Technical Committee and Subcommittee Reports

2009–2010 Report of the Technical Committee

Committee Members: D. Sedin, *Chair*; C. Benedict; J. Cornell; M. Eurich; A. Fristsch; R. Jennings; G. Kelly; K. Lakenburges; A. Porter; C. Powell; S. Thompson; and R. Foster (*senior advisor*)

Activity in 23 subcommittees was conducted by the ASBC Technical Committee and Subcommittee chairs during 2009–2010. As a result, four methods are being recommended for inclusion in the ASBC *Methods of Analysis* (MOA):

- IBU of Dry-Hopped Beer, chaired by Ruth Martin (Sierra Nevada Brewing Company)
- Malt-4 Extract Mills and Mashing, chaired by Aaron MacLeod (Canadian Grain Commission)
- EMAST Standard, chaired by Jolanta Menert (Bush Agricultural Resources LLC).
- Malt-8 Protein (Barley Standard Versus EDTA), chaired by Rebecca Jennings (Rahr Malting)

Additionally, a review of four sections of the MOA was completed:

- Wort, chaired by Mark Eurich (MillerCoors)
- Sensory Analysis, chaired by Sue Thompson (MillerCoors)
- Microbiology, chaired by Chris Powell (University of Nottingham)
- Processing Aids, chaired by Aaron Porter (Sierra Nevada Brewing Company)

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

- Solid-Phase Microextraction–Gas Chromatography/Mass Selective Detection (SPME-GC/MS) Fingerprint of Beer Volatiles and Semivolatiles, chaired by Roman Ortiz (MillerCoors)
- Wort and Beer Fermentable and Total Carbohydrates by HPLC, chaired by Mark Eurich (MillerCoors)
- ATP, chaired by Caroline Pachello (MillerCoors)
- Alpha-amylase Automated Flow Using Potassium Ferricyanide, chaired by Al Budde (USDA/ARS)
- Deoxynivalenol Analysis by ELISA, chaired by Shayne Bartlett (Cargill Malt)
- Miniature Fermentation Assay, chaired by Alex Speers (Dalhousie University)
- Sortimat, chaired by Paul Ritchie (Canada Malting)
- GC-FID Analysis for Beer Volatiles, chaired by Joe Palausky (Boulevard Brewing)

The following five methods are being recommended for collaborative study in 2010–2011:

- Iso-alpha-acids in Beer and Wort by HPLC, chair TBD, ex officio is Aaron Porter
- IBU in Wort by Spectrophotometer, chair TBD, ex officio is Bob Foster
- IBU in Wort by Segmented Flow Analysis, chair TBD, ex officio is Mark Eurich
- Beta-glucans 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)

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

- Soluble Starch, chaired by Rebecca Jennings (Rahr Malting)
- Check Services, Jim Munroe (Anheuser-Busch, retired), Sue Casey (ASBC), Stephen Kenny (Washington State University IAREC), and John Barr (North Dakota State University).
- New and Alternate Methods of Analysis, chaired by Karl Lakenburges (Anheuser-Busch InBev).
- International Methods, chaired by Dana Sedin (MillerCoors)
- Craft Brewers, chaired by Gina Kelly (New Belgium Brewing Company)
- Sensory Science, chaired by Annette Fritsch (Annette Fritsch Consulting) and Sue Thompson (MillerCoors)
- International Hop Standards Subcommittee, chaired by Bob Foster (MillerCoors)
- Packaging Methods, chaired by Chaz Benedict (Hach) and Aaron Porter (Sierra Nevada Brewing Company)

Jim Monroe (Anheuser-Busch InBev, retired) 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. Their hard work and dedication are greatly appreciated!

The ASBC Board of Directors has instituted a grant program to be administered by the ASBC Technical Committee. The program will be used to fund projects that will provide new methods or programs of value to ASBC members. The first grant of $2,000 has been awarded to Mark Zunkel for the development of a beer flavor database. The next proposed grant will be for the investigation of beer-degassing methods to determine the optimal degassing process(es) to utilize for the ASBC MOA.

I would like to thank the subcommittee chairs for their hard work and dedication in conducting their respective collaborative studies throughout 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@anheuser-busch.com*)

This is a standing subcommittee whose function is to collect, from various sources, including polling membership, 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.

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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
(*Gina Kelly, gkelly@newbelgium.com*)

The mandate of the Craft Brewers Subcommittee is to connect with the craft brewing membership of the ASBC and explore opportunities to make the Society more relevant to those individuals. Additionally, the subcommittee will develop and pursue strategies to bring craft brewers who are not members of the Society into ASBC. Accomplishments and activities in previous years include launching the Craft Brewers Check Service; roll-out of a Craft Brewers community on ASBC*net* with Ask the Expert and Forum sections; and promoting ASBC to craft brewers at their events, such as the Great American Beer Festival and the Craft Brewers Conference. This year, the subcommittee's plans include continuing the Ask the Expert and Forum interactive series on ASBC*net*; continuing a craft brewers focus group monthly conference call; and polling ASBC Local Section chairs on how to increase craft brewer membership at their level.

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

This is a standing subcommittee. It was formed on recommendation from 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 methodology and propose new methodology for collaborative testing. Activities in previous years include developing a panel performance-monitoring tool that will be included in an upcoming version of the MOA, developing a list of reference standards that can be used for training beer sensory panels, and reviewing the revised triangle test methodology with the EBC to retain International Collaborative Method status. The next project involves updating the beer flavor wheel.

Solid-Phase Microextraction–Gas Chromatography/Mass Selective Detection (SPME-GC/MS) Fingerprint of Beer Volatiles and Semivolatiles
(*Roman Ortiz, Ortiz.Roman.2@MillerCoors.com*)

This subcommittee was formed to evaluate the use of solid-phase microextraction as a sampling technique, coupled with gas chromatographic separation and mass selective detection, to yield volatile and semivolatile fingerprints of finished beer. Currently in the scientific literature, there are various methods described that employ this technique as a fingerprint methodology. Variations include type of SPME fiber utilized, whether the sampling of the beer is from the liquid or the headspace, and type of mass selective detection used (ion trap, quadrapole, or time-of-flight).

Wort and Beer Fermentable and Total Carbohydrates by HPLC
(*Mark Eurich, Mark.Eurich@MillerCoors.com*)

The subcommittee has been tasked to update ASBC methods Wort-14B and Beer-41B to methods currently employed in the brewing industry. Polling and key findings from previous years indicated great interest in these two methods. Significant technological gains would also deem that these methods be brought up to industry standards.

ATP
(*Caroline Pachello, Caroline.Pachello@MillerCoors.com*)

There are a number of different commercially available kits that can be used to determine adenosine triphosphate (ATP) bioluminescence as a means of assessing water hygiene. However, while such methods are employed by many brewers, there has been no formal evaluation of the technology. This is largely because kits produced by different manufacturers provide data that cannot be accurately compared, leading to brewery-specific criteria for the amount of ATP detected and its significance in terms of microbial loading. The objective of this collaborative is to assess whether reproducible results regarding water hygiene can be obtained across multiple laboratories with multiple instruments when utilizing a common ATP bioluminescence instrument.

Alpha-amylase Automated Flow Using Potassium Ferricyanide
(*Al Budde, Allen.Budde@ars.usda.gov*)

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
(*Shayne Bartlett, Shayne_Bartlett@cargill.com*)

Laboratories are looking for accurate methods that are easy to use and that can 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 that incorporate homogeneous assay technology. The difference is that EZ-Tox 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.

Miniature Fermentation Assay
(*Alex Speers, alex.speers@dal.ca*)

To assess the phenotypic characteristics of brewing yeast, small-scale fermentation vessels are often employed to replicate or mimic full-scale vessels. Currently, there are a number of different small-scale vessels that are widely used, ranging from 2-L EBC tall tubes to smaller conical (Erlenmeyer) shake flasks. However, despite their relatively small size, these are often still too large in volume for high-throughput analysis. Currently, a standard miniature-scale fermentation technique does not exist, and this subcommittee aims to assess alternative methods to perform and monitor such fermentations. One possible technique is the method described and used in studies of premature yeast flocculation (PYF), as published in the *ASBC Journal* by Lake et al (2008).

Sortimat
(*Paul Richie, paul.ritchie@canadamalting.com*)

Laboratories are looking for accurate methods that are easy to use and that can produce results in a quick and efficient manner. It has been recommended that ASBC look into an alternative method for assortment. There are very few Eureka-Niagara barley graders in use. Several companies are using a Sortimat grader from Pfueffer.

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

This subcommittee has existed for 14 years, previously as the International Subcommittee for Isomerized Hop Alpha-acids Standards (ISIHAS), 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 (than one using mass spectrometry) to measure esters and alcohols. Based on 2009 polling results and references in the literature, a method will be designed for collaborative testing.

Iso-alpha-acids in Beer and Wort by HPLC
(*Aaron Porter, ex officio*)

This subcommittee was formed to evaluate a high-performance liquid chromatography (HPLC) method for the analysis of iso-α-acids (IAA) in beer and wort. Many common IAA HPLC methodologies exist; this subcommittee will be tasked with evaluating available methods and determining the method best suited for collaborative testing. A chair has not been identified for this subcommittee.

IBU in Wort by Spectrophotometer
(*Bob Foster, ex officio*)

This subcommittee was formed to adapt the current method(s) used for measuring IBU in packed product for wort samples. A chair has not been identified for this subcommittee.

IBU in Wort by Segmented Flow Analysis
(*Mark Eurich, ex officio*)

This subcommittee was formed to adapt the current method(s) used for measuring IBU in packed product by segmented flow analysis for wort samples. A chair has not been identified for this subcommittee.

Beta-glucans in Wort by Segmented Flow Analysis
(*Aaron MacLeod, aaron.macleod@grainscanada.gc.ca*)

In conducting the 2009–2010 review of the Wort section of the MOA, it was recommended that segmented flow analysis for β-glucans in wort be considered for collaborative study. Segmented flow analysis is commonly utilized in industry for the analysis of β-glucans, and this would bring the MOA in line with current methodology.

Free Amino Nitrogen in Wort by Segmented Flow Analysis
(*Aaron MacLeod, aaron.macleod@grainscanada.gc.ca*)

In conducting the 2009–2010 review of the Wort section of the MOA, it was recommended that segmented flow analysis for free amino nitrogen (FAN) in wort be considered for collaborative study. Segmented flow analysis is commonly utilized in industry for the analysis of FAN, and this would bring the MOA in line with current methodology.

Packaging Methods
(*Chaz Benedict, cbenedict@hach.com*)

This is a standing subcommittee. It was formed to evaluate packaging methodology, review packaging methods within the MOA, and act as a liaison position between ASBC and other packaging-related organizations. This subcommittee has recently been tasked with developing best practice guidelines and recommendations for topics such as how to identify and troubleshoot high in-package oxygen.

Determination of Bitterness Units and Iso-α-acid Levels in Dry-Hopped Beers Using the Iso-α-acids Spectrophotometric Method

Subcommittee Members: R. Martin, *Chair*; S. Bruslind; D. Collazo; C. Geiger; C. Guy; F. Hamp; B. Jaskula-Goiris (EBC); B. Jordan; J. Jordyn; R. Juzeler; S. Krug; K. Lee; J. Mastin; K. McGivney; S. Mulqueen; A. Porter; J. Schmid; R. Schmidt; R. Smith; S. Steele; C. Taylor; S. Taylor; and R. Foster (*ex officio*)

Keywords: HPLC, IAA, IBU

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the determination of iso-α-acids (IAA), ASBC method Beer-23B (1), ranged from 4.3 to 8.0% and 20.3 to 34.7%, respectively, and were judged acceptable.
2. A *t* test was performed on the IAA spectrophotometric and HPLC methods and showed no significant difference in mean values at the 95% confidence level.
3. The IAA spectrophotometric mean better estimated the IAA levels determined by HPLC compared with the isohumulone bitterness units (IBU) spectrophotometric method (2) for dry-hopped beers.

RECOMMENDATIONS

1. The subcommittee recommends that the archived ASBC IAA spectrophotometric method used for estimating IAA levels in dry- and traditionally hopped beers be reinstated as ASBC method Beer-23B (1).
2. Discharge the subcommittee.

This was the third year of the subcommittee's evaluation of the archived IAA method compared with the IBU method and referenced with the HPLC IAA method (4). The committee was started at the request of brewers producing dry-hopped beers who have asked for a spectrophotometric method to determine the true bitterness of their beers, since some of their "dry-hopped" products have very high IBU values but do not taste that bitter from a sensory standpoint.

During the first year, a ruggedness test was performed with select collaborators to test the accuracy and feasibility of the ASBC archived IAA spectrophotometric method. During the second year, sample pairs with different production dates were not sent to collaborators, and Youden unit block testing (3) could not be performed. However, standard statistical analysis indicated the archived spectrophotometric IAA method was accurate. This year the subcommittee recommended sending sample pairs with different production dates in order to utilize the Youden unit block testing procedure (2), as well as evaluate the ruggedness of the method for dry-hopped beers covering a range of concentrations (low, medium, and high). The subcommittee members also recommended including the evaluation of a non–dry-hopped, lager beer sample pair for comparison. HPLC analysis (4) was used as a comparison with IAA measurement in this study because it is the standard method used in the industry to determine accurate IAA content in beer.

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PROCEDURE

Four sample pairs were sent to each collaborator. Each pair was from the same brand but from different production dates and was selected to cover a range of IAA concentrations, as was recommended the previous year by the subcommittee collaborators. The samples consisted of two non–dry-hopped beers (A1/A2), two low dry-hopped beers (B1/B2), two medium dry-hopped beers (C1/C2), and two high dry-hopped beers (D1/D2). Participants were asked to follow the methods as close as possible. Results were evaluated using the Youden unit block design (2).

RESULTS AND DISCUSSION

Results from 22 collaborators were received for sample pairs A1/A2, B1/B2, C1/C2, and D1/D2 for the IBU method. Results for the IAA method were received from 21 collaborators for all sample pairs. The results are summarized in Tables I and II, respectively. Outliers were identified using Dixon's ratio test described in the Youden unit block procedure (2). Both test results for the sample pairs were excluded from data analyses for the six outliers identified. In discussing the method with one of the outlier collaborators, it was found that the solvent layers had mixed after setting out for a short time. This could have affected the results because it was stated in the method that this step be done as soon as possible. One known reason for error with the outliers was pipetting inexperience, where some outliers inadvertently aspirated a small amount of the emulsion layer into the alkaline methanol. This resulted in slightly higher results. Additionally, results from four collaborators using a modified version of EBC method 7.9 (4) were received for all pairs and are summarized in Table III.

The statistical summary of the IBU and IAA data for both analytical methods is presented in Table IV. Repeatability and reproducibility coefficients of variation for the IBU method ranged from 1.8 to 5.1% and 6.8 to 9.6%, respectively, and were judged acceptable. Repeatability and reproducibility coefficients of variation for the determination of IAA, ASBC method Beer-23B (1), ranged from 4.3 to 8.0% and 20.3 to 34.7%, respectively, and were judged acceptable. The high level of reproducibility coefficients of variation for the IAA method might be due to unfamiliarity with the method, as well as insufficient separation of the solvent layers and inclusion of the emulsion layer in the sample cell.

A paired *t* test was performed to evaluate the difference in means between the spectrophotometric and HPLC methods for testing IAA levels. Table V shows that there was no significant difference between the two methods for the means at the 95% confidence level, and the archived spectrophotometric IAA procedure was judged an acceptable method. Table VI shows the differences between the samples for the IBU and IAA grand means and the reference HPLC IAA method. When only the dry-hopped IBU and IAA grand means were plotted against each other, the relationship had a linear function of $y = 0.8513\times$, with an R^2 value of 0.995 (Fig. 1).

The IAA method delivered a result that is lower than the IBU method, which was expected. Using the formula from the line created in Table VI, one can estimate the IAA level by multiplying the IBU result by 0.8513. It was determined that the archived IAA method with its new modifications qualifies as a practical spectrophotometric method for estimating IAA levels in dry-hopped beers.

TABLE I
Results for Bitterness Units Spectrophotometric Method—Beer-23A

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A1**	**A2**	**B1**	**B2**	**C1**	**C2**	**D1**	**D2**
1	33.0	34.0	25.0	24.0	43.5	49.0	89.0	89.0
2	29.5	30.0	21.5	21.0	38.5	42.5	80.0	81.0
3	31.5	33.5	24.5	24.5	44.5	48.0	88.5	87.5
4	27.0	30.0	25.5	22.5	45.0	41.0	88.5	89.0
5	29.0	29.0	20.0	20.0	41.0	37.0	81.5	80.5
6	27.0	29.0	20.0	20.5	36.5	34.0	68.0	70.0
7	33.0	33.5	25.0	23.5	44.0	48.0	88.5	89.5
8	24.5	25.5	26.0[a]	34.0[a]	49.5	48.0	87.5	87.5
9	32.0	32.0	23.0	22.0	42.0	48.0	84.5	83.5
10	31.5	31.5	23.0	23.0	43.5	47.0	86.5	86.0
11	31.5	31.5	23.5	22.5	41.5	45.5	85.0	82.0
12	32.5	35.5	25.0	23.5	43.5	47.5	88.0	90.0
13	26.0[a]	37.0[a]	25.5	19.0	33.5	35.5	77.5	78.5
14	28.0	32.0	24.0	22.0	38.5	43.0	84.5	78.5
15	28.5	29.5	22.0	20.5	38.0	42.0	80.5	81.0
16	29.0	30.5	22.0	21.5	39.5	44.5	79.5	80.0
17	24.0[a]	25.0[a]	18.0	19.5	38.0	37.5	71.0	71.5
18	36.0[a]	31.5[a]	19.0	18.5	39.0	41.5	83.0	86.5
19	28.0	30.0	24.5	21.5	40.5	46.5	78.5	81.0
20	31.5	31.5	24.0	22.5	42.0	46.0	87.5	84.0
21	33.5	34.5	25.5	24.5	42.5	46.5	85.0	85.5
22	33.0	33.5	25.0	24.0	44.5	49.0	89.5	89.5
Mean[b]	30.18	31.39	23.38	22.05	41.32	43.98	83.27	83.25
Grand mean[b]	30.79		22.71		42.65		83.26	

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

TABLE II
Results for Archived Iso-α-acids Spectrophotometric Method—Beer-23B

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A1**	**A2**	**B1**	**B2**	**C1**	**C2**	**D1**	**D2**
1	30.0	28.5	15.0	14.5	34.5	35.0	67.0	62.0
2	32.0	32.0	22.0	20.0	35.5	39.5	77.0	77.5
3	31.5	32.5	22.5	24.0	39.0	41.0	79.5	75.0
4	33.0	33.0	28.5	29.0	65.0[a]	37.5[a]	112.5	118.0
5	22.0	23.0	15.5	15.0	25.0	25.0	50.5	49.0
6	34.0	34.0	22.0	21.5	23.5	26.5	73.5	68.5
7	30.0	34.0	20.0	20.0	36.0	41.5	79.0	76.0
8	49.5	40.5	29.5	27.0	40.5[a]	25.0[a]	79.5	81.5
9	35.5	32.0	20.0	21.5	45.0	48.5	95.5	90.5
10	30.5	34.0	21.5	18.5	32.0	40.5	67.5	72.0
11	20.5[a]	51.0[a]	13.5[a]	85.0[a]	23.5[a]	40.0[a]	41.0[a]	113.0[a]
12	33.5	33.5	37.5	42.0	21.0	21.0	77.0	77.0
13	30.5	29.5	17.0	16.0	30.5	34.5	71.0	65.0
14	25.0	27.0	15.0	15.5	23.5	29.0	45.5	48.0
15	21.5	22.0	10.5	9.5	15.5[a]	19.0[a]	30.5	31.0
16	42.5	36.5	24.5	24.5	39.5	48.0	89.5	93.0
17	28.5	28.5	17.0	18.0	113.5[a]	37.5[a]	72.5	74.5
18	30.5	35.5	18.0	17.0	32.5	38.0	59.0	68.0
19	17.5	17.0	10.0	6.5	27.0	27.5	63.0	63.5
20	33.0	27.5	19.5	17.5	33.5	36.0	77.0	69.5
21	33.0	29.0	24.5	24.0	38.5	40.0	72.5	73.5
22	…	…	…	…	…	…	…	…
Mean[b]	31.18	30.48	20.50	20.08	32.28	35.72	71.95	71.65
Grand mean[b]	30.83		20.29		34.00		71.80	

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

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TABLE III
Summary of HPLC Method Results for Iso-α-acids

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A1**	**A2**	**B1**	**B2**	**C1**	**C2**	**D1**	**D2**
4	33.3	34.2	20.7	18.5	33.1	38	75.9	74.7
6	27.2	28.2	15.9	15.7	26.7	31.5	63.1	66
10	38.1	35.3	17.5	17.7	35.1	36.4	81.7	73.3
13	33.4	33.6	20.4	19.7	33.7	39.3	73.9	73.1
Mean	33.00	32.83	18.63	17.90	32.15	36.30	73.65	71.78
Grand mean	32.91		18.26		34.23		72.71	

TABLE IV
Statistical Summary of Results for Bitterness Units (BU) and Archived Iso-α-acids (IAA) Spectrophotometric Methods[a]

Method			Repeatability			Reproducibility		
Sample Pair	**No. of Labs**	**Grand Mean**	S_r	cv_r	r_{95}	S_R	CV_R	R_{95}
BU								
A1/A2	19	30.79	2.49	2.72	2.34	2.49	8.07	6.96
B1/B2	20	22.71	1.08	4.75	3.02	1.88	8.30	5.28
C1/C2	22	42.65	2.18	5.11	6.11	4.07	9.55	11.40
D1/D2	22	83.26	1.47	1.77	4.13	5.67	6.81	15.88
IAA								
A1/A2	20	30.83	2.45	7.96	6.87	6.25	20.29	17.51
B1/B2	20	20.29	1.28	6.30	3.58	7.03	34.67	19.69
C1/C2	16	34.00	1.94	5.69	5.42	7.44	21.89	20.84
D1/D2	20	71.80	3.06	4.27	8.58	17.8	24.79	49.84

[a] Calculations were made according to ASBC method Statistical Analysis-4 (2).

