Flow Analysis Using Potassium Ferricyanide for Detection

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	42.0	42.0	72.9	73.6	65.1	65.6	56.6	56.1
2	61.0	64.0	125.3	115.6	95.5	96.7	74.6	73.4
3	76.2	77.4	150.1	138.9	110.2	108.0	82.0	79.6
4	51.9	56.2	116.3	105.0	83.4	85.4	67.5	65.9
5	123.9	123.4	236.5	215.8	177.4	179.2	136.9	137.6
6	29.6	32.7	76.5	69.1	56.4	56.7	42.2	42.2
7	43.0	37.0	59.0	60.0	56.0	47.0	40.0	42.0
Mean	61.10	61.83	119.51	111.13	92.00	91.24	71.39	70.97
Grand mean	61.46		115.32		91.62		71.18	

TABLE II
α-Amylase Activity at 20° DU (as is) Determined by ASBC Method Malt-7C

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H
1	45.6	48.3	95.6	85.2	73.2	74.6	58.0	60.1
2	37.3	38.3	72.4	70.8	60.8	58.1	49.1	50.6
3	34.0	34.4	62.6	61.2	50.9	51.0	43.4	44.2
4	31.6	31.4	84.6	76.8	60.7	54.5	42.1	44.4
5	45.6	49.9	93.1	88.0	77.4	73.5	61.9	65.8
6	47.6	47.5	81.5	85.2	78.9	73.5	63.5	62.8
Mean	40.28	41.63	81.64	77.85	66.98	64.20	53.01	54.65
Grand mean	40.96		79.75		65.59		53.83	

TABLE III
Statistical Summary of Results[a]

Method Sample Pair	No. of Labs	Grand Mean	Repeatability			Reproducibility		
			S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Potassium ferricyanide								
A/B	7	61.46	2.43	4.0	6.81	31.45	51.2	88.06
C/D	7	115.33	5.33	4.6	14.94	57.68	50.1	161.50
E/F	7	91.62	2.75	3.0	7.70	43.67	47.7	122.28
G/H	7	71.18	1.05	1.5	2.94	32.81	46.1	91.87
Malt-7C								
A/B	6	40.96	1.27	3.1	3.55	7.41	18.1	20.75
C/D	6	79.75	3.58	4.5	10.02	11.50	14.4	32.19
E/F	6	65.59	2.14	3.3	5.98	11.01	16.9	30.83
G/H	6	53.83	1.09	2.0	3.06	9.44	17.5	26.44

[a] All calculations were based on Tables I and II.

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TABLE IV
α-Amylase Activity at 20° DU (as is) Determined by Segmented Flow Analysis Using Potassium Ferricyanide for Detection[a]

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E[b]	F	G	H
1	43.2	43.2	75.0	75.7	67.0	67.5	58.2	57.7
2	42.8	44.9	87.9	81.1	67.0	67.8	52.3	51.5
3	46.3	47.1	91.2	84.4	67.0	65.7	49.8	48.4
4	41.7	45.1	93.4	84.3	67.0	68.6	54.2	52.9
5	41.7	45.1	93.4	84.3	67.0	68.6	54.2	52.9
6	35.2	38.8	90.9	82.1	67.0	67.3	50.1	50.1
7	51.4	44.3	70.6	71.8	67.0	56.2	47.8	50.2
Mean	43.91	44.29	85.46	80.11	67.00	65.83	52.03	51.83
Grand mean	44.10		82.78		66.41		51.93	

[a] Data from Table I normalized.
[b] Averaged sample value used to normalize data.