TABLE V
***t* Test: Two-Sample Assuming Unequal Variances for the Archived Iso-α-acids (IAA) Spectrophotometric and HPLC Methods**

	IAA Spectrophotometric	IAA HPLC
Mean	39.2300	39.5281
Variance	434.4540	465.8979
Observations	8	8
Hypothesized mean difference	0	
df	14	
t Statistic[a]	–0.028102	
$P(T \le t)$ one-tail	0.4889887	
t Critical one-tail	1.7613101	
$P(T \le t)$ two-tail	0.9779775	
t Critical two-tail	2.1447867	

[a] Not significant at the 95% confidence level.

TABLE VI
Estimation of the Difference Between Grand Means of Bitterness Units (BU) Versus Iso-α-acids (IAA) Spectrophotometric (Spectro) Versus IAA HPLC Methods in Dry-Hopped Beers

Sample	BU Spectro	IAA Spectro	Difference	IAA HPLC
A[a]	30.79	30.83	0.04	32.91
B	22.71	20.29	–2.42	18.26
C	42.65	34.00	–8.65	34.23
D	83.26	71.80	–11.46	72.71

[a] Non–dry-hopped.

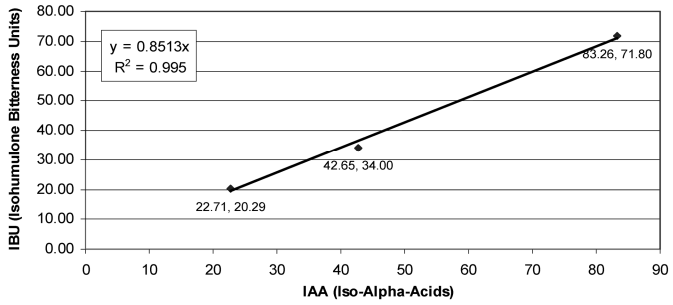

Fig. 1. Estimation of the difference between IBU and IAA (spectrophotometric) methods for dry-hopped beer.

Mill and Mashing Apparatus for Determination of Malt Extract

Subcommittee Members: A. MacLeod, *Chair*; D. Allsopp; B. Amundsen; S. Bartlett; P. Bolin; K. Churchill; R. Joy; J. McCann; P. Ritchie; A. Stern; T. Whittaker; and R. Jennings (*ex officio*)

Keywords: Coarse extract, Congress mash, Fine extract, Malt mill, Mash bath

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for fine-grind extract ranged from 0.2 to 0.3% and 0.3 to 0.4%, respectively, and were judged acceptable.
2. Repeatability and reproducibility coefficients of variation for coarse-grind extract ranged from 0.3 to 0.4% and 0.4 to 0.6%, respectively, and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends that the descriptions of the mill and mashing apparatus in the current ASBC official method for extract (Malt-4) be revised as follows:
   a. *Mill, Fine and Coarse Grind.* Laboratory mill of Buhler-type DFLU disc mill.
   b. *Mashing Apparatus.* Water bath that will firmly hold a number of mash beakers. Bath water is circulated to ensure uniformity of temperature in entire bath. Water level must be held above maximum level of mash in mash beakers. Heating arrangement for bath must be capable of raising mash temperature 1 degree Celsius/min up to 70°C. Each mashing beaker has a stirrer provided to cause an upward motion of mash. Stirring gear drives each stirrer at same speed (80–100 rpm.)
2. Discharge the subcommittee.

This is the subcommittee's first year of existence. The subcommittee was started on the recommendation of the subcommittee for Methods of Analysis Malt Review (2). Revisions were made to the official method for extract, Malt-4 (1), during the 2009 methods review to reflect current laboratory practices. The most significant changes were made to the descriptions of the malt mill and mashing apparatus. The Buhler DFLU disc mill has become the standard in the industry, replacing the Miag-Seck–type cone mill. The description of the mash bath was modified to include models that employ a magnetic stirring mechanism, which is now more common than the propeller blade (overhead stirrer) method. This collaborative study was performed to determine the repeatability and reproducibility for the updated method using modern mill and mashing equipment.

doi:10.1094/ASBCJ-2010-0902-01

PROCEDURE

A total of eight malt samples representing four sample pairs with a range of extract levels was sent to each collaborator. Sample pairs A/B and C/D were different 2-row barley varieties from different production dates, and sample pairs E/F and G/H were different 6-row barley varieties from different production dates. For each sample, the collaborator prepared a Congress mash using the Buhler DFLU disc mill and their own mashing equipment following the Malt-4 procedure. The Buhler-Miag DFLU disc mill was calibrated according to the Malt-4 method for fine and coarse grinds. Results were evaluated using the Youden unit block design (1).

RESULTS AND DISCUSSION

Twelve collaborators submitted results for all four sample pairs (A/B, C/D, E/F, and G/H). Although outliers were identified using Dixon's ratio test (1), no data were excluded from the statistical analysis since no deviations from protocol were found. Data for fine- and coarse-grind extracts are presented in Tables I and II, respectively. Mash baths using magnetic stirring devices were used by 10 collaborators, while baths employing propeller stirring were used by 2 collaborators. Although there were not enough participating laboratories for a direct statistical comparison of variations in extract results between stirring methods, no differences were observed when the stirring methods were analyzed separately.

The statistical summary of the extract data is presented in Table III. Repeatability and reproducibility coefficients of variation for fine-grind extract ranged from 0.2 to 0.3% and 0.3 to 0.4%, respectively, and were judged acceptable. Repeatability and reproducibility coefficients of variation for coarse-grind extract ranged from 0.3 to 0.4% and 0.4 to 0.6%, respectively, and were judged acceptable.

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*, 2009 ed. Malt-4 Extract; Statistical Analysis-4 Youden unit block collaborative testing procedure. The Society, St. Paul, MN, 2009.
2. American Society of Brewing Chemists. Report of the Subcommittee for Methods of Analysis Malt Review. *J. Am. Soc. Brew. Chem.* 67:262, 2009.

TABLE I
Fine-Grind Extract in Malt (%, db)

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	82.1	82.1	81.2	81.5	80.1	80.0	79.6	80.0
2	81.8	81.9	81.2	81.3	79.8	80.1	79.6	79.7
3	82.1	82.3	81.7	81.8	80.1	79.3	79.4	79.4
4	81.8	82.6	81.3	81.2	79.7	79.7	79.6	79.8
5	81.7	82.1	81.2	81.5	80.1	80.2	79.9	80.0
6	82.8	82.2	81.4	81.4	79.7	80.5	79.6	79.5
7	81.5	81.3	80.7	80.9	79.6	79.6	79.3	79.6
8	82.4	82.3	81.6	81.6	80.1	80.2	79.9	80.0
9	82.6	82.2	81.6	81.3	80.1	80.1	79.9	79.7
10	82.1	82.1	81.3	81.3	80.0	80.4	79.8	79.7
11	82.3	82.1	81.5	81.7	80.1	80.1	80.2	80.0
12	82.2	81.9	81.3	81.3	79.8	79.9	79.3	79.2
Mean	82.12	82.09	81.33	81.40	79.93	80.01	79.68	79.72
Grand mean	82.10		81.37		79.97		79.70	

TABLE II
Coarse-Grind Extract in Malt (%, db)

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	81.4	81.5	80.7	80.2	79.2	79.3	78.0	77.9
2	81.4	81.4	80.0	80.3	79.0	79.2	77.9	77.8
3	81.6	81.5	79.9	81.3	79.2	78.4	77.5	77.7
4	81.2	81.9	80.4	80.3	78.8	79.2	76.9	76.5
5	81.1	81.6	80.0	80.4	79.2	79.4	78.2	78.3
6	82.3	81.7	80.2	80.4	79.0	79.8	78.1	78.0
7	81.2	80.7	79.3	79.2	78.7	78.2	77.0	77.5
8	81.7	81.6	80.3	80.5	79.5	79.5	78.0	78.3
9	81.3	81.4	80.2	80.3	79.3	79.4	78.4	78.0
10	81.4	81.3	79.5	79.9	78.7	78.5	77.6	77.7
11	81.9	81.7	80.4	80.4	79.6	79.7	78.0	77.7
12	81.8	81.5	79.6	79.7	79.2	79.1	77.7	77.8
Mean	81.53	81.48	80.04	80.24	79.12	79.14	77.78	77.77
Grand mean	81.50		80.14		79.13		77.77	

TABLE III
Statistical Summary of Results for Extract[a]

Extract			**Repeatability**			**Reproducibility**		
Sample Pair	**No. of Labs**	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Fine grind								
A/B	12	82.10	0.26	0.32	0.74	0.35	0.42	0.97
C/D	12	81.37	0.12	0.15	0.34	0.25	0.31	0.71
E/F	12	79.97	0.26	0.33	0.73	0.28	0.35	0.78
G/H	12	79.70	0.14	0.17	0.38	0.27	0.34	0.75
Coarse grind								
A/B	12	81.50	0.26	0.32	0.73	0.32	0.40	0.91
C/D	12	80.14	0.32	0.40	0.90	0.46	0.57	1.29
E/F	12	79.13	0.29	0.37	0.81	0.42	0.53	1.16
G/H	12	77.77	0.20	0.25	0.55	0.46	0.59	1.30

[a] Calculations were made based on Youden unit block design analysis (1).

Megazyme E-MAST Standard for Segmented Flow Analysis of α-Amylase in Malt

Subcommittee Members: J. Menert, *Chair*; C. Adams; B. Amundsen; P. Bolin; A. Fox; K. French; R. Joy; A. MacLeod; 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, ASBC method Malt-7C, using E-MAST as the standard, ranged from 1.6 to 6.0% and were judged acceptable.
2. Reproducibility coefficients of variation for the determination of α-amylase by segmented flow analysis, ASBC method Malt-7C, using E-MAST as the standard, ranged from 9.8 to 19.6% and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends that the Megazyme E-MAST product be adopted for inclusion in ASBC method Malt-7C (1).
2. Discharge the subcommittee.

This was the first year of the subcommittee's existence. Based on the recommendation of the Subcommittee for Methods of Analysis Malt Review (2), this subcommittee was formed to evaluate the Megazyme E-MAST (3) product as a standard for method Malt-7C. The Megazyme E-MAST standard was tested as a replacement product for Sigma Alpha Amylase Type VIII-A (A-2771-500), which is no longer available.

doi:10.1094/ASBCJ-2010-0826-01

PROCEDURE

Collaborators were provided with 10 samples of commercial malt representing sample pairs A/B through I/J. Samples were prepared using a Buhler DFLU disc mill. Settings were determined using method Malt-4 for fine grind. Ground malt samples were sealed to prevent moisture gain and sent to each collaborator in November 2009. Sample pair A/B was produced from a blend of two- and six-row barley malts from different shipping dates. Sample pairs C/D through I/J were from the same variety but had different production dates. Sample pairs C/D, E/F, and I/J were produced from six-row barley malts, and sample pair G/H was produced from a two-row barley malt. Sample pairs were chosen to represent a range of α-amylase levels used by commercial maltsters. Collaborators were asked to have a segmented flow analyzer capable of measuring α-amylase using method Malt-7C. Collaborators obtained the E-MAST standard directly from Megazyme. Samples were prepared for analysis following method Malt-6A (1). The standard was prepared by following Megazyme's instructions. Each lab used its own calibration and was asked to determine moisture using method Malt-3 (1). A template to record results was provided by the chair of the subcommittee. Results were evaluated using the Youden unit block design (1).

TABLE I
Results for α-Amylase (Standard Reference Method) Prepared with E-MAST

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F	G	H	I	J
1	55.9	57.0	54.6	55.3	64.7	66.2	88.7	92.1	44.7	43.7
2	62.8	70.2	66.3	65.2	81.5	83.5	102.8	110.4	67.2	55.2
3	53.3	55.2	48.6	46.9	66.3	67.1	85.6	92.3	42.8	33.3
4	48.4	49.1	45.4	45.3	61.7	60.2	81.0	58.8[a]	38.3	32.6
5	50.6	52.3	50.9	48.1	61.5	59.8	77.3	80.6	46.1	37.9
6	52.1	54.3	49.4	48.6	61.5	60.1	79.1	79.6	40.4	36.9
7	51.6	57.3	55.6	52.7	71.1	71.6	90.4	99.4	52.0	41.4
8	58.1	62.2	58.2	56.8	78.5	75.8	102.5	104.5	46.3	37.4
9	51.7	53.1	49.2	48.3	68.5	64.0	83.7	85.6	36.3	31.8
Mean[b]	53.85	56.74	53.14	51.91	68.36	67.59	88.77	93.01	45.99	38.91
Grand mean[b]	55.30		52.53		67.98		90.91		42.45	

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

TABLE II
Statistical Summary of Results for E-MAST[a]

			Repeatability			Reproducibility		
Sample Pair	No. of Labs	Grand Mean	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
A/B	9	55.30	1.64	2.96	4.58	5.40	9.77	15.12
C/D	9	52.53	0.82	1.57	2.30	6.33	12.06	17.34
E/F	9	67.98	1.51	2.22	4.22	7.76	11.41	21.73
G/H	8	90.91	2.20	2.39	6.08	12.78	14.42	29.12
I/J	9	42.45	2.57	6.04	7.19	8.31	19.58	23.28

[a] All calculations were made based on Youden unit block design analysis (1).

RESULTS AND DISCUSSION

Results from nine collaborators were received for the five sample pairs. Results for sample H from lab 4 was considered outliers; therefore, results for sample pair G/H from lab 4 were excluded from the calculations. The results for α-amylase using E-MAST as the standard are presented in Table I. Outliers were identified using Dixon's ratio test (1).

The statistical summary of the α-amylase data for the Megazyme E-MAST standard using method Malt-7C are presented in Table II. Repeatability and reproducibility coefficients of variation ranged from 1.6 to 6.0% and 9.8 to 19.6%, respectively, and were judged acceptable. The E-MAST standard is viscous, and usage of a positive-displacement pipette is necessary. Even though the coefficient of variation is high, it is comparable to the original method and standard.

LITERATURE CITED

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3. Megazyme International Ireland Ltd. *Data Booklet on Malt α-Amylase/β-Amylase Standard (Lot 70304), E-MAST 05/07*. Megazyme, Bray, Ireland, 2007.

Ethylenediaminetetraacetate Acid (EDTA) Versus Barley as a Calibration Standard for Determining Protein in Malt Using Combustion Analysis

Subcommittee Members: R. Jennings, *Chair/Ex officio*; K. Allder (EBC); S. Bartlett; A. Budde; T. Chicos; G. Fox (EBC); J. Kahle; A. MacLeod; D. Pickett; M. Schmitt (EBC); and T. Whittaker

Keywords: EDTA, Nitrogen

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for determination of protein by combustion analysis using National Institute of Standards and Technology (NIST)-certified ethylenediaminetetraacetate acid (EDTA) as the calibration standard ranged from 0.328 to 1.739% and 0.512 to 1.932%, respectively, and were judged acceptable.
2. Repeatability and reproducibility coefficients of variation for determination of protein by combustion analysis using NIST-certified barley as the calibration standard ranged from 0.544 to 1.098% and 0.770 to 1.243%, respectively, and were judged acceptable.
3. Based on the paired *t* test for differences in means for protein analyses, the NIST-certified EDTA and barley standards were significantly different at the 95% confidence level.

RECOMMENDATIONS

1. The subcommittee recommends the NIST-certified barley standard be adopted for inclusion in ASBC method Malt-8B.
2. Discharge the subcommittee.

This was the first year of the subcommittee's existence. Based on the recommendation of the Subcommittee for Methods of Analysis Malt Review (2), this subcommittee was formed to evaluate the NIST-certified barley standard as an alternative to the NIST-certified EDTA standard for protein analysis as listed in method Barley-7C (1).

PROCEDURE

Collaborators were provided with 10 samples of commercial malt, representing sample pairs A/B through I/J, and one sample each of NIST-certified EDTA and barley. Sample pairs were chosen to represent malt with varying protein levels. Samples were prepared using a Buhler DFLU disc mill with settings determined by method Malt-4 for fine grind. Ground malt samples were sealed to prevent moisture gain and sent to each collaborator in October 2009. Sample pair A/B was produced from a blend of two 2-row barley varieties, sample pairs C/D and G/H were produced from different 2-row barley varieties, sample pair E/F was produced from distiller's material, and sample pair I/J was produced from 6-row barley of the same variety. All sample pairs were from different production dates. The instruments were set to the manufacturer's recommended settings and calibrated with NIST-certified EDTA for one set of results and with NIST-certified barley for another set of results. Results were evaluated using the Youden unit block design (1) and Minitab statistical software for the paired *t* test for differences in means at the 95% confidence level.

RESULTS AND DISCUSSION

Results from 11 collaborators were received for sample pairs A/B, C/D, E/F, G/H, and I/J. Results from two collaborators were excluded prior to statistical analysis because of known deviations from the prescribed experimental protocol. The results for protein determination using NIST-certified barley and EDTA are summarized in Tables I and II, respectively. Outliers were determined using Dixon's ratio test (1).

The statistical summary of the protein data for both NIST-certified EDTA and barley used in determination of protein by combustion analysis is presented in Table III. Repeatability and reproducibility coefficients of variation for NIST-certified EDTA ranged from 0.328 to 1.739% and 0.512 to 1.932%, respectively, and were judged acceptable. Repeatability and reproducibility coefficient of variations for NIST-certified barley ranged from 0.544 to 1.098% and 0.770 to 1.243%, respectively, and were judged acceptable.

The results of the paired *t* test for comparison of differences in protein analyses means when using NIST-certified EDTA or barley (combustion method, Barley-7C) are presented in Table IV. The paired *t* test results indicate the two standards were significantly different at the 95% confidence level for all samples, except C, G, and E. All of the EDTA protein levels were higher than the barley protein levels. EDTA is a chemical synthesized from ethylenediamine (1,2-diaminoethane), formaldehyde, and sodium cyanide. It was originally designed to sequester metal ions. At the time that method Barley-7C was collaboratively studied, EDTA was what was commonly available for use. EDTA results in protein levels in the range typically seen for meat products. Since combustion analysis does not actually measure protein, but rather the nitrogen that is given off during combustion, the protein in EDTA and barley is derived by multiplying the nitrogen reading by 6.25. Verification of the protein level for NIST-certified barley has been performed using method Barley-7A (1). However, the protein level for EDTA has not been determined using this method. Even though the *t* test results showed a significant difference between the two standards, this can be explained by the consistently higher values that EDTA provides for protein.

LITERATURE CITED

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2. American Society of Brewing Chemists. Report of the Subcommittee for Methods of Analysis Malt Review. *J. Am. Soc. Brew. Chem.* 67:262, 2009.

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TABLE I
Percent Protein in Malt Determined by the Combustion Method Using NIST-certified Barley as the Calibration Standard

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
1	11.20	11.20	10.90	10.80	13.43	13.90	11.50	11.40	12.70	12.60
2	11.22	11.13	10.65	10.79	13.46	13.61	11.51	11.32	12.72	12.79
3	11.16	11.22	10.78	10.51	13.61	14.19	11.50	11.53	12.90	12.55
4	11.33	11.47	11.02	10.76	13.62	13.85	11.47	11.66	13.10	12.79
5	11.30	11.20	10.70	10.50	13.80	13.90	11.30	11.40	12.70	12.50
6	11.21	11.21	10.82	10.54	13.58	13.98	11.30	11.46	12.82	12.80
7	11.29	11.37	10.92	10.77	13.39	13.77	11.48	11.47	12.55	12.55
8	11.19	11.20	10.88	10.55	13.42	13.60	11.53	11.36	12.66	12.59
9	11.31	11.20	10.66	10.75	13.45	13.60	11.45	11.31	12.70	12.70
Mean	11.246	11.244	10.814	10.663	13.529	13.822	11.448	11.434	12.761	12.652
Grand mean	11.245		10.739		13.676		11.441		12.707	

TABLE II
Percent Protein in Malt Determined by the Combustion Method Using NIST-certified EDTA as the Calibration Standard

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
1	11.51	11.48	10.75	10.96	13.86	13.86	11.48	11.55	13.08	12.98
2	11.51	11.48	10.89	10.89	13.82	13.65	11.50	11.41	12.85	12.90
3	11.28	11.38	11.02	10.74	13.69	14.52	11.59	11.65	13.06	12.86
4	11.44	11.61	10.95	10.82	13.70	13.98	11.43[a]	11.69[a]	13.17	12.86
5	11.40	11.40	10.90	10.90	13.60	14.20	11.50	11.50	13.20	12.80
6	11.34	11.43	10.84	10.73	13.66	14.34	11.51	11.55	13.18	12.87
7	11.45	11.38	10.82	10.91	13.28	13.70	11.55	11.58	12.48[a]	12.50[a]
8	11.69	11.71	11.14[a]	11.06[a]	13.96	14.42	11.72[a]	11.83[a]	13.11	12.86
9	11.51	11.48	10.85	10.85	13.80	13.81	11.50	11.51	12.95	12.91
Mean[b]	11.459	11.483	10.878	10.850	13.708	14.053	11.519	11.536	13.075	12.880
Grand mean[b]	11.471		10.864		13.881		11.527		12.978	

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

TABLE III
Statistical Summary of Results for Combustion Method Using NIST-certified Barley or EDTA as the Calibration Standard[a]

			Repeatability			Reproducibility		
Standard Sample Pair	**No. of Labs**	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
NIST-certified barley								
A/B	9	11.245	0.061	0.544	0.171	0.087	0.770	0.243
C/D	9	10.739	0.118	1.098	0.330	0.130	1.213	0.365
E/F	9	13.676	0.119	0.872	0.334	0.170	1.243	0.476
G/H	9	11.441	0.102	0.889	0.285	0.100	0.872	0.279
I/J	9	12.707	0.104	0.816	0.290	0.141	1.110	0.395
EDTA								
A/B	9	11.471	0.056	0.485	0.156	0.115	0.998	0.321
C/D	8	10.864	0.105	0.964	0.293	0.082	0.759	0.231
E/F	9	13.881	0.241	1.739	0.676	0.268	1.932	0.751
G/H	7	11.527	0.038	0.328	0.106	0.059	0.512	0.165
I/J	8	12.978	0.108	0.836	0.304	0.094	0.721	0.262

[a] Calculations were made based on Youden unit block design analysis (1).

TABLE IV
Comparison of NIST-certified EDTA and Barley for the Determination of Protein in Malt Using the Paired *t* Test for Differences in Means[a]

Statistical Parameter	**Protein**
Number of sample pairs (N)	83
Mean of differences (D)	0.147
Standard error of differences (SD)	1.236
Calculated t	–8.76[b]
$t_{0.05}$	1.99

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

Methods of Analysis Wort Review

Subcommittee Members: M. Eurich, *Chair*; A. Budde; R. Jennings; K. Lakenburges; A. MacLeod; and A. Porter

CONCLUSIONS

1. The subcommittee reviewed the ASBC *Methods of Analysis* (MOA) Wort section. Many of the methods have alternatives that are used in the industry, and many need modifications. The methods were reviewed for accuracy of citations and availability of described supplies.
2. Several of the MOA Wort methods require additional work, which would include alternative methods. These methods may not be modified completely until additional input is provided.

RECOMMENDATIONS

1. The subcommittee recommends that revisions to the MOA Wort methods be adopted for inclusion in the 2010 ASBC MOA.
2. The subcommittee recommends Wort-11, Reducing Sugars (Copper Reducing Substances) be archived.
3. The subcommittee recommends a collaborative study to evaluate Wort-13, Viscosity (International Method) for an alternative using instruments such as that manufactured by Anton Paar and other manufacturers.
4. The subcommittee recommends that segmented flow analysis be evaluated by collaborative study for the following methods:
   a. Wort-12, Free Amino Nitrogen (International Method)
   b. Wort-18, β-Glucan in Congress Wort by Fluorescence
   c. Beer-23, Beer Bitterness (add wort analysis to the current method)
5. The subcommittee recommends evaluation of Wort-17 for determination of protein in hopped worts, as in unhopped worts.
6. The subcommittee recommends updates to the Wort Glossary. The subcommittee also recommends that for any future changes to the Wort Glossary all title words be explained or expounded on in the method itself.

This is the first year of the subcommittee's existence. Based on member polling, the Technical Committee recommended formation of this subcommittee to update the Wort section of the MOA. Current methods used in the industry may not coincide with those listed in the MOA. These differences are most likely due to technological gains made since these methods were published.

PROCEDURE

A short note was sent out to potential subcommittee members to determine interest in joining the MOA Wort Review Subcommittee. Subcommittee members were provided with each Wort method or those that they are familiar with and asked to review them for accuracy (i.e., spelling, calculations, conformity to laboratory practices, etc.). Subcommittee members participated in two conference calls for open discussion of the methods and glossary.

Following the review process all minor revisions and updates will be made to the methods. An additional list of new methods may be generated based on new technology or methods currently used within the industry.

RESULTS AND DISCUSSION

The results of the MOA Wort methods review are listed by method.

1. Wort-1, Sampling
   a. In footnote, update filter paper to include "currently available filter paper or Ahlstrom, which is located in Helsinki, Finland." Remove Shliecher and Schuell Inc.
2. Wort-2, Specific Gravity
   a. Minor grammatical changes only.
3. Wort-3, Extract
   a. Add an update to the method in order to reference the use of a density meter to obtain Plato value using a density meter programmed with a Plato table.
4. Wort-4, Apparent Extract by Hydrometer
   a. No changes recommended.
5. Wort-5, Yeast Fermentable Extract
   a. Update to include instrumental methods listed in Beer-4.
   b. Add definition in Calculation section for real degree of fermentation (RDF)—A measure of the extent to which the extract (dissolved solids) has been fermented. It is roughly the difference of the extract of the original wort and the real extract of the beer divided by the original extract.
   c. Add definition in Calculation section for real extract (RE)—The actual extract (dissolved solids) of a beer devoid of alcohol and carbon dioxide. For wort, since no alcohol or carbon dioxide is present, it is proportional to the carbohydrate concentration of the wort.
6. Wort-6, Iodine Reaction
   a. Method archived.
7. Wort-7, Total Acidity
   a. Minor grammatical changes only.
8. Wort-8, pH (Hydrogen Ion Concentration)
   a. Recommend that the method be changed to report results to nearest 0.01 rather than 0.05.
9. Wort-9, Method for Preparation of Wort for Color Determination
   a. Recommend the addition that "color measurement should be performed within 30 min to ensure accuracy, as evaporation of the sample will cause false high color results."
   b. Replace Johns Manville as supplier with World Minerals and Sigma.
   c. Other minor grammatical changes only.
10. Wort-10, Protein
    a. Replace result of 0.126 in N % by wt value in Example with correct value of 0.015. Replace 0.126 with 0.015 in protein % by wt calculation and with corrected result of 0.09 in place of 0.79.
    b. Other minor grammatical changes only.
11. Wort-11, Reducing Sugars (Copper Reducing Substances)
    a. Recommend method be archived.
12. Wort-12, Free Amino Nitrogen (International Method)
    a. Recommend this method for collaborative study using segmented flow analysis (SFA) as an alternative.
    b. Recommend adding CAS registry number for all suppliers.

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   c. Add concentration units of mg/L directly after "= 150" under Examples.

13. Wort-13, Viscosity (International Method)
   a. Recommend removing chromic acid as a viable cleaning solution throughout entire MOA. Replace all references to chromic acid with Nochromix or other similar type of cleaning agent as an alternative.
   b. A collaborative study is recommended to evaluate any instruments, such as that manufactured by Anton Paar.
14. Wort-14, Fermentable Saccharides by Chromatography
   a. Under method A, Reagents (d), replace Nutritional Biochemicals Co. as resource with "CAS# 1464-44-4."
   b. Under method A, Reagents (e) and (f), and under method B, Reagents (e) and (f), add "J. T." prior to "Baker."
   c. Under method B, Apparatus (a), replace the city of "Richmond" with "Hercules."
   d. Under method B, Apparatus (h), add the word "Classic" directly after "Sep-Pak."
   e. Under method B, Apparatus (h), replace "Associates" with "Technologies Corp., Milford, MA." Also replace "part No 91910" with "WAT051910."
   f. Other minor grammatical changes only.
15. Wort-15, Magnesium by Atomic Absorption Spectrophotometry
   a. Under Reagents, add an additional section (d), with "Reagent water."
   b. Other minor grammatical changes only.
16. Wort-16, Zinc by Atomic Absorption Spectrophotometry
   a. Under Reagents, add an additional section (d), with "Reagent water."
17. Wort-17, Protein in Unhopped Wort by Spectrophotometry
   a. Under Reagents (a), replace all wording after "0.5% w/v." with "Accurately perform a 100-old dilution using the 0.5% w/v reagent."
   b. Under Apparatus (a), add "grating spectrophotometers" directly after "calibrate."
   c. Most laboratories no longer perform Kjeldahl analyses. It is recommended that Beer-11B be referenced as the preferred method and list the Kjeldahl method, Beer-11A, as an alternative. Recommend removal of all wording with regard to the Kjeldahl method.
   d. Recommend this method for collaborative study using hopped worts.
18. Wort-18, β-Glucan in Congress Wort by Fluorescence
   a. Under Reagents (d), add Megazyme as the supplier and remove Polysciences.
   b. Under Reagents, add an additional section (f), with "Reagent water."
   c. Under Apparatus, add an additional section (I), with "Amber bottle."
   d. Under Apparatus, remove sections (c) and (d) and refer to Reagents (a) for dilution.
   e. Recommend this method for collaborative study using segmented flow analysis (SFA) as an alternative.
   f. Other minor grammatical changes only.
19. Wort-19, Fermentable Carbohydrates by Cation Exchange HPLC
   a. Recommend potentially moving this method to Wort-14 as an alternative and listing it as method C.
20. Wort-20, Elemental Analysis by Inductively Coupled Plasma-Atomic Emission Spectroscopy
   a. No changes recommended.
21. Wort-21, Thiobarbituric Acid Index
   a. No changes recommended.
22. Wort Glossary
   a. Recommend updates.

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*, 9th ed. The Society, St. Paul, MN, 2009.

Methods of Analysis Sensory Analysis Review

Subcommittee Members: S. Thompson, *Chair*; A. Benson; G. Conley; A. Fritsch; C. Haddock; J. Helber; T. Horner; L. Salazar; and K. Zigich

CONCLUSIONS

1. The subcommittee reviewed the Sensory Analysis section of the ASBC *Methods of Analysis* (MOA). The methods were reviewed for accuracy of citations and applicability to the current state of sensory science.
2. One of the Sensory Analysis methods requires additional work.

RECOMMENDATIONS

1. The subcommittee recommends that revisions to the Sensory Analysis section of the MOA be adopted for inclusion in the 2010 ASBC MOA.
2. Maintain the subcommittee to revise Sensory Analysis-12, Flavor Terminology and Reference Standards.
3. There are no methods the subcommittee is recommending be archived.

This subcommittee was formed based on the recommendation of the ASBC Technical Committee to evaluate the accuracy of the Sensory Analysis section of the MOA.

PROCEDURE

Subcommittee members were provided with 4–5 of the 13 Sensory Analysis methods and asked to review them for accuracy (i.e., spelling, calculations, conformity to sensory practices, etc.). Following the review process, all minor revisions and updates were made to the methods. Significant changes to the methods are listed in the Results and Discussion section with an explanation of why the change is required.

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RESULTS AND DISCUSSION

The results of the MOA Sensory Analysis review are listed by method.

1. Sensory Analysis-1, Terms and Definitions
   Difference-from-control test was added as a term and defined because Sensory Analysis-13 was added to the MOA in 1999, after the writing of Sensory Analysis-1.
2. Sensory Analysis-2, Test Room, Equipment, Conduct of Test
   Reference to a digitizer and the associated reference article were removed because computers have replaced the use of digitizers for data capture in sensory laboratories. The note for specific ruby-colored glassware was deleted because that glassware is no longer available through any supplier. The figure of a sensory booth was updated to include a computer.
3. Sensory Analysis-3, Choice of Method
   Reference to the triangular test being appropriate where only 6, 7, or 8 assessors are available was deleted because the current view is that a triangular test should not be conducted with so few assessors.
4. Sensory Analysis-7, Triangular Test
   EBC published a revised triangular test method that ASBC participated in reviewing in 2008. This revised method will replace the Triangular Test Method in the current MOA.
5. Sensory Analysis-10, Descriptive Analysis
   Additional statistical analysis procedures utilized for analyzing descriptive data, which are detailed in Sensory Analysis-13, were included. A reference for these statistical procedures was added.
6. Sensory Analysis-12, Flavor Terminology and Reference Standards
   It is recommended that the committee work on revising the method on Flavor Terminology and Reference Standards, including updating the flavor wheel.

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis,* 2009 ed. Sensory Analysis section. The Society, St. Paul, MN, 2009.

Methods of Analysis Filter Aid Review

Subcommittee Members: A. Porter, *Chair/Ex officio*; K. Bacigalupo; R. Martin; and B. Sheffield

CONCLUSIONS

1. The subcommittee reviewed the *Methods of Analysis* (MOA) Filter Aid section. These methods have no alternatives that are used in the industry, but some may need modification. The methods were reviewed for accuracy of citations and availability of described supplies.
2. There are currently only four Filter Aid methods. The subcommittee found only minor grammatical errors, one update for a reference method, and some questions to answer as a group.

RECOMMENDATIONS

1. The subcommittee recommends the revisions to the MOA Filter Aid methods be adopted for inclusion in the 2010 ASBC MOA.
2. Maintain the subcommittee for additional review or testing after Technical Committee recommendations.
3. The subcommittee recommends the following changes be made to the Filter Aid methods:
   a. Correct some misspellings.
   b. Add Beer-45 (ICP method) as an additional method for iron determination.
   c. Have the ASBC Technical Committee decide if it's necessary to include similar tests for PVPP and/or silica gel.
   d. For Filter Aid-3, change the sample size from 32 to 12 oz of beer and change the filter aid addition to beer from 2.5 to 1.0 g.
4. At this time the subcommittee has no recommendations for collaborative studies.
5. There are no methods the subcommittee is recommending be archived.

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This is the first year of the subcommittee's existence. This subcommittee was formed based on the recommendation of the ASBC Technical Committee to evaluate the accuracy of the Filter Aid section of the MOA. This subcommittee was also tasked with suggesting additional elements to be added to the methodology, as well as archiving methods no longer in use.

PROCEDURE

A short note was sent out to potential subcommittee members to determine interest in joining the MOA Filter Aid Review Subcommittee. Subcommittee members were provided with each Filter Aid method and asked to review it for accuracy (i.e., spelling, calculations, conformity to laboratory practices, etc.). Subcommittee members were asked to participate in one meeting for the discussion of methods. Recommended changes and questions about the methods are listed in the Results and Discussion section with an explanation of why the change is required.

RESULTS AND DISCUSSION

The results of the MOA Filter Aid review are listed by method.

1. Filter Aid-1, Sampling
   Question: Do we want to include silica gel and PVPP (for all four methods)?
2. Filter Aid-2, pH of Water Suspension
   It is recommended that 0.2*M* disodium phosphate (Na_2HPO_4) be changed to 0.2*M* disodium hydrogen phosphate (Na_2HPO_4) and that "stirrir" be changed to "stirrer."
3. Filter Aid-3, Effects of Odor and Taste
   It is recommended that 32-oz bottles of beer be changed to 12-oz bottles of beer and that "recrown" be changed to "re-crown."
4. Filter Aid-4, Iron Pickup by Beer
   It is recommended that Beer-45 be added as an alternative method for iron determination. We also recommend that it be noted that the control beer should undergo the same procedure as the test beer minus the filter aid addition.

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*, 2009 ed. Filter Aid section. The Society, St. Paul, MN, 2009.

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

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

Keywords: Adenosine triphosphate, ATP, Rapid test, Water

CONCLUSIONS

1. For measuring adenosine triphosphate (ATP) in water containing yeast, 54 of 60 data sets within a laboratory had 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 72.2% of the variability in the yeast in water data set was captured in the model and was considered to be acceptable.
3. For measuring ATP in water containing bacteria, 39 of 44 data sets within a laboratory had a coefficient of variation of <10% and were considered to be acceptable.
4. Analysis of data between different laboratories using a linear regression model indicated that 16.2% of the variability in the bacteria in water data set was captured by the model and was considered to be unacceptable.

RECOMMENDATIONS

1. The current data suggest that the evaluated method can be used for the accurate assessment of yeast in water samples. However, the subcommittee recommends that further analysis be performed for the assessment of samples containing bacteria.
2. An alternative protocol for culturing bacterial microbes will be used in an attempt to standardize the metabolic state of bacteria, leading to an improved correlation in ATP results between laboratories.