RESULTS AND DISCUSSION

Results from seven collaborators using SFA with potassium ferricyanide for detection were received for the four sample pairs and are presented in Table I. The sample pairs were also analyzed using method Malt-7C, and these results are presented in Table II. Potential outliers were identified using Dixon's ratio test (1). The statistical summary for the determination of α-amylase by potassium ferricyanide and iodine are presented in Table III. Repeatability coefficients of variation for the determination of α-amylase by SFA using potassium ferricyanide ranged from 1.5 to 4.6% and were judged acceptable. Reproducibility coefficients of variation for the determination of α-amylase by SFA using potassium ferricyanide ranged from 46.1 to 51.2% and were judged unacceptable. The repeatability and reproducibility coefficients of variation for the determination of α-amylase by Malt-7C ranged from 2.0 to 4.5% and 14.4 to 18.1%, respectively.

It appears that calibration using the Megazyme malt amylase standard E-MAST may have caused a considerable amount of the reproducibility error. The correlation values between labs ranged between 0.94 and 0.99 (data not shown), indicating a strong similarity in the analytical response to the samples, but the difference in the magnitude of the results between the collaborators contributed to the deterioration in reproducibility. This difference strongly suggests variation in calibration from one lab to another. The data from Tables I and II were normalized to the averaged value of sample E as determined by Malt-7C (activity at 20° DU = 66.98) and are presented in Tables IV and V, respectively. The normalized results were subjected to statistical analysis (1) and are reported in Table VI. The normalized data showed that the determination of α-amylase by potassium ferricyanide can be reproducible and accurate. Normalization of the data generated by Malt-7C analyses also improved the reproducibility between labs. The normalized grand means for the two methods of detection were very similar and yielded a correlation coefficient of 0.99, demonstrating that both methods produced nearly identical responses, with the exception of the calibration bias.

TABLE V
α-Amylase Activity at 20° DU (as is) Determined by ASBC Method Malt-7C[a]

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E[b]	F	G	H
1	41.7	44.2	87.5	78.0	67.0	68.3	53.1	55.0
2	41.1	42.2	79.8	78.0	67.0	64.0	54.1	55.8
3	44.8	45.3	82.5	80.5	67.0	67.2	57.2	58.1
4	34.9	34.6	93.4	84.7	67.0	60.1	46.5	49.0
5	39.5	43.2	80.6	76.2	67.0	63.6	53.6	56.9
6	40.4	40.3	69.2	72.3	67.0	62.4	53.9	53.3
Mean	40.38	41.64	82.13	78.27	67.00	64.26	53.05	54.69
Grand mean	41.01		80.20		65.62		53.87	

[a] Data from Table II normalized.
[b] Averaged sample value used to normalize data.

TABLE VI
Statistical Summary of Normalized Results[a]

Method	No. of	Grand	Repeatability			Reproducibility		
Sample Pair	Labs	Mean	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Potassium ferricyanide								
A/B	7	44.10	2.60	5.9	7.28	4.07	9.2	11.40
C/D	7	82.78	3.12	3.8	8.72	7.12	8.6	19.94
E/F	7	66.41	3.06	4.6	8.57	3.06	4.6	8.57
G/H	7	51.93	0.92	1.8	2.58	3.19	6.1	8.94
Malt-7C								
A/B	6	41.01	1.10	2.7	3.08	3.55	8.7	9.93
C/D	6	80.20	3.34	4.2	9.36	6.46	8.1	18.08
E/F	6	65.62	2.13	3.3	5.98	2.13	3.3	5.98
G/H	6	53.87	0.97	1.8	2.71	3.40	6.3	9.51

[a] All calculations were based on Tables IV and V.

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Headspace Gas Chromatography–Flame Ionization Detector Analysis for Major Beer Volatiles (International Method)

Subcommittee Members: J. Palausky, *Chair*; C. Brodie; M. Christopherson; V. Kellner (EBC); R. Ortiz; F. Sitjas (EBC); K. Taylor; and K. Lakenburges (*ex officio*)

Keywords: Alcohols, Aldehydes, Esters, FID, GC

CONCLUSIONS

1. Repeatability coefficients of variation for the determination of acetaldehyde by headspace gas chromatography–flame ionization detector (GC-FID) analysis ranged from 2.9 to 7.2% and were judged acceptable. Reproducibility coefficients of variation for the determination of acetaldehyde by headspace GC-FID analysis ranged from 51 to 57% and were judged unacceptable.
2. Repeatability coefficients of variation for the determination of ethyl acetate by headspace GC-FID analysis ranged from 2.1 to 3.4% and were judged acceptable. Reproducibility coefficients of variation for the determination of ethyl acetate by headspace GC-FID analysis ranged from 10 to 12% and were judged acceptable.
3. Repeatability coefficients of variation for the determination of isoamyl acetate by headspace GC-FID analysis ranged from 3.9 to 6.1% and were judged acceptable. Reproducibility coefficients of variation for the determination of isoamyl acetate by headspace GC-FID analysis ranged from 17 to 24% and were judged unacceptable.
4. Repeatability coefficients of variation for the determination of isoamyl alcohol by headspace GC-FID analysis ranged from 2.2 to 2.4% and were judged acceptable. Reproducibility coefficients of variation for the determination of isoamyl alcohol by headspace GC-FID analysis ranged from 7.4 to 25% and were judged unacceptable.

RECOMMENDATIONS

1. The current data suggest good intralaboratory precision but poor interlaboratory precision. The difference in the precision of results was most likely attributable to the preparation of calibration standards. The subcommittee recommends that future collaborative analysis be performed after re-examination of the standard preparation section of the protocol.
2. Due to the unacceptable statistical data, the subcommittee recommends that the collaborative study be repeated in 2011–2012 with a focus on calibration and standard preparation.

This was the second year of this subcommittee's existence. Based on polling by the Subcommittee on the Coordination of New and Alternative Methods (2), this subcommittee was formed to evaluate the applicability of headspace GC-FID analysis for the determination of a select set of volatile organic compounds in beer. In the first year, collaborative analysis showed unacceptable statistical data for three of four compounds tested (3). Following ruggedness analysis, minor modifications were made to the sample preparation portion of the collaborative protocol. Changes included specifying a pipette type and establishing an equilibration time and beer temperature prior to sample aliquoting.

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PROCEDURE

Three sample pairs of commercial beers were sent to each collaborator. Each pair was of the same brand but from different production times. All sample pairs were commercially available light beers selected to cover a broad range of volatile concentrations. Calibration was accomplished by standard additions of volatiles, with 1-butanol as an internal standard. Results were evaluated using the Youden unit block design (1).

RESULTS AND DISCUSSION

Results from seven collaborators were received for the three sample pairs. Results for one collaborator were excluded prior to statistical analysis because of known deviations from the prescribed experimental protocol. Data for acetaldehyde, ethyl acetate, isoamyl acetate, and isoamyl alcohol are presented in Tables I–IV, respectively. Outliers were identified using Dixon's ratio test (1). A statistical summary of the volatile data is shown in Table V.

The repeatability coefficients of variation were judged acceptable for all compounds tested. The reproducibility coefficients of variation were judged unacceptable for three of four compounds tested. The reproducibility coefficients of variation for ethyl acetate ranged from 10 to 12% and were judged acceptable. The reproducibility coefficients of variation for acetaldehyde, isoamyl acetate, and isoamyl alcohol ranged from 51 to 57%, 17 to 24%, and 7.4 to 25%, respectively, and were judged unacceptable.

TABLE I
Determination of Acetaldehyde (mg/L) in Beer by Headspace Gas Chromatography–Flame Ionization Detector Analysis

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F
1	0.81	0.72	1.31	1.22	2.37	2.85
2	1.13	1.32	2.32	2.09	3.41	3.97
3	1.09	1.02	1.47	1.52	2.53	2.63
4	2.81	2.87	4.91	4.92	7.34	8.70
5	1.90	1.84	2.93	2.90	5.06	6.00
6	1.02	1.05	1.64	1.54	2.62	3.28
Mean	1.459	1.470	2.430	2.366	3.888	4.571
Grand mean	1.465		2.398		4.230	

TABLE II
Determination of Ethyl Acetate (mg/L) in Beer by Headspace Gas Chromatography–Flame Ionization Detector Analysis