The aim of this subcommittee is to evaluate the use of bioluminescence to detect adenosine triphosphate (ATP) as a means of assessing water hygiene. This was the second year for the subcommittee, which was formed based on polling at the 2008 ASBC Annual Meeting. During the first year, insufficient collaborators were obtained to initiate collaborative trials. However, polling was performed to ascertain the range of equipment and test methodologies currently employed in breweries. Based on this information, the subcommittee decided to assess the 3M Clean-Trace total ATP water tests and the Clean-Trace NG luminometer during the second year. It should be noted that similar instruments and test reagents are available from a number of other manufacturers. While each brand may vary in sensitivity, precision, and cost, the main focus of this collaborative study was to assess the ability of ATP technology to detect yeast and bacteria in water samples.

PROCEDURE

A bacteria strain (*Lactobacillus brevis*) and a lager yeast culture (*Saccharomyces pastorianus*) were sent to each of 12 collaborators. Participants were also provided with universal beer agar (UBA) plates made with low-hopped beer and supplemented with Tween 80 for the purpose of cultivating each microorganism. Collaborators grew microbes aerobically for 3–5 days at 28–31°C and subsequently analyzed each fresh culture. Care was taken to ensure that cultures were not allowed to overgrow, in order to maintain cell vitality. Stationary phase cultures were diluted in sterile distilled water to achieve a stock suspension with a target absorbance of approx. 0.05 for yeast and 0.06 for bacteria at a wavelength of 600 nm. A series of dilutions was performed on each stock to enable ATP measurements to be obtained at varying cell concentrations. Dilutions for yeast included 1:2, 1:10, 1:100, 1:500, and 1:1,000, while dilutions for bacteria included 1:2, 1:10, and 1:100. Since bacteria have less ATP per unit cell compared to yeast, the higher dilution rates were eliminated. Each culture was divided and analyzed in triplicate for detectable ATP, utilizing the 3M Clean-Trace NG luminometer and Clean-Trace total ATP water test, and data were expressed in arbitrary relative light units (RLU). To provide an estimate of the number of colony forming units (CFU) present in the original cultures, each solution was also membrane filtered and cultivated on UBA for 5–7 days at 28–31°C prior to enumeration.

RESULTS AND DISCUSSION

Results for the analysis of water samples containing either yeast or bacteria using ATP bioluminescence were obtained from 12 collaborators (Table I). Data produced by collaborator 11 for yeast in water were excluded because an accurate representation of CFU could not be obtained (Table II). Results from analyses of yeast and bacteria in water from collaborator 12 were excluded due to contamination of cultures either during shipment or cultivation. Each remaining collaborator was able to detect ATP in diluted samples of yeast and bacteria in water (Table I). The ATP data reported were used to determine the coefficient of variation (CV) (Table III), and the estimated CFU present in the original culture, along with the measured RLU, were used to produce linear regression models (Figs. 1 and 2).

The CV is a measure of relative variability given by standard deviation/mean. The CV is a unitless measure that allows one to compare 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 yeast, 54 of 60 data sets exhibited a CV of <10%, indicating good repeatability of the method within laboratories, with a maximum CV of 29.3% observed. It should be noted that variation was more common as the yeast concentration decreased, as indicated by the increasing frequency at which a CV value of >10% occurred (Table III). For analysis of ATP in water samples containing bacteria, 39 of 44 data sets exhibited a CV of <10%, indicating good repeatability of the method within individual laboratories (Table III), with a maximum CV value of 13.0% observed. Similar to the data obtained for yeast samples, the frequency of CV values of >10% increased as bacteria concentrations decreased.

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 utilized to calculate predictive models of ATP in water for yeast and bacteria, as measured by RLU compared with CFU. The linear equa-

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tion for the model for yeast in water was calculated as $\hat{y} = 0.572 + 0.816x_1$, in which x is defined as CFU per milliliter (ln transformed) and $\hat{y}$ is the calculated value for RLU per 50-µL sample (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) (1). The R^2 value also provides an indication of the strength of a linear relationship (2): 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 (2). Analysis of interlaboratory reproducibility using the line calculated by the linear regression model (Fig. 1) indicated that 72.2% ($R^2 = 0.722$) of the variability in ln(RLU) was explained by ln(CFU). For small-scale testing that involves analysis of cultures that may have variable ATP per unit cell (due to physiological differences), a 72.2% explanation of variation in the response was deemed sufficient to call this an acceptable model given the variation seen in the data from all laboratories. This suggests that the data were reproducible between laboratories, i.e., all laboratories were able to replicate similar results. The range in ln(RLU) values for yeast that could be viable based on the model of the data generated from all laboratories is given by a 95% confidence level calculated using the equation $\ln RLU = (0.572 + 0.816x_1) \pm (1.96)(1.4485)$, in which 1.4485 is the variability (standard deviation) of the linear regression model and 1.96 is the z score associated with providing a 95% confidence interval.

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

	ATP Relative Light Units (RLU)											
Dilution	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Yeast												
Stock	74,085	16,438	73,659	17,569	562,329	403,921	213,212	138,890	130,697	190,153	26,546	10,243
	83,107	16,208	136,910	19,876	380,609	403,260	256,286	90,444	84,246	123,810	4,591	9,082
	57,226	18,925	93,374	17,321	523,496	398,628	189,477	79,570	109,040	176,086	12,221	9,522
1:2	48,056	16,224	100,266	14,657	212,551	211,151	132,695	71,116	54,568	107,796	16,121	633
	30,045	12,780	121,703	14,870	194,269	217,428	137,381	66,806	72,933	42,624	6,432	712
	42,474	14,295	44,320	15,789	229,132	216,249	119,697	35,546	61,793	16,315	10,278	591
1:10	6,697	3,905	22,243	4,109	59,575	47,976	28,725	10,711	13,058	24,834	3,358	5
	8,015	3,640	10,957	4,246	26,545	49,956	19,189	11,471	9,596	22,681	3,735	25
	6,818	3,520	12,640	4,003	79,490	50,294	22,307	8,442	12,287	24,737	3,448	6
1:100	317	1,010	1,520	909	3,525	3,277	2,284	1,116	957	1,328	381	23
	389	920	1,464	875	6,091	3,184	2,428	932	935	1,348	424	11
	129	1,230	1,542	1,020	4,995	3,674	3,381	1,199	1,138	1,440	308	21
1:500	46	160	347	356	1,015	556	403	241	243	240	110	3
	36	270	395	376	770	556	190	226	262	511	654	18
	116	524	329	425	392	557	371	239	229	481	120	12
1:1,000	46	12	185	15	689	330	283	154	164	191	137	0
	121	17	221	21	592	360	251	83	152	217	75	2
	67	70	61	25	284	372	183	89	123	446	67	0
Bacteria												
Stock	1,474	126	448	119	9,287	9,671	53	4,987	750	1,335	1,344	335
	1,598	136	2,474	137	3,672	13,718	47	3,246	1,404	2,087	3,328	471
	2,234	146	1,550	156	8,046	10,435	63	4,396	1,599	1,750	2,429	381
1:2	980	118	754	130	3,577	6,403	27	5,095	845	1,218	875	226
	1,097	94	1,073	126	1,843	6,431	29	2,331	813	2,252	1,730	190
	1,683	106	948	121	3,448	6,374	32	1,308	1,079	1,060	1,728	301
1:10	390	61	196	71	670	876	20	771	77	247	232	62
	225	67	97	75	577	858	17	406	110	271	357	19
	370	57	131	62	996	877	17	645	235	228	248	11
1:100	51	19	43	22	39	181	16	66	52	75	67	54
	35	20	62	29	39	179	10	33	24	41	34	1
	46	14	32	31	50	176	13	67	24	52	74	21

TABLE II
Detection of Yeast and Bacteria in Water Using Adenosine Triphosphate (ATP)

	Colony Forming Units (CFU)											
Dilution	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Yeast												
Calculated stock/mL	14×10^4	19×10^5	15×10^4	25×10^5	56×10^4	40×10^4	53×10^4	20×10^4	89×10^3	71×10^4	NA	26×10^5
10^{-2}	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	1	TNTC
10^{-3}	141	TNTC	151	TNTC	TNTC	TNTC	≈230	196	89	TNTC	0	TNTC
10^{-4}	31	520	3	470	56	40	53	14	6	71	0	260
10^{-5}	4	19	1	25	2	2	2	1	0	10	0	100
10^{-6}	0	8	3	6	1	0	3	0	0	0	0	100
Bacteria												
Calculated stock/mL	39×10^2	65×10^6	17×10^6	72×10^6	10×10^6	16×10^5	59×10^2	67×10^5	36×10^5	41×10^6	47×10^5	22×10^3
10^{-2}	39	TNTC	TNTC	TNTC	TNTC	TNTC	59	TNTC	TNTC	TNTC	TNTC	220
10^{-3}	9	TNTC	TNTC	TNTC	TNTC	TNTC	14	TNTC	TNTC	TNTC	TNTC	200
10^{-4}	0	TNTC	TNTC	TNTC	TNTC	165	1	TNTC	TNTC	TNTC	TNTC	120
10^{-5}	0	484	168	395	105	25	0	67	36	≈330	47	0
10^{-6}	0	65	18	72	6	1	0	9	4	41	3	0

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

Dilution	Collaborator	Mean	Median	Min	Max	SD	CV (%)
Bacteria							
None	1	7.46	7.38	7.30	7.71	0.22	3.0
	2	4.91	4.91	4.84	4.98	0.07	1.5
	3	7.09	7.35	6.10	7.81	0.88	12.5
	4	4.92	4.92	4.78	5.05	0.14	2.8
	5	8.78	8.99	8.21	9.14	0.50	5.7
	6	9.32	9.25	9.18	9.53	0.18	2.0
	7	3.99	3.97	3.85	4.14	0.15	3.7
	8	8.33	8.39	8.09	8.51	0.22	2.6
	9	7.08	7.25	6.62	7.38	0.40	5.7
	10	7.44	7.47	7.20	7.64	0.23	3.0
	11	7.70	7.80	7.20	8.11	0.46	6.0
1:2	1	7.11	7.00	6.89	7.43	0.29	4.0
	2	4.66	4.66	4.54	4.77	0.11	2.4
	3	6.82	6.85	6.63	6.98	0.18	2.6
	4	4.83	4.84	4.80	4.87	0.04	0.7
	5	7.95	8.15	7.52	8.18	0.37	4.7
	6	8.76	8.76	8.76	8.77	0.00	0.1
	7	3.38	3.37	3.30	3.47	0.09	2.5
	8	7.82	7.75	7.18	8.54	0.68	8.7
	9	6.81	6.74	6.70	6.98	0.15	2.3
	10	7.26	7.10	6.97	7.72	0.40	5.5
	11	7.23	7.45	6.77	7.46	0.39	5.4
1:10	1	5.81	5.91	5.54	5.97	0.23	4.0
	2	4.12	4.11	4.04	4.20	0.08	2.0
	3	4.91	4.88	4.57	5.28	0.35	7.2
	4	4.24	4.26	4.13	4.32	0.10	2.3
	5	6.59	6.51	6.36	6.90	0.28	4.3
	6	6.77	6.78	6.75	6.78	0.01	0.2
	7	2.89	2.83	2.83	3.00	0.09	3.2
	8	6.37	6.47	6.01	6.65	0.33	5.2
	9	4.83	4.70	4.34	5.46	0.57	11.8
	10	5.51	5.51	5.43	5.60	0.09	1.6
	11	5.61	5.51	5.45	5.88	0.23	4.1
1:100	1	3.77	3.83	3.56	3.93	0.19	5.2
	2	2.86	2.94	2.64	3.00	0.19	6.7
	3	3.78	3.76	3.47	4.13	0.33	8.8
	4	3.30	3.37	3.09	3.43	0.18	5.5
	5	3.75	3.66	3.66	3.91	0.14	3.8
	6	5.19	5.19	5.17	5.20	0.01	0.3
	7	2.55	2.56	2.30	2.77	0.24	9.2
	8	3.96	4.19	3.50	4.20	0.40	10.2
	9	3.44	3.18	3.18	3.95	0.45	13.0
	10	3.99	3.95	3.71	4.32	0.30	7.6
	11	4.01	4.20	3.53	4.30	0.42	10.6
Yeast							
None	1	11.17	11.21	10.95	11.33	0.19	1.7
	2	9.75	9.71	9.69	9.85	0.09	0.9
	3	11.49	11.44	11.21	11.83	0.31	2.7
	4	9.81	9.77	9.76	9.90	0.08	0.8
	5	13.09	13.17	12.85	13.24	0.21	1.6
	6	12.90	12.91	12.90	12.91	0.01	0.1
	7	12.29	12.27	12.15	12.45	0.15	1.2
	8	11.51	11.41	11.28	11.84	0.29	2.5
	9	11.57	11.60	11.34	11.78	0.22	1.9
	10	11.99	12.08	11.73	12.16	0.23	1.9

(*continued on next page*)

[a] Valid $N = 3$.

With respect to the analysis of ATP in water samples containing bacteria, although CV values indicated good repeatability of the method within a laboratory a comparison of the linear relationship between CFU and RLU between laboratories (Fig. 2) indicated that only 16.2% ($R^2 = 0.162$) of the variability of ln(RLU) for bacteria was explained, due to a significant number of outliers and a high dispersion of data between laboratories, and was deemed to be unacceptable.

Although data from analysis of ATP in water containing bacteria did not yield a good statistically predictive linear regression model, it should be noted that an increase in RLU was observed with increasing cell concentrations within individual data sets. This indicates there is an increase in ATP as a consequence of greater numbers of bacteria, even though each collaborator reported variation in the RLU obtained. It is suggested that the variability in the data may be the result of differences in terms of cellular energy between

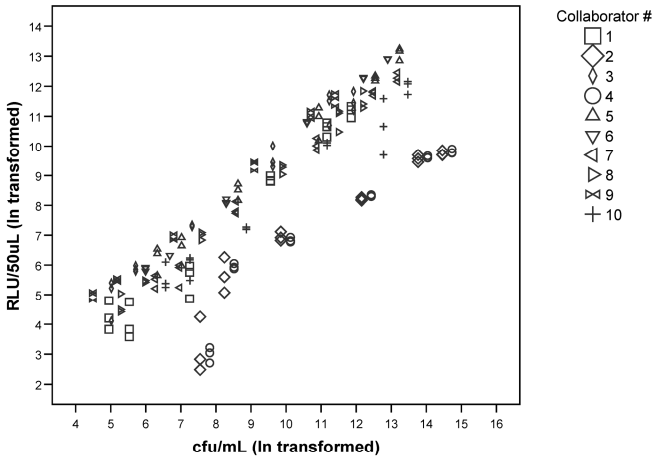
TABLE III
(*continued from preceding page*)[a]

Dilution	Collaborator	Mean	Median	Min	Max	SD	CV (%)
Yeast							
1:2	1	10.58	10.66	10.31	10.78	0.24	2.3
	2	9.57	9.57	9.46	9.69	0.12	1.2
	3	11.31	11.52	10.70	11.71	0.54	4.7
	4	9.62	9.61	9.59	9.67	0.04	0.4
	5	12.26	12.27	12.18	12.34	0.08	0.7
	6	12.28	12.28	12.26	12.29	0.02	0.1
	7	11.77	11.80	11.69	11.83	0.07	0.6
	8	10.92	11.11	10.48	11.17	0.38	3.5
	9	11.05	11.03	10.91	11.20	0.15	1.3
	10	10.65	10.66	9.70	11.59	0.94	8.9
1:10	1	8.88	8.83	8.81	8.99	0.10	1.1
	2	8.21	8.20	8.17	8.27	0.05	0.6
	3	9.59	9.44	9.30	10.01	0.37	3.9
	4	8.32	8.32	8.29	8.35	0.03	0.4
	5	10.82	10.99	10.19	11.28	0.57	5.3
	6	10.81	10.82	10.78	10.83	0.03	0.2
	7	10.05	10.01	9.86	10.27	0.20	2.0
	8	9.22	9.28	9.04	9.35	0.16	1.7
	9	9.35	9.42	9.17	9.48	0.16	1.7
	10	10.09	10.12	10.03	10.12	0.05	0.5
1:100	1	5.53	5.76	4.86	5.96	0.59	10.6
	2	6.95	6.92	6.82	7.11	0.15	2.1
	3	7.32	7.33	7.29	7.34	0.03	0.4
	4	6.84	6.81	6.77	6.93	0.08	1.2
	5	8.47	8.52	8.17	8.71	0.28	3.3
	6	8.12	8.09	8.07	8.21	0.08	0.9
	7	7.88	7.79	7.73	8.13	0.21	2.7
	8	6.98	7.02	6.84	7.09	0.13	1.9
	9	6.91	6.86	6.84	7.04	0.11	1.6
	10	7.22	7.21	7.19	7.27	0.04	0.6
1:500	1	4.06	3.83	3.58	4.75	0.62	15.2
	2	5.65	5.60	5.08	6.26	0.59	10.5
	3	5.87	5.85	5.80	5.98	0.09	1.6
	4	5.95	5.93	5.87	6.05	0.09	1.5
	5	6.51	6.65	5.97	6.92	0.49	7.5
	6	6.32	6.32	6.32	6.32	0.00	0.0
	7	5.72	5.92	5.25	6.00	0.41	7.20
	8	5.46	5.48	5.42	5.48	0.03	0.6
	9	5.50	5.49	5.43	5.57	0.07	1.2
	10	5.96	6.18	5.48	6.24	0.42	7.0
1:1,000	1	4.28	4.20	3.83	4.80	0.49	11.4
	2	3.19	2.83	2.48	4.25	0.93	29.3
	3	4.91	5.22	4.11	5.40	0.70	14.2
	4	2.99	3.04	2.71	3.22	0.26	8.7
	5	6.19	6.38	5.65	6.54	0.47	7.7
	6	5.87	5.89	5.80	5.92	0.06	1.1
	7	5.46	5.53	5.21	5.65	0.23	4.1
	8	4.65	4.49	4.42	5.04	0.34	7.3
	9	4.98	5.02	4.81	5.10	0.15	3.0
	10	5.58	5.38	5.25	6.10	0.46	8.2

sample groups. This is likely to have arisen due to variation in the physiological condition of each culture between laboratories. Consequently, it is suggested that the evaluation of water containing bacteria should be repeated with more precise directions regarding the growth, maintenance, and handling of bacterial cultures. It is anticipated that this will lead to improved physiological consistency of bacterial cultures and more precise data.

LITERATURE CITED

1. Bender, F. E., Douglas, L. W., and Kramer, D. S. *Statistical Methods for Food and Agriculture*. The AVI Publishing Company, Inc., Westport, CT. Pp.169-190, 1982.
2. Kiemele, M. J., and Schmidt, S. R. *Basic Statistics: Tools for Continuous Improvement*, 3rd ed. Air Academy Press, Colorado Springs, CO. Pp. 21-22, 1993.



Model Summary[a]

Model	*R*	R^2	Adjusted R^2	SE of Estimate
1	0.850[b]	0.722	0.720	1.44850

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

ANOVA[a]

Model 1	Sum of Squares	df	Mean Square	*F*	Sigma
Regression	968.348	1	968.48	461.526	0.000[b]
Residual	373.470	178	2.098		
Total	1,341.818	179			

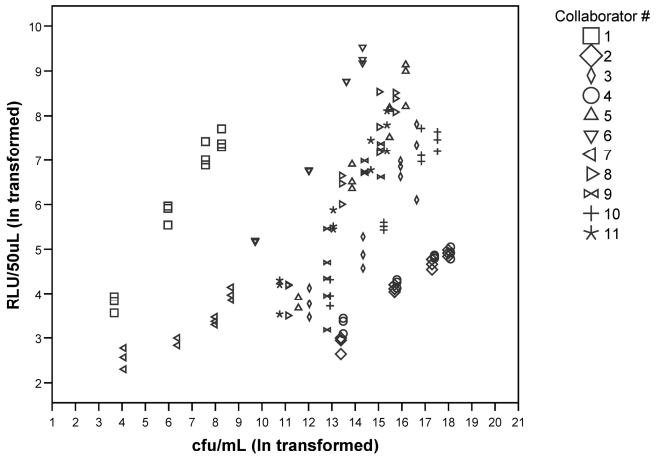
[a] Dependent variable: lnRLU.
[b] Predictors: (constant), lnCFU.

Coefficients[a]

	Unstandardized Coefficients		Standardized Coefficients			95% Confidence Interval for *B*	
Model 1	*B*	SE	Beta	*t*	Sigma	Lower Bound	Upper Bound
(Constant)	0.572	0.376		1.521	0.130	–0.170	1.313
lnCFU	0.816	0.038	0.850	21.483	0.000	0.741	0.891

[a] Dependent variable: lnRLU.

Fig. 1. Yeast regression model.



Model Summary[a]

Model	*R*	R^2	Adjusted R^2	SE of Estimate
1	0.402[b]	0.162	0.156	1.71063

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

ANOVA[a]

Model 1	Sum of Squares	df	Mean Square	*F*	Sigma
Regression	73.540	1	73.540	25.131	0.000[b]
Residual	380.415	130	2.926		
Total	453.955	131			

[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)	2.979	0.550		5.416	0.000	1.891	4.068
lnCFU	0.203	0.041	0.402	5.013	0.000	0.123	0.284

[a] Dependent variable: lnRLU.

Fig. 2. Bacteria regression model.

Miniature Fermentation Method

Subcommittee Members: A. Speers, *Chair*; D. Bendiak; J. Caudill; D. Cook; S. E. Eck; B. Fernandez; B. Gibson; A. MacLeod; J. Monroe; N. Parker; A. Porter; R. Schuba; S. Walker (EBC); L. White; M. Voetz (EBC); and C. Powell (*ex officio*)

Keywords: Apparent degree of fermentation, In vitro fermentation

CONCLUSIONS

1. Selected testing times reported in the method below are appropriate to provide estimates of original extract (OE) and apparent extract (AE) using SMA and He-Bru yeast strains and a nonlinear logistic model.
2. A *t* test revealed no significant difference between the apparent degree of fermentation (ADF) values for the two yeast strains (mean value of 0.83). The ADF within-lab error or repeatability values averaged 0.019 and 0.022 for the SMA and He-Bru strains, respectively. The reproducibility or between-lab error (standard deviation) for the ADF values was 0.028 and 0.022 for the SMA and He-Bru strains, respectively.

RECOMMENDATIONS

1. The method appears to be suitable for the determination of ADF, especially when examining the effect of different yeast strains and fermentation conditions.
2. It is proposed that the chair investigate the feasibility of supplying the collaborators with a Microsoft Excel template to undertake the modeling of the logistic equation to easily determine the best-fit curve and, thus, good estimates of OE, AE, and ADF values.
3. It is further proposed that this study should be continued for a second year using either malt or wort samples (to be decided after polling of the subcommittee).

This is the first year of the subcommittee's existence. The subcommittee was initiated after discussions during the 2009 ASBC Annual Meeting. 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. The aim of this subcommittee is to evaluate an alternative mini-fermentation method (4). To assess the new method's potential to identify yeast defects, two strains with different flocculence were selected by the committee: SMA and He-Bru. A commercial malt extract was used as the fermentation medium. During the first year of the collaborative study, the committee focused on determining appropriate sampling times for commercial laboratories and developing an appropriate statistical analysis for the mini-fermentation procedure (1). It is expected that a variety of malt types will be examined in future years.

PROCEDURE

SMA and He-Bru yeast strains (VLB) were precultivated on YEPD agar and grown aerobically in YEPD broth (Difco Laboratories) for 24 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 (Yeast-4). Subsequently, the yeast was pitched into synthetic wort (150 g of malt extract [Difco] per liter to yield a solution of approx. 14°P) to provide an initial concentration of 1.5×10^7 cells/mL. Immediately, 15-mL aliquots of this pitched wort were aseptically distributed into 40 individual fermentation tubes and maintained at 21°C. Fermentations were monitored by destructive sampling, whereby experimental tubes were removed for analysis at regular time points over a 78-hr fermentation period. In each instance, yeast growth was monitored by analysis of turbidity at 600 nm, and fermentation progression was determined by gravity (degrees Plato) analysis. Sampling was performed in quadruplicate, and in each instance, the collected gravity data were analyzed using a nonlinear logistic model as described previously (3,4).

RESULTS AND DISCUSSION

Results from 13 collaborators were received. All collaborators provided fermentation data similar to that shown in Figure 1, using both the SMA and He-Bru lager strains. While most collaborators undertook quadruplicate testing at the time periods recommended, a few supplied single, duplicate, or triplicate readings, as shown in Tables I and II. The absorbance data were difficult to meaure and

doi:10.1094/ASBCJ-2010-0929-01

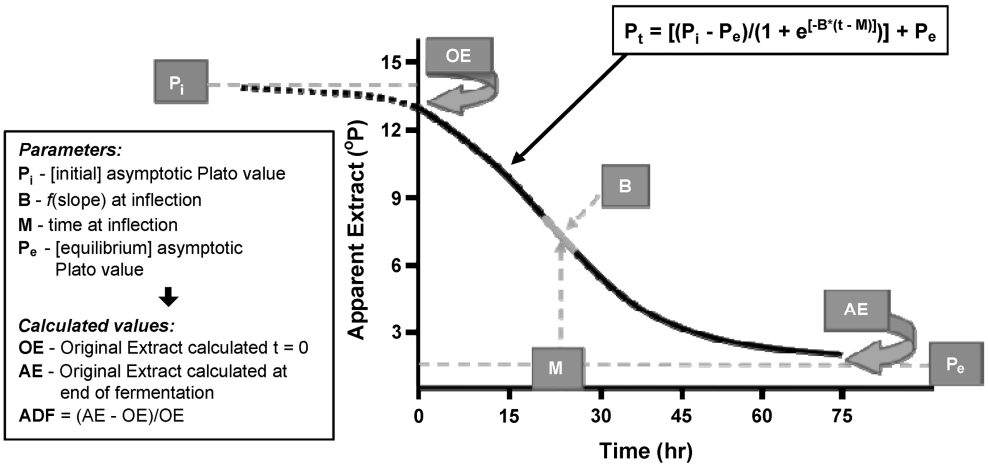


Fig. 1. Estimation of original and apparent extract (OE and AE, respectively) from the fit of the logistic model.

TABLE I
Fermentation Gravity (°P) for SMA Yeast Strain[a]

	Collaborator												
Time (hr)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
0	13.683	14.1	14	13.1	14.5	14.162	15	12.6	13.4	14.09	12.9	14.08	13.710
0	13.683	14.1	14	13.1	…	14.162	15	…	13.4	…	12.9	…	13.705
0	13.683	14.1	14	13.1	…	14.162	15	…	13.3	…	12.9	…	13.710
0	13.683	14.1	14	13.1	…	14.162	15	…	13.4	…	12.9	…	13.703
5	13.207	…	…	…	…	…	…	…	…	13.44	…	…	…
5	13.232	…	…	…	…	…	…	…	…	…	…	…	…
5	13.18	…	…	…	…	…	…	…	…	…	…	…	…
5	13.097	…	…	…	…	…	…	…	…	…	…	…	…
6	…	13.1	13.3	12.5	…	13.414	…	…	12.9	…	12	14.18	13.151
6	…	13.2	13.3	12.5	…	13.811	…	…	12.7	…	12.1	…	13.158
6	…	13.2	13.3	12.4	…	14.109	…	…	12.7	…	12.1	…	13.163
6	…	13.1	13.3	12.4	…	13.977	…	…	12.5	…	12	…	13.147
8	…	…	…	…	…	…	…	11.62	…	…	…	…	…
8	…	…	…	…	…	…	…	…	…	…	…	…	…
8	…	…	…	…	…	…	…	…	…	…	…	…	…
8	…	…	…	…	…	…	…	…	…	…	…	…	…
18	…	…	…	…	11.6	…	…	…	…	…	…	…	…
18	…	…	…	…	…	…	…	…	…	…	…	…	…
18	…	…	…	…	…	…	…	…	…	…	…	…	…
18	…	…	…	…	…	…	…	…	…	…	…	…	…
19	…	…	…	…	…	…	7.6	…	…	…	…	…	…
19	…	…	…	…	…	…	7.4	…	…	…	…	…	…
19	…	…	…	…	…	…	7.6	…	…	…	…	…	…
19	…	…	…	…	…	…	8.8	…	…	…	…	…	…
20.5	…	…	…	…	…	…	…	…	…	…	…	…	7.022
20.5	…	…	…	…	…	…	…	…	…	…	…	…	7.042
20.5	…	…	…	…	…	…	…	…	…	…	…	…	7.125
20.5	…	…	…	…	…	…	…	…	…	…	…	…	7.059
22	6.712	4	5.7	5.2	11.1	10.414	…	…	5.8	9.71	7.6	12.75	…
22	5.993	4.3	5.6	5.4	…	10.629	…	…	5.5	…	5.6	…	…
22	5.803	3.9	5.6	5.3	…	10.58	…	…	5.5	…	6.6	…	…
22	8.362	4	5.8	5.4	…	10.701	…	…	5.8	…	6.5	…	…
24	…	…	…	…	…	…	5.1	7.33	…	…	…	…	…
24	…	…	…	…	…	…	5.4	9.37	…	…	…	…	…
24	…	…	…	…	…	…	5.7	8.28	…	…	…	…	…
24	…	…	…	…	…	…	6.3	…	…	…	…	…	…
25	…	…	…	…	…	…	…	…	…	…	…	…	5.861
25	…	…	…	…	…	…	…	…	…	…	…	…	5.625
25	…	…	…	…	…	…	…	…	…	…	…	…	…
25	…	…	…	…	…	…	…	…	…	…	…	…	…
26	…	3.3	…	…	10.3	9.381	…	…	7.1	8.95	…	…	…
26	…	3.4	…	…	…	10.14	…	…	3.7	…	…	…	…
26	…	3.3	…	…	…	9.922	…	…	5.3	…	…	…	…
26	…	3.5	…	…	…	9.507	…	…	5.5	…	…	…	…
29	4.357	…	…	…	…	…	…	…	…	8.06	…	…	…
29	5.219	…	…	…	…	…	…	…	…	…	…	…	…
29	4.375	…	…	…	…	…	…	…	…	…	…	…	…
29	4.647	…	…	…	…	…	…	…	…	…	…	…	…
30	…	1.8	2.5	2.7	…	6.68	…	…	2.6	…	8.9	11.18	4.887
30	…	1.8	2.8	2.9	…	6.73	…	…	2.7	…	6.6	…	4.776
30	…	1.8	2.5	3	…	10.139	…	…	2.8	…	4.1	…	4.987
30	…	1.8	2.8	2.9	…	7.974	…	…	3.1	…	6.5	…	4.974
34	…	…	…	…	…	…	…	6.07	…	…	…	…	…
34	…	…	…	…	…	…	…	8.35	…	…	…	…	…
34	…	…	…	…	…	…	…	5.47	…	…	…	…	…
34	…	…	…	…	…	…	…	…	…	…	…	…	…
34.5	…	…	…	…	…	…	…	…	…	…	…	…	3.639
34.5	…	…	…	…	…	…	…	…	…	…	…	…	3.896
34.5	…	…	…	…	…	…	…	…	…	…	…	…	3.725
34.5	…	…	…	…	…	…	…	…	…	…	…	…	4.004
43	…	…	…	…	…	…	2.2	…	…	…	…	…	…
43	…	…	…	…	…	…	2.2	…	…	…	…	…	…
43	…	…	…	…	…	…	2.3	…	…	…	…	…	…
43	…	…	…	…	…	…	2.2	…	…	…	…	…	…
44	2.155	…	…	…	…	…	…	…	…	…	…	…	…
44	2.336	…	…	…	…	…	…	…	…	…	…	…	…
44	2.191	…	…	…	…	…	…	…	…	…	…	…	…
44	3.096	…	…	…	…	…	…	…	…	…	…	…	…
46	…	1.8	2	2.2	5.2	9.005	…	…	2.3	4.25	3.5	6.35	…
46	…	1.8	2	2.3	…	3.316	…	…	2.1	…	3.6	…	…
46	…	1.8	2	2.3	…	3.641	…	…	2.2	…	3.3	…	…
46	…	1.8	2	2.2	…	8.585	…	…	2.1	…	…	…	…
47	…	…	…	…	…	…	…	…	…	…	…	…	…
47	…	…	…	…	…	…	…	…	…	…	…	…	…
47	…	…	…	…	…	…	…	…	…	…	…	…	…
47	…	…	…	…	…	…	…	…	…	…	…	…	…
48	2.137	…	…	…	…	…	2.1	5.74	…	…	…	…	3.308
48	2.81	…	…	…	…	…	2.1	5.23	…	…	…	…	3.377
48	2.429	…	…	…	…	…	2.3	4.53	…	…	…	…	3.270
48	2.25	…	…	…	…	…	2.2	…	…	…	…	…	3.951
50	…	1.8	2	2.3	…	3.605	…	…	2.2	4.19	4	5.82	…
50	…	1.8	2	2.2	…	3.64	…	…	2.2	…	3	…	…

(*continued on next page*)

[a] Note, not all extract values were measured at identical times. "…" indicates data not supplied.

TABLE I
***(continued from preceding page)*[a]**

	Collaborator												
Time (hr)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
50	…	1.8	1.9	2.3	…	3.106	…	…	2.1	…	2	…	…
50	…	1.8	1.9	2.3	…	3.055	…	…	2.2	…	2.9	…	…
51	…	…	…	…	…	…	…	…	…	…	…	…	…
51	…	…	…	…	…	…	…	…	…	…	…	…	…
51	…	…	…	…	…	…	…	…	…	…	…	…	…
51	…	…	…	…	…	…	…	…	…	…	…	…	…
52	2.191	…	…	…	3.2	…	…	…	…	…	…	…	…
52	2.206	…	…	…	…	…	…	…	…	…	…	…	…
52	2.086	…	…	…	…	…	…	…	…	…	…	…	…
52	2.149	…	…	…	…	…	…	…	…	…	…	…	…
53	…	…	…	…	…	…	…	5.67	…	4.15	…	…	…
53	…	…	…	…	…	…	…	7.37	…	…	…	…	…
53	…	…	…	…	…	…	…	6.14	…	…	…	…	…
53	…	…	…	…	…	…	…	…	…	…	…	…	…
54	…	1.8	2	2.4	…	4.575	…	…	2.2	…	…	4.63	3.035
54	…	1.8	1.9	2.2	…	4.082	…	…	2.2	…	…	…	3.068
54	…	1.8	1.9	2.2	…	2.273	…	…	2.1	…	…	…	3.248
54	…	1.8	1.9	2.2	…	3.74	…	…	2.2	…	…	…	3.179
56	…	…	…	…	2.2	…	…	…	…	…	1.9	…	…
56	…	…	…	…	…	…	…	…	…	…	2	…	…
56	…	…	…	…	…	…	…	…	…	…	1.9	…	…
56	…	…	…	…	…	…	…	…	…	…	1.9	…	…
57	…	…	…	…	…	…	…	…	…	…	…	…	…
57	…	…	…	…	…	…	…	…	…	…	…	…	…
57	…	…	…	…	…	…	…	…	…	…	…	…	…
57	…	…	…	…	…	…	…	…	…	…	…	…	…
58	…	…	…	…	…	…	…	6.31	…	…	…	…	…
58	…	…	…	…	…	…	…	4.21	…	…	…	…	…
58	…	…	…	…	…	…	…	5.92	…	…	…	…	…
58	…	…	…	…	…	…	…	…	…	…	…	…	…
61	…	…	…	…	3.3	…	…	…	…	…	…	…	…
61	…	…	…	…	…	…	…	…	…	…	…	…	…
61	…	…	…	…	…	…	…	…	…	…	…	…	…
61	…	…	…	…	…	…	…	…	…	…	…	…	…
67.5	…	…	…	…	…	…	2.1	…	…	…	…	…	…
67.5	…	…	…	…	…	…	2.1	…	…	…	…	…	…
67.5	…	…	…	…	…	…	2.3	…	…	…	…	…	…
67.5	…	…	…	…	…	…	2.2	…	…	…	…	…	…
68	3.58	…	…	…	…	…	…	…	…	…	…	…	…
68	2.26	…	…	…	…	…	…	…	…	…	…	…	…
68	3.075	…	…	…	…	…	…	…	…	…	…	…	…
68	2.213	…	…	…	…	…	…	…	…	…	…	…	…
70	…	1.9	1.9	2.1	…	4.481	…	…	2.4	2.85	…	3.13	3.217
70	…	1.9	2	2.2	…	1.967	…	…	2.3	…	…	…	2.916
70	…	1.9	1.9	2.2	…	2.196	…	…	2.3	…	…	…	2.785
70	…	1.9	2	2.2	…	2.889	…	…	2.3	…	…	…	3.260
71	…	…	…	…	…	…	…	…	…	…	1.9	…	…
71	…	…	…	…	…	…	…	…	…	…	3	…	…
71	…	…	…	…	…	…	…	…	…	…	2.4	…	…
71	…	…	…	…	…	…	…	…	…	…	4.5	…	…
71.5	…	…	…	…	…	…	2.1	…	…	…	…	…	…
71.5	…	…	…	…	…	…	2.1	…	…	…	…	…	…
71.5	…	…	…	…	…	…	2.2	…	…	…	…	…	…
71.5	…	…	…	…	…	…	2.2	…	…	…	…	…	…
72	…	…	…	…	…	…	…	3.13	…	…	…	…	…
72	…	…	…	…	…	…	…	4.26	…	…	…	…	…
72	…	…	…	…	…	…	…	5.2	…	…	…	…	…
72	…	…	…	…	…	…	…	…	…	…	…	…	…
73	2.101	…	…	…	…	…	…	…	…	…	…	…	…
73	2.078	…	…	…	…	…	…	…	…	…	…	…	…
73	2.087	…	…	…	…	…	…	…	…	…	…	…	…
73	2.09	…	…	…	…	…	…	…	…	…	…	…	…
74	…	1.9	1.9	2.2	…	2.98	…	…	2.2	2.28	1.8	2.01	…
74	…	1.9	1.9	2.2	…	1.962	…	…	2.2	…	3.5	…	…
74	…	1.9	1.9	2.2	…	2.024	…	…	…	…	3.9	…	…
74	…	1.9	1.9	2.2	…	3.237	…	…	…	…	4.1	…	…
75	…	…	…	…	…	…	2.1	…	…	…	…	…	…
75	…	…	…	…	…	…	2.1	…	…	…	…	…	…
75	…	…	…	…	…	…	2.1	…	…	…	…	…	…
75	…	…	…	…	…	…	2.1	…	…	…	…	…	…
76	…	…	…	…	2.2	…	…	…	…	…	…	…	…
76	…	…	…	…	…	…	…	…	…	…	…	…	…
76	…	…	…	…	…	…	…	…	…	…	…	…	…
76	…	…	…	…	…	…	…	…	…	…	…	…	…
77	2.107	…	…	…	…	…	…	1.66	…	2.65	…	…	2.927
77	2.136	…	…	…	…	…	…	5.27	…	…	…	…	2.353
77	2.13	…	…	…	…	…	…	4.61	…	…	…	…	2.353
77	2.13	…	…	…	…	…	…	…	…	…	…	…	2.534
78	…	1.9	1.9	2.1	3.0	1.859	…	2.8	2.2	…	…	1.93	…
78	…	1.9	1.9	2.3	…	2.016	…	3.13	2.2	…	…	…	…
78	…	1.9	1.9	2.3	…	3.654	…	3.9	…	…	…	…	…
78	…	1.9	1.9	2.2	…	3.058	…	…	…	…	…	…	…
78.5	…	…	…	…	…	…	…	…	…	…	4.1	…	…
78.5	…	…	…	…	…	…	…	…	…	…	2.6	…	…
78.5	…	…	…	…	…	…	…	…	…	…	3.3	…	…
78.5	…	…	…	…	…	…	…	…	…	…	…	…	…