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F
1	17.2	16.4	20.1	21.4	25.9	23.7
2	13.3	12.4	16.3	17.8	20.4	18.7
3	13.9	13.9	17.5	17.2	21.6	19.7
4	15.9	16.5	20.7	21.7	24.7	22.7
5	16.2	16.1	19.7	20.9	24.9	22.7
6	13.4	13.3	16.3	15.7	20.5	20.0
Mean	14.98	14.76	18.42	19.11	23.68	21.92
Grand mean	14.87		18.76		22.80	

TABLE III
Determination of Isoamyl Acetate (mg/L) in Beer by Headspace Gas Chromatography–Flame Ionization Detector Analysis

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
1	1.31	1.24	1.34	1.33	1.76	1.57
2	1.44	1.60	1.83	2.08	3.15	2.49
3	1.52	1.56	1.93	2.04	3.66	3.00
4	1.24	1.25	1.77	1.83	2.80	2.27
5	1.46	1.41	1.83	1.95	3.33	2.61
6	1.01	0.93	1.17	1.17	2.19	1.91
Mean	1.330	1.332	1.645	1.733	2.815	2.307
Grand mean	1.331		1.689		2.561	

TABLE IV
Determination of Isoamyl Alcohol (mg/L) in Beer by Headspace Gas Chromatography–Flame Ionization Detector Analysis

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
1	77.0	72.5	93.0[a]	101[a]	172[a]	136[a]
2	42.6	42.3	43.3	44.9	55.5	48.6
3	45.5	44.3	51.4	50.2	61.6	56.5
4	51.5[a]	34.3[a]	50.1	52.7	64.9	57.2
5	51.5	50.6	53.4	53.7	70.8	62.6
6	47.2	47.0	48.7	48.4	63.3	59.5
Mean[b]	52.76	51.33	49.37	49.99	63.23	56.88
Grand mean[b]	52.05		49.68		60.05	

[a] Outlier at $P \leq 0.05$ based on totals and/or differences (1).
[b] Calculated excluding outliers.

The current data showed good repeatability but poor reproducibility. The method included analysis of highly volatile compounds that presented challenges for preparation of calibration standards. The poor reproducibility may be due to chemical purity, storage conditions, or the age of the standard used for calibration.

The subcommittee recommends that future collaborative analysis be conducted after re-examination of the standard preparation section of the protocol. Alternate ideas include providing reference materials (either the neat chemicals used to produce calibration standards, per the existing protocol, or a concentrated mix) and changing the standard preparation to use more easily measured volumes and techniques common to the majority of collaborators.

TABLE V
Statistical Summary of Results[a]

			Repeatability			Reproducibility		
Compound Sample Pair	**No. of Labs**	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Acetaldehyde								
A/B	6	1.465	0.075	5.1	0.210	0.77	53	2.16
C/D	6	2.398	0.070	2.9	0.197	1.37	57	3.84
E/F	6	4.230	0.303	7.2	0.848	2.17	51	6.08
Ethyl acetate								
A/B	6	14.87	0.40	2.7	1.13	1.74	12	4.86
C/D	6	18.76	0.64	3.4	1.78	2.27	12	6.37
E/F	6	22.11	0.45	2.1	1.27	2.25	10	6.30
Isoamyl acetate								
A/B	6	1.331	0.064	4.8	0.179	0.220	17	0.616
C/D	6	1.689	0.066	3.9	0.185	0.352	21	0.985
E/F	6	2.561	0.157	6.1	0.439	0.626	24	1.750
Isoamyl alcohol								
A/B	5	52.05	1.27	2.4	3.57	13.1	25	36.7
C/D	5	49.68	1.07	2.2	3.01	3.67	7.4	10.3
E/F	5	60.05	1.31	2.2	3.65	5.35	8.9	15.0

[a] All calculations were made based on Tables I–IV.