TABLE II
Fermentation Gravity (°P) for He-Bru Yeast Strain[a]

	Collaborator											
Time (hr)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**13**
0	13.683	14.1	14	13.1	15.3	14.166	15	12.6	11.4	14.18	13.1	13.785
0	13.683	14.1	14	13.1	…	14.166	15	…	…	…	13.1	13.795
0	13.683	14.1	14	13.1	…	14.166	15	…	…	…	13.2	13.795
0	13.683	14.1	14	13.1	…	14.166	15	…	…	…	13.3	13.795
5	13.053	…	…	…	…	…	…	…	…	13.63	…	…
5	13.169	…	…	…	…	…	…	…	…	…	…	…
5	13.138	…	…	…	…	…	…	…	…	…	…	…
5	13.229	…	…	…	…	…	…	…	…	…	…	…
6	…	12.8	13.4	12.2	…	14.15	…	…	10.3	…	12.3	13.343
6	…	12.7	13.4	12.4	…	14.58	…	…	10.3	…	12.3	13.359
6	…	12.7	13.4	12.5	…	13.871	…	…	10.1	…	12.3	13.352
6	…	12.7	13.4	12.4	…	13.62	…	…	10.6	…	12.3	13.354
8	…	…	…	…	…	…	…	11.54	…	…	…	…
8	…	…	…	…	…	…	…	11.51	…	…	…	…
8	…	…	…	…	…	…	…	…	…	…	…	…
8	…	…	…	…	…	…	…	…	…	…	…	…
18	…	…	…	…	12.2	…	…	…	…	…	…	…
18	…	…	…	…	…	…	…	…	…	…	…	…
18	…	…	…	…	…	…	…	…	…	…	…	…
18	…	…	…	…	…	…	…	…	…	…	…	…
19	…	…	…	…	…	…	6.8	…	…	…	…	…
19	…	…	…	…	…	…	6.5	…	…	…	…	…
19	…	…	…	…	…	…	8.3	…	…	…	…	…
19	…	…	…	…	…	…	7.7	…	…	…	…	…
20.5	…	…	…	…	…	…	…	…	…	…	…	6.795
20.5	…	…	…	…	…	…	…	…	…	…	…	6.676
20.5	…	…	…	…	…	…	…	…	…	…	…	6.708
20.5	…	…	…	…	…	…	…	…	…	…	…	6.659
22	8.076	3.3	5	5.4	11.9	10.725	…	…	2.8	8.99	6.5	…
22	6.994	3.5	5	5.5	…	10.658	…	…	3.8	…	6.5	…
22	8.086	3	5.2	5.4	…	9.403	…	…	3.5	…	…	…
22	7.996	3.2	5.1	5.4	…	9.198	…	…	3.1	…	…	…
24	…	…	…	…	…	…	4	8.33	…	…	…	…
24	…	…	…	…	…	…	3.9	8.39	…	…	…	…
24	…	…	…	…	…	…	5.2	7.16	…	…	…	…
24	…	…	…	…	…	…	4.3	…	…	…	…	…
25	…	…	…	…	…	…	…	…	…	…	…	4.809
25	…	…	…	…	…	…	…	…	…	…	…	4.789
25	…	…	…	…	…	…	…	…	…	…	…	…
25	…	…	…	…	…	…	…	…	…	…	…	5.046
26	…	3.5	…	…	10.7	10.532	…	…	1.7	8.99	…	…
26	…	3.3	…	…	…	9.217	…	…	1.7	…	…	…
26	…	3.3	…	…	…	6.87	…	…	1.7	…	…	…
26	…	3.5	…	…	…	7.883	…	…	2.4	…	…	…
29	5.95	…	…	…	…	…	…	…	…	7.04	…	…
29	7.259	…	…	…	…	…	…	…	…	…	…	…
29	7.032	…	…	…	…	…	…	…	…	…	…	…
29	8.592	…	…	…	…	…	…	…	…	…	…	…
30	…	1.8	2.5	3	…	9.288	…	…	1.6	…	4.5	3.820
30	…	1.8	2.6	3	…	9.205	…	…	1.4	…	5	3.848
30	…	1.8	2.6	3.1	…	6.33	…	…	1.5	…	3.4	3.573
30	…	1.8	2.4	3.1	…	…	…	…	1.5	…	4.1	3.490
34	…	…	…	…	…	…	…	8.32	…	…	…	…
34	…	…	…	…	…	…	…	7.46	…	…	…	…
34	…	…	…	…	…	…	…	7.07	…	…	…	…
34	…	…	…	…	…	…	…	…	…	…	…	…
34.5	…	…	…	…	…	…	…	…	…	…	…	2.170
34.5	…	…	…	…	…	…	…	…	…	…	…	2.714
34.5	…	…	…	…	…	…	…	…	…	…	…	2.208
34.5	…	…	…	…	…	…	…	…	…	…	…	2.198
43	…	…	…	…	…	…	2.2	…	…	…	…	…
43	…	…	…	…	…	…	2.2	…	…	…	…	…
43	…	…	…	…	…	…	2.2	…	…	…	…	…
43	…	…	…	…	…	…	2.2	…	…	…	…	…
44	2.419	…	…	…	…	…	…	…	…	…	…	…
44	4.515	…	…	…	…	…	…	…	…	…	…	…
44	3.926	…	…	…	…	…	…	…	…	…	…	…
44	5.692	…	…	…	…	…	…	…	…	…	…	…
46	…	1.8	2	2.4	5.7	6.955	…	…	1.7	3.21	3.8	…
46	…	1.8	2	2.3	…	4.911	…	…	1.7	…	3.7	…
46	…	1.8	2	2.4	…	2.387	…	…	1.6	…	2.8	…
46	…	1.8	2	2.3	…	3.214	…	…	1.6	…	2.6	…
47	…	…	…	…	…	…	…	…	…	…	…	…
47	…	…	…	…	…	…	…	…	…	…	…	…
47	…	…	…	…	…	…	…	…	…	…	…	…
47	…	…	…	…	…	…	…	…	…	…	…	…
48	2.268	…	…	…	…	…	2.3	5.48	…	…	…	2.091
48	2.152	…	…	…	…	…	2.3	6.6	…	…	…	2.091
48	3.24	…	…	…	…	…	2.3	6.98	…	…	…	2.188
48	3.231	…	…	…	…	…	2.3	…	…	…	…	2.084
50	…	1.8	2	2.4	…	2.866	…	…	1.7	3.83	4	…
50	…	1.8	2	2.3	…	3.225	…	…	1.6	…	3.4	…

(*continued on next page*)

[a] Note, not all extract values were measured at identical times. "…" indicates data not supplied.

TABLE II
(*continued from preceding page*)[a]

	Collaborator											
Time (hr)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**13**
50	…	1.8	2	2.3	…	7.453	…	…	1.6	…	3.3	…
50	…	1.8	1.9	2.3	…	2.036	…	…	1.6	…	3.4	…
51	…	…	…	…	…	…	…	…	…	…	…	…
51	…	…	…	…	…	…	…	…	…	…	…	…
51	…	…	…	…	…	…	…	…	…	…	…	…
51	…	…	…	…	…	…	…	…	…	…	…	…
52	4.81	…	…	…	4.5	…	…	…	…	…	…	…
52	2.326	…	…	…	…	…	…	…	…	…	…	…
52	3.174	…	…	…	…	…	…	…	…	…	…	…
52	7.103	…	…	…	…	…	…	…	…	…	…	…
53	…	…	…	…	…	…	…	7.34	…	2.23	…	…
53	…	…	…	…	…	…	…	6.07	…	…	…	…
53	…	…	…	…	…	…	…	4.46	…	…	…	…
53	…	…	…	…	…	…	…	…	…	…	…	…
54	…	1.8	2	2.4	…	4.488	…	…	1.8	…	…	2.058
54	…	1.8	1.9	2.3	…	4.278	…	…	1.6	…	…	2.063
54	…	1.8	2	2.3	…	3.03	…	…	1.6	…	…	2.112
54	…	1.8	2	2.4	…	4.756	…	…	1.6	…	…	2.066
56	…	…	…	…	2.3	…	…	…	…	…	4.1	…
56	…	…	…	…	…	…	…	…	…	…	3.5	…
56	…	…	…	…	…	…	…	…	…	…	3.5	…
56	…	…	…	…	…	…	…	…	…	…	3.3	…
57	…	…	…	…	…	…	…	…	…	…	…	…
57	…	…	…	…	…	…	…	…	…	…	…	…
57	…	…	…	…	…	…	…	…	…	…	…	…
57	…	…	…	…	…	…	…	…	…	…	…	…
58	…	…	…	…	…	…	…	4.53	…	…	…	…
58	…	…	…	…	…	…	…	5.69	…	…	…	…
58	…	…	…	…	…	…	…	5	…	…	…	…
58	…	…	…	…	…	…	…	…	…	…	…	…
61	…	…	…	…	3.7	…	…	…	…	…	…	…
61	…	…	…	…	…	…	…	…	…	…	…	…
61	…	…	…	…	…	…	…	…	…	…	…	…
61	…	…	…	…	…	…	…	…	…	…	…	…
67.5	…	…	…	…	…	…	2.2	…	…	…	…	…
67.5	…	…	…	…	…	…	2.2	…	…	…	…	…
67.5	…	…	…	…	…	…	2.2	…	…	…	…	…
67.5	…	…	…	…	…	…	2.2	…	…	…	…	…
68	5.749	…	…	…	…	…	…	…	…	…	…	…
68	2.171	…	…	…	…	…	…	…	…	…	…	…
68	2.147	…	…	…	…	…	…	…	…	…	…	…
68	2.128	…	…	…	…	…	…	…	…	…	…	…
70	…	1.8	1.9	2.4	…	2.316	…	…	1.7	2.77	…	2.053
70	…	1.9	1.9	2.4	…	1.918	…	…	1.8	…	…	2.058
70	…	1.8	1.9	2.4	…	5.1	…	…	1.7	…	…	2.061
70	…	1.9	1.9	2.3	…	3.584	…	…	1.7	…	…	2.056
71	…	…	…	…	…	…	…	…	…	…	2.5	…
71	…	…	…	…	…	…	…	…	…	…	2	…
71	…	…	…	…	…	…	…	…	…	…	1.9	…
71	…	…	…	…	…	…	…	…	…	…	1.8	…
71.5	…	…	…	…	…	…	2.2	…	…	…	…	…
71.5	…	…	…	…	…	…	2.2	…	…	…	…	…
71.5	…	…	…	…	…	…	2.2	…	…	…	…	…
71.5	…	…	…	…	…	…	2.2	…	…	…	…	…
72	…	…	…	…	…	…	…	5.89	…	…	…	…
72	…	…	…	…	…	…	…	6.1	…	…	…	…
72	…	…	…	…	…	…	…	4	…	…	…	…
72	…	…	…	…	…	…	…	…	…	…	…	…
73	2.077	…	…	…	…	…	…	…	…	…	…	…
73	2.091	…	…	…	…	…	…	…	…	…	…	…
73	2.109	…	…	…	…	…	…	…	…	…	…	…
73	3.223	…	…	…	…	…	…	…	…	…	…	…
74	…	1.9	1.9	2.4	…	2.05	…	…	…	2.8	3.5	…
74	…	1.9	1.9	2.4	…	3.14	…	…	…	…	2.9	…
74	…	1.9	1.9	2.4	…	3.873	…	…	…	…	2	…
74	…	1.9	1.9	2.4	…	2.921	…	…	…	…	1.8	…
75	…	…	…	…	…	…	2	…	…	…	…	…
75	…	…	…	…	…	…	2.1	…	…	…	…	…
75	…	…	…	…	…	…	2.1	…	…	…	…	…
75	…	…	…	…	…	…	2.1	…	…	…	…	…
76	…	…	…	…	2.3	…	…	…	…	…	…	…
76	…	…	…	…	…	…	…	…	…	…	…	…
76	…	…	…	…	…	…	…	…	…	…	…	…
76	…	…	…	…	…	…	…	…	…	…	…	…
77	2.14	…	…	…	…	…	…	5.9	…	2.68	…	1.880
77	2.105	…	…	…	…	…	…	4	…	…	…	1.837
77	2.151	…	…	…	…	…	…	4.04	…	…	…	1.888
77	2.106	…	…	…	…	…	…	…	…	…	…	1.885
78	…	1.9	2	2.3	2.6	1.962	…	5.18	1.8	…	…	…
78	…	1.9	2	2.2	…	1.842	…	5.64	1.7	…	…	…
78	…	1.9	2	2.3	…	2.139	…	5.9	1.7	…	…	…
78	…	1.9	2	2.3	…	1.828	…	…	1.7	…	…	…
78.5	…	…	…	…	…	…	…	…	…	…	1.7	…
78.5	…	…	…	…	…	…	…	…	…	…	1.7	…
78.5	…	…	…	…	…	…	…	…	…	…	1.7	…
78.5	…	…	…	…	…	…	…	…	…	…	1.7	…

TABLE III
Individual Collaborative Study Results of a Nonlinear Analysis and Determination of Original Extract (OE), Apparent Extract (AE), and Apparent Degree of Fermentation (ADF) for Fermentations Using the SMA Yeast Strain

	Collaborator											
Code[a]	1	2	3	4	5	6	7	9	10	11	12	13
P_e	2.253	1.823	1.919	2.217	2.281	2.310	2.096	2.162	2.235	2.779	1.753	3.012
P_i	14.580	14.480	14.130	13.320	14.900	15.600	15.970	13.900	16.120	15.130	14.530	15.030
B	–0.145	–0.225	–0.222	–0.217	–0.099	–0.079	–0.154	–0.175	–0.073	–0.095	–0.093	–0.141
M	18.440	15.470	18.260	17.590	30.590	28.080	16.830	17.990	24.520	16.480	40.930	16.460
df	36	40	36	36	6	40	28	36	7	34	6	34
r^2	0.989	0.998	1.000	1.000	0.987	0.930	0.996	0.983	0.998	0.932	0.997	0.994
Abs. SS	8.546	2.117	0.270	0.207	2.632	59.280	2.204	12.010	0.457	38.570	0.679	3.802
AE	2.256	1.823	1.919	2.217	2.436	2.623	2.098	2.163	2.581	2.826	2.267	3.015
OE	13.782	14.104	13.923	13.079	14.315	14.309	14.996	13.419	14.118	13.004	14.254	13.952
ADF	0.836	0.871	0.862	0.830	0.830	0.817	0.860	0.839	0.817	0.783	0.841	0.784
ADF within-lab error	0.011	0.004	0.001	0.001	0.037	0.042	0.006	0.012	0.021	0.051	0.023	0.009
Avg. ADF	0.831											
Between-lab error[b]	0.019											

[a] P_e = upper limit to which the function asymptotically approaches; P_i = lower limit to which the function asymptotically approaches; and B = value proportional to the slope at the time of inflection (M).
[b] Standard deviation.

TABLE IV
Individual Collaborative Study Results of a Nonlinear Analysis and Determination of Original Extract (OE), Apparent Extract (AE), and Apparent Degree of Fermentation (ADF) for Fermentations Using the He-Bru Yeast Strain

	Collaborator										
Code[a]	1	2	3	4	5	6	7	9	10	11	13
P_e	2.194	1.846	1.951	2.333	2.038	2.467	2.187	1.645	2.579	2.568	2.002
P_i	18.290	14.810	14.180	13.380	16.170	16.180	15.220	10.540	14.790	15.590	14.430
B	–0.067	–0.217	–0.236	–0.206	–0.082	–0.079	–0.237	–0.558	–0.115	–0.109	–0.178
M	14.700	13.450	17.450	17.360	31.320	24.620	17.180	19.350	24.620	14.710	18.070
df	36	40	36	36	6	39	28	33	7	34	35
r^2	0.934	0.996	1.000	1.000	0.987	0.926	0.995	0.993	0.989	0.974	0.998
Abs. SS	47.000	3.657	0.113	0.165	2.757	61.450	3.201	2.241	2.207	16.260	1.800
AE	2.472	1.846	1.951	2.333	2.415	2.716	2.187	1.645	2.615	2.586	2.002
OE	13.914	14.148	13.984	13.079	15.174	14.471	15.001	10.540	14.117	13.406	13.953
ADF	0.822	0.870	0.860	0.822	0.841	0.812	0.854	0.844	0.815	0.807	0.856
ADF within-lab error	0.073	0.005	0.001	0.001	0.045	0.044	0.006	0.006	0.027	0.027	0.004
Avg. ADF	0.837										
Between-lab error[b]	0.022										

[a] P_e = upper limit to which the function asymptotically approaches; P_i = lower limit to which the function asymptotically approaches; and B = value proportional to the slope at the time of inflection (M).
[b] Standard deviation.

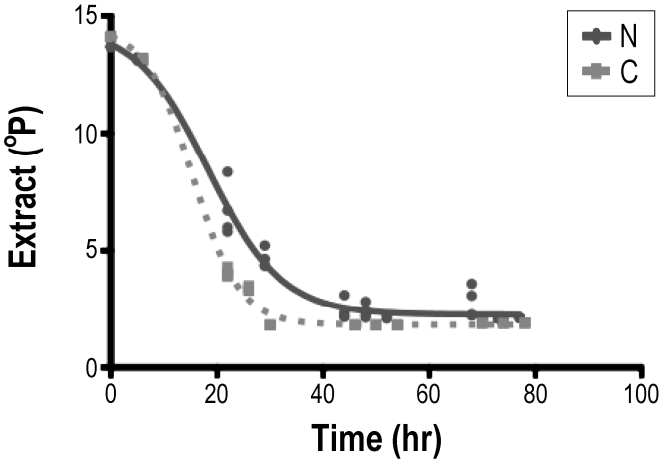


Fig. 2. Example fit of SMA collaborator data sets 1 (solid line) and 2 (dashed line) with the fit of the logistic model.

sure and model due to the high particulate matter present in the malt extract and the high number of data points needed to model the change in absorbance with time. However, the extract data were successfully fit with a nonlinear regression routine (Prism 5.0c, Graph Pad Software) used to obtain the four parameters of the logistic equation

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

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

The results of fitting equation 1 using nonlinear regression analysis (4) are displayed in Tables III and IV. One data set (collaborator 8) did not converge and was eliminated from further analysis. Collaborator 12 only completed the SMA yeast fermentation. Using the parameter fits displayed in Tables III and IV, OE and AE were calculated at 0 and 75 hr, respectively, from the nonlinear fit. Figure 2 depicts a typical fit for two SMA data sets from collaborators 1 and 2. Although individual curves displayed differences during fermentation, nonlinear curve fitting allowed more precise calculation of AE and OE than simple measurement at 0 and 75 hr.

To compare the within- and between-lab errors of the fermentations, ADF was calculated using the following equation:

$$\text{ADF} = (\text{OE} - \text{AE})/\text{OE} \quad (2)$$

ADF was compared for each collaborator and each yeast strain. To estimate the individual ADF within-lab error, a calculation of the propagation of error (2) was undertaken. The algorithm developed to calculate this error used the asymptotic standard errors of the P_i and P_e values derived from the nonlinear regression analysis. The validity of this approach was confirmed by a full calculation of the propagation error from equation 1. These ADF error values were confirmed with a web-based error propagation program (5), one of a number listed by Wikipedia (http://en.wikipedia.org/wiki/List_of_uncertainty_propagation_software).

The individual ADF values, as well as their averages and standard errors (between-lab errors), and the individual ADF errors (within-lab errors) are displayed in Tables III and IV. Examination of the data indicates that the within-lab error values averaged 0.019 and 0.22 for the SMA and He-Bru strains, respectively. The between-lab error or standard deviation of the ADF values was 0.028 and 0.022 for the SMA and He-Bru strains, respectively. It was notable that an unpaired t test of the SMA and He-Bru ADF values from Tables III and IV exhibited no significant difference ($P > 0.05$) between strains. If one considers an average ADF value of 0.83 and the worst errors encountered (approx. 0.03), the highest coefficient of variation noted was <3.7%. The method, thus, was successful in this first year of the collaborative study and proved suitable for monitoring flocculation defects in yeast.

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Enzyme-Linked Immunosorbent Assay Method for Deoxynivalenol Analysis in Malt

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Keywords: DON, ELISA

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the determination of deoxynivalenol (DON) using the Neogen Veratox enzyme-linked immunosorbent assay (ELISA) kit ranged from 6.277 to 45.80% and 13.790 to 78.798%, respectively, and were judged unacceptable.
2. Repeatability and reproducibility coefficients of variation for the determination of DON using the Diagnostix EZ-Tox ELISA kit ranged from 0.000 to 20.702% and 8.958 to 162.617%, respectively, and were judged unacceptable.
3. Based on the *t* test assuming unequal variances, Malt-13 and the Neogen Veratox ELISA kit were significantly different at the 95% confidence level for all samples.
4. Based on the *t* test assuming unequal variances, Malt-13 and the Diagnostic EZ-Tox ELISA kit were significantly different at the 95% confidence level only for samples G and H.
5. Based on the *t* test assuming unequal variances, the Neogen Veratox and Diagnostix EZ-Tox ELISA kits were significantly different at the 95% confidence level for all samples, except A.

RECOMMENDATIONS

1. The subcommittee recommends repeating this collaborative study for a second year.
2. The subcommittee recommends adding barley samples to this collaborative study.

This was the first year of the subcommittee's existence. Based on the recommendation of the Subcommittee for Methods of Analysis Malt Review (2), this subcommittee was formed to evaluate two ELISA kits as alternatives to Malt-13 for analysis of DON (1). Levels of DON in grain are regulated by the USDA and FDA. Food products for consumption by humans cannot contain more than 1 ppm DON.

PROCEDURE

Collaborators were provided with eight samples of commercial malt, representing sample pairs A/B through G/H. Sample pairs were chosen to represent malts with varying levels of DON. Samples were prepared using a Buhler DFLU disc mill with settings determined by method Malt-4 for fine grind. Ground malt samples were sealed to prevent moisture gain and sent to each collaborator in late November 2009. Sample pair A/B was produced from a blend of two 2-row varieties. Sample pair C/D was the same 2-row variety with different production dates. Sample pair E/F was produced from a blend of two 6-row varieties, and sample pair G/H was a 6-row variety with different production dates. Results were evaluated using the Youden unit block design (1) and the *t* test assuming unequal variances at the 95% confidence level.

RESULTS AND DISCUSSION

Results for the Neogen Veratox ELISA kit were received from 11 collaborators for sample pairs A/B, C/D, E/F, and G/H; 7 collaborators reported results for the Diagnostix EZ-Tox ELISA kit; and 4 collaborators reported results for the Malt-13 method. Results from one collaborator using EZ-Tox ELISA were excluded prior to statistical analysis because of known deviations from the prescribed experimental protocol. The results for DON levels measured by Veratox ELISA, Malt-13, and EZ-Tox ELISA are summarized in Tables I–III, respectively. Outliers were determined using Dixon's ratio test; however, no outliers were removed from statistical analysis. Outliers were not excluded because of the very small values that were observed, and no known deviations from protocol were noted.

The statistical summary of the DON data for both the Veratox and EZ-Tox ELISA kits are presented in Table IV. The repeatability and reproducibility coefficients of variation for the determination of DON using the Veratox ELISA kit ranged from 6.277 to 45.80% and 13.790 to 78.798%, respectively, and were judged unacceptable. The repeatability and reproducibility coefficients of variation for the determination of DON using the EZ-Tox ELISA kit ranged from 0.000 to 20.702% and 8.958 to 162.617%, respectively, and were judged unacceptable. Samples A and B had very small DON values, leading to extremely large coefficients of variation. Even if samples A and B were thrown out, the repeatability and reproducibility coefficients of variation would still be higher than is acceptable. Results for the four sample pairs indicated an increasing percentage for repeatability and reproducibility coefficients of variation. As the sample value decreased, the coefficients of variation increased. This was taken into consideration when evaluating this method. For future study it is recommended that the collaborative study exclude samples that have 0 ppm DON.

The results of the *t* test assuming unequal variances are presented in Tables V–VII. The *t* test assuming unequal variances was used for comparison of the different methods due to the different number of collaborators for each method. Based on the *t* test assuming unequal variances, Malt-13 and EZ-Tox ELISA were significantly different at the 95% confidence level only for samples G and H; Malt-13 and Veratox ELISA were significantly different at the 95% confidence level for all samples; and Veratox and EZ-Tox ELISA kits were significantly different at the 95% confidence level for all samples, except A.

The recommendation to repeat this collaborative study for another year is strictly due to the sample set. Samples need to conform to USDA and FDA regulations for grain for human consumption.

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TABLE I
Neogen Veratox Enzyme-Linked Immunosorbent Assay Method for Detecting Deoxynivalenol (ppm) in Malt

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	0.20	0.20	0.40	0.40	0.90	0.70	3.20	2.70
2	0.20	0.10	0.40	0.30	1.00	0.90	3.00	2.20
3	0.20	0.20	0.60	0.50	0.80	0.80	3.50	3.10
4	0.10	0.20	0.60	0.50	0.80	0.80	3.00	2.80
5	0.10	0.00	0.40	0.30	0.90	1.00	3.70	3.60
6	0.10	0.10	0.60	0.50	1.20	1.00	4.10	3.50
7	0.00	0.00	0.40	0.40	0.90	1.00	4.20	3.40
8	0.00	0.10	0.30	0.30	0.60	0.70	3.80	3.50
9	0.10	0.10	0.50	0.40	0.60	0.70	3.00	3.10
10	0.70	0.10	0.40	0.44	1.00	1.10	3.00	2.80
11	0.00	0.00	0.20	0.30	0.70	0.90	3.10	2.90
Mean	0.154	0.100	0.436	0.395	0.855	0.873	3.418	3.055
Grand mean	0.127		0.415		0.864		3.236	

TABLE II
GC Assay Method (Malt-13) for Detecting Deoxynivalenol (ppm) in Malt

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
19	0.00	0.00	0.28	0.31	0.67	0.79	2.44	2.40
20	0.03	0.02	0.16	0.19	0.46	0.48	1.92	1.66
21	0.00	0.00	0.22	0.21	0.58	0.61	2.10	1.90
22	0.00	0.00	0.21	0.23	0.54	0.46	1.80	1.90
Mean	0.008	0.005	0.218	0.235	0.563	0.585	2.065	1.965
Grand mean	0.006		0.226		0.574		2.015	

TABLE III
Diagnostix EZ-Tox Enzyme-Linked Immunosorbent Assay Method for Detecting Deoxynivalenol (ppm) in Malt

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
12	0.01	0.01	0.20	0.10	0.50	0.51	2.56	3.00
13	0.01	0.01	0.35	0.31	0.62	0.59	2.39	2.63
14	0.00	0.00	0.28	0.29	0.55	0.52	2.89	2.64
15	0.01	0.01	0.14	0.17	0.49	0.59	3.00	2.54
16	0.01	0.01	0.31	0.28	0.49	0.65	2.40	2.34
17	0.01	0.01	0.10	0.10	0.58	0.62	2.66	2.32
18	0.10	0.10	0.19	0.30	0.64	0.55	2.80	2.74
Mean	0.021	0.021	0.224	0.221	0.553	0.576	2.671	2.601
Grand mean	0.021		0.223		0.564		2.636	

TABLE IV
Statistical Summary of Results for Neogen Veratox and Diagnostix EZ-Tox Enzyme-Linked Immunosorbent Assay (ELISA) Methods[a]

ELISA Method			**Repeatability**			**Reproducibility**		
Sample Pair	**No. of Labs**	**Grand Mean**	S_r	cv_r	r_{95}	S_R	CV_R	R_{95}
Veratox								
A/B	11	0.099	0.045	45.801	0.126	0.078	78.798	0.218
C/D	11	0.415	0.051	12.316	0.143	0.109	26.187	0.305
E/F	11	0.864	0.094	10.873	0.263	0.163	18.833	0.455
G/H	11	3.236	0.203	6.277	0.569	0.446	13.790	1.250
EZ-Tox								
A/B	7	0.021	0.000	0.000	0.000	0.035	162.617	0.098
C/D	7	0.223	0.046	20.702	0.129	0.093	41.945	0.262
E/F	7	0.564	0.060	10.675	0.169	0.057	10.169	0.161
G/H	7	2.636	0.226	8.578	0.633	0.236	8.958	0.661

[a] Calculations were made based on Youden unit block design analysis (1).

TABLE V
***t* Test Comparison of Neogen Veratox Enzyme-Linked Immunosorbent Assay and GC Assay (Malt-13) Methods for Detecting Deoxynivalenol in Malt[a]**

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H
Mean over two methods	0.082	0.053	0.327	0.315	0.709	0.729	2.742	2.510
N for Veratox	11	11	11	11	11	11	11	11
N for GC	4	4	4	4	4	4	4	4
Degrees of freedom	10	11	13	9	11	5	9	7
Calculated *t*	2.459[b]	3.978[b]	4.764[b]	4.359[b]	4.180[b]	3.298[b]	6.850[b]	5.392[b]
$t_{0.05}$	2.228	2.201	2.160	2.262	2.201	2.571	2.262	2.365

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

TABLE VI
***t* Test Comparison of Diagnostix EZ-Tox Enzyme-Linked Immunosorbent Assay and GC Assay (Malt-13) Methods for Detecting Deoxynivalenol in Malt[a]**

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H
Mean over two methods	0.015	0.013	0.221	0.228	0.558	0.581	2.368	2.283
N for EZ-Tox	7	7	7	7	7	7	7	7
N for GC	4	4	4	4	4	4	4	4
Degrees of freedom	9	8	9	9	5	3	6	5
Calculated *t*	0.919[b]	1.166[b]	0.159[b]	–0.305[b]	–0.194[b]	–0.118[b]	3.659[c]	3.551[c]
$t_{0.05}$	2.262	2.306	2.262	2.262	2.571	3.182	2.447	2.571

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

TABLE VII
***t* Test Comparison of Neogen Veratox and Diagnostix EZ-Tox Enzyme-Linked Immunosorbent Assay Methods for Detecting Deoxynivalenol in Malt[a]**

Parameter	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H
Mean over two methods	0.088	0.061	0.330	0.308	0.704	0.724	3.045	2.828
N for EZ-Tox	7	7	7	7	7	7	7	7
N for Veratox	11	11	11	11	11	11	11	11
Degrees of freedom	11	15	16	12	13	14	16	16
Calculated *t*	2.190[b]	2.930[c]	4.075[c]	3.930[c]	5.074[c]	6.311[c]	4.491[c]	2.894[c]
$t_{0.05}$	2.201	2.131	2.120	2.179	2.160	2.145	2.120	2.120

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

Headspace Gas Chromatography–Flame Ionization Detector Analysis for Beer Volatiles

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Keywords: Alcohols, Aldehydes, Esters, FID, GC

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the determination of acetaldehyde by headspace gas chromatography–flame ionization detector (GC-FID) analysis ranged from 0.70 to 12% and 14 to 28%, respectively, and were judged unacceptable.
2. Repeatability and reproducibility coefficients of variation for the determination of ethyl acetate by headspace GC-FID analysis ranged from 0.96 to 4.3% and 8.2 to 8.9%, respectively, and were judged acceptable.
3. Repeatability coefficients of variation for the determination of isoamyl acetate by headspace GC-FID analysis ranged from 0.42 to 10% and were judged acceptable. Reproducibility coefficients of variation for the determination of isoamyl acetate by headspace GC-FID analysis ranged from 14 to 32% and were judged unacceptable.
4. Repeatability and reproducibility coefficients of variation for the determination of isoamyl alcohol by headspace GC-FID analysis ranged from 3.9 to 12% and 12 to 18%, respectively, and were judged unacceptable.

RECOMMENDATION

1. Due to the unacceptable statistical data, the subcommittee recommends that the collaborative study be repeated in 2010–2011 following ruggedness evaluation.

Based on polling by the Subcommittee for Coordination of New and Alternative Methods of Analysis (2), this subcommittee was formed to evaluate the applicability of headspace GC-FID analysis for the determination of volatile organic compounds in beer. The volatile target compounds were selected because they represent a range of chemical functionality (e.g., alcohols, aldehydes, and esters) and they are present in all beers.

PROCEDURE

Four sample pairs of commercial beers were sent to each collaborator. Each pair was of the same brand but from different production times. Two sample pairs were commercially available ales (A/B and C/D), and two sample pairs were commercial light beers (E/F and G/H) 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 eight collaborators were received for the four sample pairs. Results for ethyl acetate, isoamyl acetate, and isoamyl alcohol from one collaborator were excluded prior to statistical analyses 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).

The statistical summary of the volatile data is shown in Table V. The repeatability coefficients of variation were judged acceptable for two of four compounds tested. The repeatability coefficients of variation for ethyl acetate and isoamyl acetate ranged from 0.96 to 4.3% and 0.42 to 10%, respectively, and were judged acceptable. The repeatability coefficients of variation for acetaldehyde and isoamyl alcohol ranged from 0.70 to 12% and 3.9 to 12%, respectively, and were judged unacceptable.

Repeatability for acetaldehyde may have been affected by the nature of the ales provided for testing. The ales were bottle-conditioned, which can produce different levels of acetaldehyde depending on the length of time between production and analysis. Future collaborative tests should utilize non–bottle-conditioned products.

Repeatability for isoamyl alcohol may have been affected by chromatographic coelution of two amyl alcohols on the selected column. Ruggedness testing should be conducted to determine the potential effect of chromatographic coelution.

The reproducibility coefficients of variation were judged unacceptable for three of four compounds tested. The reproducibility coefficients of variation for ethyl acetate ranged from 8.2 to 8.9% and were judged acceptable. The reproducibility coefficients of variation for acetaldehyde, isoamyl acetate, and isoamyl alcohol ranged from 14 to 28%, 14 to 32%, and 12 to 18%, respectively, and were judged unacceptable.

Reproducibility of results for acetaldehyde, isoamyl acetate, and isoamyl alcohol may have been affected by sampling errors. A few collaborators indicated difficulty with sampling carbonated beverages using glass pipettes. The sampling technique should be evaluated by ruggedness testing for inclusion in future collaborative testing. Future sample testing will attempt to cover a broader range of analytes.

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

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	4.89	5.67	2.76	3.50	1.82	1.77	6.12	5.96
2	7.67[a]	9.41[a]	6.69[a]	6.85[a]	3.58[a]	3.87[a]	8.21[a]	6.39[a]
3	5.63	6.90	3.17	4.80	1.08	0.95	4.73	4.61
4	5.24	4.05	3.58	3.66	2.40	2.18	5.78	5.69
5	7.59[a]	13.9[a]	4.91[a]	8.58[a]	3.00[a]	2.68[a]	8.11[a]	7.35[a]
7	6.92	8.85	4.54	6.39	1.89	1.75	5.87	5.67
8	6.80	8.14	4.87	6.19	2.05	1.93	5.78	5.72
9	5.01	5.93	2.87	3.88	1.38	1.21	4.29	4.20
Mean[b]	5.747	6.590	3.631	4.736	1.769	1.631	5.428	5.308
Grand mean[b]	6.168		4.184		1.700		5.368	

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

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

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	19.9	19.7	16.8	15.4	16.9	16.1	16.4	19.7
2	…[a]	…	…	…	…	…	…	…
3	17.7	17.8	15.8	16.9	15.2	14.7	14.0	16.7
4	27.2[b]	23.8[b]	23.9[b]	25.0[b]	23.7[b]	21.3[b]	21.8[b]	21.2[b]
5	19.0	18.8	17.8	18.8	17.3	16.7	16.1	19.2
7	18.6	19.3	16.7	18.0	16.9	15.8	14.8	17.8
8	17.2	16.9	15.6	16.6	14.6	14.0	13.9	17.0
9	21.7	21.2	19.0	20.5	19.1	18.3	16.9	21.1
Mean[c]	19.02	18.94	16.95	17.69	16.66	15.92	15.35	18.57
Grand mean[c]	18.98		17.32		16.29		16.96	

[a] Data were deleted from all calculations due to known deviation from protocol.
[b] Outlier at $P < 0.05$ based on totals and/or differences (1).
[c] Calculated excluding outliers.

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

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	1.24	1.22	1.15	1.19	1.25	1.24	1.48	1.43
2	…[a]	…	…	…	…	…	…	…
3	0.615	0.645	0.650	0.680	1.41	1.45	1.39	1.85
4	1.41[b]	1.28[b]	1.45[b]	1.45[b]	2.68[b]	2.75[b]	2.67[b]	2.99[b]
5	0.574	0.592	0.618	0.658	1.32	1.37	1.32	1.74
7	0.733	0.823	0.771	0.802	1.74	1.73	1.68	2.24
8	0.568	0.600	0.621	0.654	1.24	1.27	1.27	1.71
9	0.738	0.747	0.749	0.790	1.62	1.67	1.54	2.16
Mean[c]	0.7443	0.7712	0.7598	0.7951	1.429	1.454	1.447	1.854
Grand mean[c]	0.7577		0.7774		1.442		1.651	

[a] Data were deleted from all calculations due to known deviation from protocol.
[b] Outlier at $P < 0.05$ based on totals and/or differences (1).
[c] Calculated excluding outliers.

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

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	31.8[a]	33.7[a]	33.2[a]	29.6[a]	69.5[a]	70.0[a]	71.4[a]	97.7[a]
2	…[b]	…	…	…	…	…	…	…
3	32.9	32.3	31.1	33.0	29.7	29.8	34.7	32.9
4	42.7	37.1	39.9	38.8	41.3	41.1	39.3	38.5
5	45.8	46.5	35.1	44.3	30.5	45.8	35.1	37.5
7	33.2	33.0	33.2	36.5	32.6	31.6	35.9	34.7
8	43.9	41.8	40.8	42.8	43.3	42.4	51.1	47.7
9	45.3	44.5	41.8	43.2	45.6	45.0	51.0	47.1
Mean[c]	40.63	39.19	36.98	39.76	37.16	39.28	41.18	39.73
Grand mean[c]	39.91		38.37		38.22		40.45	

[a] Outlier at $P < 0.05$ based on totals and/or differences (1).
[b] Data were deleted from all calculations due to known deviation from protocol.
[c] Calculated excluding outliers.