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Coordination of New and Alternate Methods of Analysis

Subcommittee Members: K. Lakenburges, *Chair*; C. Benedict; S. Brendecke; J. Cornell; M. Eurich; R. Foster; A. Fritsch; R. Jennings; A. Porter; C. Powell; and D. Sedin (*ex officio*)
Associate Members: J. Angres; R. Duffy-Krywicki; J. Masschelin (TTB); and T. Nielsen
Corresponding Members: K. Harayama (BCOJ); and E. Welten (EBC)
Keywords: Accelerated aging, Amino acids, Gluten, HPLC, PCR, Propylene glycol, Volatile sulfur compounds, Wort viscosity

RECOMMENDATIONS

1. Conduct online polling to obtain input on new and alternate methods.

The function of this subcommittee is to collect, from various sources, new and alternate methods of analysis that may be useful to the industries our Society serves. These methods are reviewed to establish their merit and usefulness, and a recommendation regarding collaborative testing is made to the Technical Committee. The subcommittee tracks and records the disposition of each method considered. The subcommittee is also charged with the responsibility of periodically reviewing existing methods for accuracy and usefulness.

STATUS OF SUBCOMMITTEE

Membership and Meetings

Given the very close ties this subcommittee has with the Technical Committee, it has been decided to make the Subcommittee on New and Alternate Methods of Analysis an integral part of the Technical Committee's activities and align membership of the two groups. Additional subject matter experts will be added to this subcommittee or consulted with on an as needed basis.

The subcommittee meeting at the 2010 ASBC Annual Meeting in Providence, RI, was well attended. Topics of interest and discussion included

- Viscosity methods.
- UPLC as an alternative for HPLC.
- The process for evaluating methods through the Technical Committee.
- A poster presented by Joyce Carr and Tim Kostelecky regarding reduced solvent usage.
- The IBU limit of the spectrophotometric method.
- A simple method for hop oil analysis (follow up with Bob Smith).
- Poster 47 by Sylvie Deckers et al on a method determining gushing potential.
- MEBAK publication on in-line technology to be available in English.

The subcommittee meeting at the 2011 ASBC Annual Meeting in Ft. Myers, FL, was well attended. Topics of interest and discussion included

- Measurement of β-glucan in wort by modifying a FIA/SFA method (using Calcofluor fluorescence) to use microtiter plates and microplate readers rather than flow systems. This reportedly allows for greater sample throughput and increased experimental flexibility. This could be an option for labs lacking FIA/SFA infrastructure (Mark Schmitt) (7,10). This is a potential topic for polling next year.
- Small-scale mashing methods (Mark Schmitt) (8,9).
- Determination of gelatinization temperatures for adjuncts and malts using a rapid visco analyzer. This is a method published in MEBAK (Martin Zarnkow) and is a potential topic for polling next year.
- A new method for determining IBU involving the use of SPE developed by Tom Shellhammer's group at OSU. Aaron Porter volunteered to look into potential formation of a subcommittee to evaluate this method.
- Phenomenex EZ:faast kit for determination of amino acids.
- Evaluation of in-line instrumentation. How could a subcommittee perform collaborative testing?
- New statistical method for looking at differences, "least squares method" (Karen DeVries).
- Osmolyte concentration—a method developed by Cynthia Henson that reportedly correlates well with some malt parameters (Mark Schmitt).
- TPO—EBC is moving forward with a collaborative study for TPO measurement. Headspace and dissolved oxygen will be measured separately. ASBC members are encouraged to participate (Frank Verkoelen).
- Yeast vitality was discussed by several members. Creating and distributing identical samples is a major challenge and obstacle for future collaborative studies (Bettie Lodolo, Chris Powell, and Chris Baugh).

Topics for Polling

Questions were developed for online polling to gather information on potential new methods for collaborative study. These questions were formatted into a web-based survey with assistance and administration by ASBC staff. The topics in the online poll, along with background information, are described below. Results from the poll can be found in the Appendix of this report.

Input on New and Alternate Methods. This subcommittee and the Technical Committee receive input on potential new and alternate methods throughout the year. Much of the input comes through the ASBC Annual Meeting, but the online poll is another valuable tool for gathering additional information.

Amino Acids in Wort and/or Beer by HPLC. Six people signed up and expressed interest in a potential subcommittee at the 2007 ASBC Annual Meeting in Victoria, BC. Online polling in 2011 yielded six positive responses for labs currently performing this analysis. Four respondents indicated an interest in participating in a collaborative study (3).

Wort Viscosity. Suggestions for methods utilizing an automated instrument came from the 2010 review of the Wort section of the ASBC *Methods of Analysis*. The 2011 poll yielded 11 positive responses for labs currently performing wort viscosity analysis: three using a capillary tube, six using a falling ball instrument, and two using a rotational instrument. Six respondents indicated an interest in participating in a collaborative study. Aaron MacLeod volunteered to chair this subcommittee.

Identification of Brewing Strains or Wild Yeast. These questions for the poll originated from a Technical Committee discus-

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sion regarding additional uses for PCR technology. Five respondents indicated they have performed yeast identification testing. Four indicated the use of PCR methods and also expressed an interest in participating in a collaborative study. Polling questions also collected additional information on the use of PCR for detection of yeast and bacteria.

Volatile Sulfur Compounds in Beer. This topic has been of interest for several years. Roman Ortiz has volunteered to chair the subcommittee. He will work to identify a method and coordinate collaborative testing. Collaborators will be needed to serve on the subcommittee (4–6).

Packaging Methods. In an effort to update the Packages and Packaging Materials section of the ASBC *Methods of Analysis*, the Technical Committee is seeking to evaluate and update packaging methods. Methods are being reviewed, and questions are being developed to include in future polling.

Topics to Archive

Accelerated Aging of Seed (Viability, Vigor Testing). The final year of the NDSU study was due to be completed in 2008, but results have not been published.

Labeling/Allergens in Beer. This topic will be monitored by the Emerging Issues Committee.

Propylene Glycol. This method was suggested by Roman Ortiz at the fall 2008 Technical Committee meeting. Propylene glycol can be measured as a fermentation by-product or a contaminant from defective heat exchangers. Currently there appears to be little interest from members in pursuing collaborative testing.

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APPENDIX

Summarized Results from 2011 Online Polling

Top Line Results

- 57 responses were received
- 11 respondents submitted information on new and alternate methods
- 20 respondents provided their contact information
- 6 respondents answered questions regarding amino acid analysis
- 9 respondents answered questions regarding wort viscosity analysis
- 5 respondents answered questions regarding the use of PCR

Information Provided Regarding New or Alternate Methods

- Measuring types and amounts of hop products in beer using very little sample preparation (1)
- Detecting beer spoilers
- Spectrophotometric method for rho, tetra, and hexa; modification of ASBC Method Hops-8B (3) to use methanol (current industry standard)
- Hop acids in hops, hop products, and beer; method provides results faster with at least the same accuracy as HPLC and uses less solvent
- Barley, malt, and wort β-glucan using a microtiter plate
- Hop aroma
- SO_2, DTNB allowing use of nontoxic reagents
- Replacing the antiquated EBC mash method
- Augmentation of ASBC Methods Beer-10A and -10C to give full color information irrespective of illuminant, path, or observer (2)
- Analyzing characteristic flavor of beer
- Gelatinization temperature—helpful to adjust mashing process with regard to attenuation limit and fermentable carbohydrates

Measurement of Volatile Aldehydes in Beer by Solid-Phase Microextraction–Gas Chromatography/Mass Spectrometry

Subcommittee Members: R. Ortiz, *Chair*; V. Kellner (EBC); M. Qian; K. Stenerson; and J. Cornell (*ex officio*)

Keywords: Beer staling, Benzaldehyde, Carbonyl, Flavor stability, 2-Furaldehyde, GC/MS, 2-Methyl-butanal, 3-Methyl-butanal, 2-Methyl-propanal, *trans*-2-Nonenal, Phenylacetaldehyde, SPME

CONCLUSIONS

1. Standard deviations for the measurement of seven compounds in packaged American lager beer by solid-phase microextraction–gas chromatography/mass spectrometry (SPME GC/MS) were determined. With limited results, the standard deviations (SD) and percent relative standard deviations (%RSD) for the seven compounds studied for fresh beer sample pair C/D ranged from 0.0283 to 26.69 µg/L and 20.48 to 85.26%, respectively, and were judged unacceptable.
2. With limited results, the SD and %RSD for the seven compounds studied for aged beer sample pair A/B ranged from 0.0941 to 651.7 µg/L and 23.84 to 96.79%, respectively, and were judged unacceptable.

RECOMMENDATIONS

1. The subcommittee chair recommends the collaborative study be terminated due to the limited availability of collaborators and the resultant lack of data to perform a standard ASBC statistical analysis.
2. Discharge the subcommittee.

This is the second interlaboratory collaborative study conducted since the formation of the subcommittee. Based on membership polling, the Technical Committee recommended forming this subcommittee in 2008 to evaluate methods for measuring beer volatiles using SPME GC/MS. Three collaborators returned results from the first study (1,2), and therefore, typical Youden unit block statistical analysis could not be performed. However, the results did give insight into the need to refine the instructions given to collaborators on performing the method, improve the beer force-aging method, and seek out additional collaborators.

The method used for the collaborative study is based on several published methods (3,5,6), as well as the method used by the subcommittee chair's laboratory. The aldehydes selected for quantification using this method are only a fraction of the aldehydes and carbonyl compounds that are present in beer, but they have been observed to have characteristics indicative of changes in flavor stability and the resulting changes in the taste and aroma of packaged beer (4). The aldehydes measured using this method were 2-methylpropanal (or isobutyraldehyde), 2-methyl-butanal (or 2-methylbutyraldehyde), 3-methyl-butanal (or isovaleraldehyde), 2-furaldehyde, benzaldehyde, phenylacetaldehyde, and *trans*-2-nonenal.

PROCEDURE

Collaborators were provided with four cans of American light lager beer: A, B, C, and D. Samples C and D were the same brand but were from different packaging runs; they were stored at 4°C until shipping. Samples A and B were created by subsampling from the C and D cartons, respectively, and were force aged at 50°C for 5 weeks. This created sample pairs C/D (fresh) and A/B (aged). Sample pair A/B was subjected to an elevated temperature for 5 weeks to force age the beer samples and increase the levels of the staling aldehydes studied. In contrast, sample pair C/D was stored at 4°C to maintain fresh beer flavor and likely correspondingly lower levels of the aldehydes studied.

The samples were shipped to the collaborators cold and were packed with cold packs in thermally protected containers. Collaborators were instructed to place the samples in a refrigerator upon receipt and to store them there until ready to analyze to minimize any changes in aldehyde concentrations prior to analysis. Collaborators were instructed to analyze samples within 2 weeks of receipt and report results using a data sheet provided by the subcommittee chair.

RESULTS AND DISCUSSION

Results were received from four of seven collaborators. The results for each of the measured aldehydes for sample pairs A/B and C/D are listed in Tables I–VII. With limited data available, Youden block statistical analysis could not be performed. Instead, SD and %RSD were determined for the results. The range of SD and %RSD for the seven compounds studied for fresh beer sample pair C/D ranged from 0.0283 to 26.69 µg/L and 20.48 to 85.26%, respectively, and were judged unacceptable. The range of SD and %RSD for the seven compounds studied for aged beer sample pair A/B ranged from 0.0941 to 652 µg/L and 23.84 to 96.79%, respectively, and were judged unacceptable.

TABLE I
2-Methyl-propanal (µg/L) Measured in Beer by Solid-Phase Microextraction–Gas Chromatography/Mass Spectrometry

	Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**
1	175	128	24.1	16.9
2	47.0	60.1	4.63	3.05
3	53.4	41.4	6.72	3.21
4	76.9	53.9	13.3	7.31
Mean	88.07	70.85	12.19	7.617
Grand mean	79.46		9.902	
SD	59.36	38.88	8.759	6.495
%RSD	67.39	54.88	71.87	85.26

TABLE II
2-Methyl-butanal (µg/L) Measured in Beer by Solid-Phase Microextraction–Gas Chromatography/Mass Spectrometry

	Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**
1	25.5	18.5	2.69	1.92
2	25.0	33.4	2.01	1.58
3	30.9	23.6	3.04	1.57
4	43.3	30.4	4.87	2.38
Mean	31.17	26.47	3.152	1.862
Grand mean	28.825		2.507	
SD	8.513	6.714	1.222	0.3814
%RSD	27.31	25.36	38.77	20.48

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TABLE III
3-Methyl-butanal (µg/L) Measured in Beer by Solid-Phase Microextraction–Gas Chromatography/Mass Spectrometry

	Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**
1	22.5	24.4	7.50	6.11
2	22.5	46.7	5.94	6.31
3	21.8	24.2	7.05	5.04
4	34.3	35.7	13.5	9.14
Mean	25.27	32.75	8.497	6.650
Grand mean	29.01		7.574	
SD	6.026	10.74	3.399	1.751
%RSD	23.84	32.80	40.00	26.33

TABLE IV
Furaldehyde (µg/L) Measured in Beer by Solid-Phase Microextraction–Gas Chromatography/Mass Spectrometry

	Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**
1	1,029	798	75.9	33.8
2	1,726	2,202	134	59.6
3	936	902	85.0	12.1
4	1,438	1,042	114.2	22.3
Mean	1,282	1,236	102	31.9
Grand mean	1,259		67.09	
SD	367.9	651.7	26.69	20.45
%RSD	26.69	52.73	26.11	64.02

TABLE V
Benzaldehyde (µg/L) Measured in Beer by Solid-Phase Microextraction–Gas Chromatography/Mass Spectrometry

	Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**
1	1.38	1.16	0.983	0.841
2	5.87	4.71	2.50	1.61
3	0.959	1.04	0.674	0.613
4	1.42	1.44	1.40	1.46
Mean	2.407	2.087	1.389	1.131
Grand mean	2.247		1.260	
SD	2.318	1.756	0.7980	0.4796
%RSD	96.29	84.14	57.44	42.41

TABLE VI
Phenylacetaldehyde (µg/L) Measured in Beer by Solid-Phase Microextraction–Gas Chromatography/Mass Spectrometry

	Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**
1	24.5	21.5	6.54	4.13
2	56.9	153	20.7	18.9
3	31.3	30.7	12.8	8.96
4	54.3	46.5	20.2	12.0
Mean	41.74	62.90	15.06	11.02
Grand mean	52.32		13.04	
SD	16.27	60.88	6.731	6.228
%RSD	38.96	96.79	44.69	56.50

TABLE VII
***trans*-2-Nonenal (µg/L) Measured in Beer by Solid-Phase Microextraction–Gas Chromatography/Mass Spectrometry**

	Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**
1	0.233	0.242	0.124	0.173
2	0.406	0.890	0.099	0.108
3	0.188	0.244	0.0615	0.222
4	0.287	0.346	0.071	0.068
Mean	0.278	0.4305	0.0889	0.1427
Grand mean	0.3545		0.1158	
SD	0.0941	0.3102	0.0283	0.0682
%RSD	33.80	72.05	31.85	47.84

The method clearly showed increases in the levels of staling aldehydes in force-aged beer compared with fresh beer. For some compounds there was also good agreement in the sample pair results for a given laboratory, indicating the potential utility of the method from a single laboratory perspective. However, in most cases there was high variation in the reported concentrations among laboratories. This method is quite complex with regard to sample preparation and calibration and involves the measurement of compounds, some of which are highly reactive, at low parts-per-billion levels. These factors combine to increase the difficulty of achieving good reproducibility of results.

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