TABLE V
Statistical Summary of Results for Determination of Volatile Organic Compounds in Beer by Headspace Gas Chromatography–Flame Ionization Detector Analysis[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	6.168	0.759	12	2.124	1.392	23	3.899
C/D	6	4.184	0.455	11	1.273	1.104	26	3.092
E/F	6	1.700	0.040	2.4	0.112	0.468	28	1.311
G/H	6	5.368	0.037	0.70	0.105	0.728	14	2.040
Ethyl acetate								
A/B	6	18.98	0.308	1.6	0.863	1.557	8.2	4.361
C/D	6	17.32	0.741	4.3	2.074	1.547	8.9	4.331
E/F	6	16.29	0.156	0.96	0.437	1.552	9.5	4.345
G/H	6	16.96	0.348	2.1	0.976	1.505	8.9	4.213
Isoamyl acetate								
A/B	6	0.7577	0.026	3.5	0.074	0.244	32	0.684
C/D	6	0.7774	0.003	0.42	0.009	0.202	26	0.566
E/F	6	1.442	0.019	1.3	0.053	0.205	14	0.573
G/H	6	1.651	0.170	10	0.475	0.240	15	0.671
Isoamyl alcohol								
A/B	6	39.91	1.580	4.0	4.425	5.967	15	16.71
C/D	6	38.37	2.449	6.4	6.857	4.442	12	12.44
E/F	6	38.22	4.573	12	12.81	6.951	18	19.46
G/H	6	40.45	1.596	3.9	4.470	7.076	17	19.81

[a] All calculations were made based on Youden unit block design analysis (1).

Coordination of New and Alternate Methods of Analysis

Subcommittee Members: K. Lakenburges, *Chair*; C. Benedict; J. Cornell; M. Eurich; B. Foster; A. Fritsch; R. Jennings; G. Kelly; A. Porter; C. Powell; S. Thompson; 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, Allergens, Amino acids, Bitterness, Decarbonation, FAN, Free amino nitrogen, β-Glucan, Gluten, HPLC, Iso-α-acids, Propylene glycol, Segmented flow analysis, Sensory, SFA, Volatile sulfur compounds, Wort viscosity

RECOMMENDATIONS

1. Conduct online polling to obtain input on new and alternative methods.
2. Gather additional information on methods for the determination of amino acids in wort to determine interest in the formation of a subcommittee and the execution of collaborative testing.

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 for the Coordination of New and Alternate Methods 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 2009 Annual Meeting in Tucson, AZ, was very well attended, with 50+ members in attendance. Topics of interest and discussion included

- Iso-α-acids analysis in beer by high-performance liquid chromatography (HPLC) (adapting the EBC method for hop extracts for analysis in wort and/or beer)
- Allergen analysis, with special interest in wheat
- Use of test kits
- Use of a tannometer for tannoids haze
- Hach spectrophotometer methods
- Reducing sugars method
- Amino acids methods (by HPLC)

Topics for Polling

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

Segmented Flow Analysis (Bitterness [BU], Free Amino Nitrogen [FAN], β-Glucan). This idea stems from the six responses for the determination of sulfur dioxide (SO_2) in beer in 2007 given at the annual meeting in Victoria, BC, Canada, and the resulting subcommittee formation followed by successful collaborative testing. Polling in 2009 yielded responses from 17 members, 14 of whom use segmented flow (SFA) for nonmalt analysis. The 2010 poll yielded responses from 15 members who currently use SFA for nonmalt analysis: bitterness (6 responses), FAN (12 responses), β-glucan (10 responses), and SO_2 (2 responses). A total of 11 respondents expressed their willingness to participate in ASBC subcommittee collaborative evaluations of the methods: bitterness (6 respondents), FAN (10 respondents) and β-glucan (9 respondents).

Iso-α-acids in Wort and Beer Determined by HPLC. This topic was suggested in response to the 2009 poll and was briefly discussed at the subcommittee meeting at the 2009 annual meeting in Tucson. Adaptation of an EBC method for determination in hop extracts was suggested, and the 2010 poll received 16 responses from labs currently using an HPLC method for iso-α-acids analysis. A total of 17 respondents expressed their willingness to participate in an ASBC subcommittee collaborative evaluation of the method.

Amino Acids in Wort Determined by HPLC. Six people expressed interest in a potential subcommittee at the annual meeting in Victoria in 2007. Additional polling online may provide details on methods in use and potential modifications developed in light of past and possible future shortages of acetonitrile (3).

Researching Methods/Subcommittee Chairs Needed

Volatile Sulfur Compounds in Beer. This topic has been of interest for several years, but a subcommittee chair is needed to identify a method and coordinate collaborative testing. This topic will be kept as a current need but will not be advanced as a subcommittee until such time as a suitable chair can be found (4–6).

Decarbonation of Beer. Several methods are used for the decarbonation of beer. Further, many analytical methods require a degassing step for beer samples prior to analysis. Not all methods for degassing are compatible with all of the analytical methods that require a decarbonation step, so there is a need to determine acceptable combinations of the two. The subcommittee is seeking a volunteer to

- Take on a review of the current key published work on decarbonation of beer and summarize the findings (1,2,7)
- Provide a recommendation to the Technical Committee for a collaborative study that tests selected decarbonation methods against common ASBC methods that require degassing

Packaging Methods. In an effort to update the Packaging Methods section of the ASBC *Methods of Analysis* (MOA), 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.

Propylene Glycol. This method was suggested at the fall 2008 Technical Committee meeting. Propylene glycol can be measured as a fermentation by-product or as a contaminant from defective heat

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exchangers. Method(s) are needed, and this is a possible future polling topic and posting at the annual meeting.

Wort Viscosity Determined by Automated Instrument. This idea came from the 2010 review of the Wort section of the MOA.

Sensory. ASBC and EBC are working toward updating the flavor wheel.

Lab Work/Publication Required

Accelerated Aging of Seed (Viability, Vigor Testing). The final year of the NDSU study was due to be completed in 2008. Polling will be conducted or a subcommittee established once the method has been published.

Topics to Continue to Monitor

Labeling/Allergens in Beer. The subcommittee will continue to monitor this topic, along with the Emerging Issues Committee. Potential labeling requirements and method selection for gluten in beer continue to be key items to watch.

Topics to Archive

None.

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APPENDIX

Summarized Results from 2010 Online Polling

Top Line Results

- 156 responses were received
- 37 respondents (24%) submitted ideas for new and alternative methods
- 78 respondents (50%) provided their contact information
- 17 respondents answered questions regarding iso-α-acids analysis by HPLC
- 14 respondents answered questions regarding segmented flow analysis

Suggestions and Input for New Methods

- Revision of Congress mash
- Mycotoxins in raw materials, wort, and beer
- Malt/beer gushing inducer
- Microorganism identification
- KOH for gram staining
- Yeast vitality (3 responses)
- Measuring dissolved oxygen in bottled and canned products
- Measurement of DP enzymes
- Monitoring yeast physiological state during fermentation by quantitative cell morphogenesis analysis
- Zinc in wort and beer
- Amino acids in wort and beer (2 responses)
- Rapid methods for beer-spoilage bacterium (2 responses)
- Correlation of fermentation technology in the brewing industry to fermentation in the distillation industry
- Use of an ebulliometer to measure alcohol
- Distilled spirits profile analysis: GC, color, and other analytes that can be measured by spectrophotometer
- SO_2 in beer by headspace GC
- Phenolic acids
- TPO, differential measurement of headspace oxygen and dissolved oxygen
- Flow cytometry assays
- Hop analysis
- Organic certification
- Gluten level assessment
- Shelf-life prediction
- SO_2 analysis
- Homogeneity of barley
- LPT proteins that are more involved in foam formation
- Flavor stability
- β-Glucans in beer
- Anthocyanogens in beer
- Coagulable nitrogen
- SPME GC/MS
- Beer flavor analysis
- Gelatinization temperature of barley and malt using RVA
- Malt lipoxygenase
- Arabinoxylans (2 responses) in barley, malt, and wort
- β-Glucans determined by HPLC
- Crease mold in or on barley
- Health
- Indole
- Microbiology

Method for Determining Alpha-Amylase in Malt by Segmented Flow Analysis Using Potassium Ferricyanide

Subcommittee Members: A. Budde, *Chair*; J. Barr; S. Bartlett; T. Chicos; K. Churchill; D. Frey; A. MacLeod; M. Maurice; J. Menting; A. Stern; and R. Jennings (*ex officio*)

Keywords: SFA

CONCLUSIONS

1. Repeatability coefficients of variation for the determination of α-amylase using potassium ferricyanide ranged from 0.86 to 5.01% and were judged acceptable.
2. Reproducibility coefficients of variation for the determination of α-amylase using potassium ferricyanide ranged from 2.55 to 15.17% and were judged unacceptable.

RECOMMENDATIONS

1. The subcommittee recommends that collaborative testing be repeated.
2. Attempts should be made to develop a protocol that yields more consistent results.
3. An attempt should be made to involve more standard reference method (SRM) collaborators. If no more are available, it is recommended that this method be tested against Malt-7C, Segmented Flow Analysis for Alpha-Amylase Using Iodine.

This was the first year of this subcommittee's evaluation of an alternative method for determining α-amylase activities in malt by segmented flow analysis utilizing potassium ferricyanide for detection. The subcommittee was formed based on the recommendation of Subcommittee for the Methods of Analysis Malt Review (2). Some members had expressed concern that the current approved method (Malt-7C) for segmented 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.

Previous attempts to establish α-amylase activity by segmented flow analysis experienced high reproducibility coefficients of variation (3). Since most analytical labs were performing segmented flow analyses and the numbers of labs routinely using the SRM were decreasing, an effort was made to include only collaborators using the most prevalent methodology (segmented flow analysis with iodine for detection). Satisfactory results from this collaborative study resulted in approval of Malt-7C as a method for determination of α-amylase (4). Segmented flow analysis utilizing potassium ferricyanide to determine α-amylase activity might allow for more accurate assessment of very low or very high activities compared with Malt-7C, while yielding similar results in the 40–60 DU range. It is recommended that for future studies the potassium ferricyanide method be compared with Malt-7C.

PROCEDURE

A total of 10 malt samples representing commercial varieties malted on separate days (similar but distinct) were ground on a Buhler DFLU disc mill according to Malt-4 for fine grind, vacuum packed, and sent to each collaborator. The 10 malts consisted of 5 sample pairs (A/B to I/J) that spanned a wide range of α-amylase levels. These samples were identical to the malts used by the subcommittee examining the Megazyme E-MAST standard as a replacement for the Sigma product that is no longer available. Calibration of the method was via D-glucose solutions, as described in Malt-6C, and sample A was used as the internal standard to anchor the experimental values. Raw analytical results were generated from the linear regression determined by the glucose standards, with the final values determined by multiplying results of the measured raw value by the averaged SRM value of sample A divided by the averaged raw values of sample A used as an internal standard. Results were evaluated using the Youden unit block design (1).

RESULTS AND DISCUSSION

Results from eight collaborators were received for the five sample pairs and are presented in Table I. The sample pairs were also analyzed by Malt-7A and -7B (SRM); the results are presented in Table II. Outliers were identified using Dixon's ratio test (1).

The statistical summary for the determination of α-amylase using potassium ferricyanide are presented in Table III. Repeatability coefficients of variation for the determination of α-amylase using potassium ferricyanide ranged from 0.86 to 5.01% and were judged acceptable. Reproducibility coefficients of variation for the determination of α-amylase using potassium ferricyanide ranged from 2.55 to 15.17% and were judged unacceptable.

Values for sample pair A/B were very consistent, as would be expected when using sample A as an internal standard. Of concern were the results of analyses of the sample pairs representing low and high activities (sample pair I/J had low activity based on SRM, while G/H had high activity based on SRM). The reproducibility coefficients of variation for these pairs were high at 10.88 and 15.17%, respectively, while the grand mean values for these two pairs did not reflect the fact that these malts had the lowest and highest activities. The premise that the potassium ferricyanide method has a more dynamic range than the iodine method should be examined more closely. A comparison of the SRM and α-amylase determined using potassium ferricyanide could not be performed due to the lack of SRM collaborators. It is hypothesized that the use of the internal standard smoothed out the values for each sample. It is recommended that the true values from each collaborator be used for future studies.

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

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
1	51.3	48.8	56.5	56.1	60.8	56.7	59.7	63.9	50.2	52.2
2	50.0	49.6	50.6	50.2	63.3	61.8	64.8	66.8	46.5	44.5
3	50.9	47.8	49.2	47.7	60.6	60.1	46.4	44.6	55.4	48.3
4	52.1	52.0	55.7	54.3	68.2	62.6	66.0	69.2	49.6	51.1
5	51.1	51.1	56.6	56.7	64.8	61.5	62.7	67.4	54.8	55.1
6	48.9	49.6	53.1	52.1	53.4	54.0	48.6	48.3	41.0	40.3
7	50.4	47.6	55.8	54.7	58.2[a]	37.4[a]	53.3	53.3	54.1	56.1
8	50.3	48.7	57.3	55.9	59.7	58.5	50.8	52.8	52.9	58.0
Mean[b]	50.64	49.40	54.35	53.47	61.50	59.3	56.53	58.30	50.56	50.69
Grand mean[b]	50.02		53.91		60.42		57.41		50.63	

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

TABLE II
α-Amylase Activity (20 DU, as is) Determined Using Malt-7A and -7B

Collaborator/ Method	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
1/Malt-7A	44.1	46.8	41.3	40.4	54.0	53.3	73.8	75.3	32.0	28.2
2/Malt-7B	57.6	58.0	54.9	53.7	65.8	64.2	67.9	68.3	47.3	42.2
Mean	50.87	52.39	48.09	47.05	59.91	58.73	70.87	71.79	39.64	35.22
Grand mean	51.63		47.58		59.33		71.33		37.43	

TABLE III
Statistical Summary of Results for the Determination of α-Amylase Using Potassium Ferricyanide[a]

		Repeatability			Reproducibility		
Sample Pair	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
A/B	50.02	1.02	2.04	2.85	1.28	2.55	3.57
C/D	53.91	0.41	0.86	1.14	3.11	5.78	8.72
E/F	60.42	1.54	2.55	4.31	3.95	6.54	11.06
G/H	57.41	1.63	2.83	4.55	8.71	15.17	24.39
I/J	50.63	2.54	5.01	7.10	5.51	10.88	15.43

[a] All calculations were based on the Youden unit block design (1).

Assortment of Malt Using the Pfeuffer Sortimat

Subcommittee Members: P. Ritchie, *Chair*; J. Barr; S. Bartlett; J. Boucek; A. Budde; T. Chicos; K. Churchill; G. Fox; R. Joy; A. MacLeod; M. Maurice; and R. Jennings (*ex officio*)

Keywords: Eureka-Niagara, Imperial, Metric

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for malt assortment using S. Howe Co., Inc. Eureka-Niagara sample grader imperial screens ranged from 0.65 to 38.68% and 1.57 to 37.18%, respectively, and were judged unacceptable.
2. Repeatability and reproducibility coefficients of variation for malt assortment using Pfeuffer Sortimat imperial screens ranged from 0.52 to 28.43% and 1.55 to 39.74%, respectively, and were judged unacceptable.
3. Repeatability and reproducibility coefficients of variation for Pfeuffer Sortimat metric screens ranged from 1.52 to 56.82% and 2.12 to 50.80%, respectively, and were judged unacceptable.
4. Based on the paired *t* test for differences in means for malt assortment, the Eureka-Niagara sample grader and Sortimat grader were significantly different at the 95% confidence level for all screens.

RECOMMENDATIONS

1. The subcommittee recommends repeating this collaborative study for a second year.
2. The tolerances for the slot width for ASBC-supplied screens used on the Eureka-Niagara sample grader and for the Sortimat-supplied screens should be updated.
3. A larger survey of slot widths is recommended for both types of supplied screens.
4. A larger number of participants for future study is recommended.

This was the first year of the subcommittee's existence. Based on the recommendation of the Subcommittee for Methods of Analysis Malt Review (2), this subcommittee was formed to evaluate the Pfeuffer Sortimat grader as an alternative method for assortment of malt as listed in method Malt-2B (1). The Sortimat is becoming a standard instrument for assortment that is used to cut down on time and error.

PROCEDURE

Collaborators were provided with six sample pairs (A/B through K/L). Samples A and B were 6-row Tradition malts from different production dates and barley sources from the fall of 2009. Samples C and D were 2-row Copeland malts produced from the same lot of barley but malted in different compartments. Samples E and F were from the same 2-row/6-row customer blend, but each sample was taken from different rail cars. Samples G and H were from a customer 2-row/6-row blend from different load dates. Samples I and J were 6-row Legacy malts produced from the same lot of barley but were malted in different compartments. Samples K and L were 2-row Copeland malts produced from the same lot of barley but were malted in different compartments Samples A–J were from fall production. Samples K and L were from winter production. Collaborators were asked to have either a Eureka-Niagara sample grader or a Sortimat grader with imperial screens of $^{7}/_{64}$, $^{6}/_{64}$, and $^{5}/_{64}$ in. Collaborators were asked to verify the slot widths of the screens with a precision gauge. This information was used to draw conclusions for tolerance levels only. Each sample was tested in duplicate. If the collaborator had the capability to measure the sample on both types of graders, they were asked to do so. Collaborators with metric screen sets for the Sortimat (2.80, 2.50, and 2.20 mm) also tested samples A–K in duplicate. Results were evaluated using the Youden unit block design (1) and paired *t* test for difference in means at the 95% confidence level.

RESULTS AND DISCUSSION

Results from 11 collaborators were received for sample pairs A/B through K/L. Six collaborators used the Eureka-Niagara sample grader, and seven used the Sortimat grader with both imperial and metric screens. Samples were tested twice on each grader. 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 imperial screens are presented in Tables V–VIII. Results from the Sortimat metric screens are presented in Tables IX–XII. Outliers were determined using Dixon's ratio test (1).

A statistical summary of the assortment data for the Eureka-Niagara sample grader is presented in Table XIII. A statistical summary of the assortment data for the Sortimat with imperial screens is presented in Table XIV. A statistical summary of the assortment for the Sortimat with metric screens is presented in Table XV. Repeatability and reproducibility coefficients of variation for the Eureka-Niagara imperial screens ranged from 0.65 to 38.68% and 1.57 to 37.18%, respectively, and were judged unacceptable. Repeatability and reproducibility coefficients of variation for the Sortimat imperial screens ranged from 0.52 to 28.43% and 1.55 to 39.74%, respectively, and were judged unacceptable. Repeatability and reproducibility coefficients of variation for the Sortimat metric screens ranged from 1.52 to 56.82% and 2.12 to 50.80%, respectively, and were judged unacceptable.

Subcommittee members were requested to measure approx. 45 slot widths per screen. As per method Malt-2B, an ASBC $^{7}/_{64}$-in. screen should have an absolute slot width of 0.1094 in., with a maximum width of 0.1099 in. and a minimum width of 0.1089 in. Measurements submitted by subcommittee members yielded results with minimum slots widths of 0.1020 in. and maximum slot widths of 0.1151 in. for an ASBC $^{7}/_{64}$-in. (0.1094-in.) screen, for an average of 0.1079 in. For comparison purposes, the Sortimat $^{7}/_{64}$-in. (0.1094-in.) screen slot width was also measured. The minimum slot width was 0.1070 in., and the maximum slot width was 0.1095 in., for an average of 0.1084 in. Based on the results submitted by subcommittee members, the $^{7}/_{64}$-in. (0.1094-in.) screens failed the criteria set out by method Malt-2B for both the ASBC- and Sortimat-supplied screens. Slot width results obtained for the $^{6}/_{64}$-in. (0.0938-in.) and $^{5}/_{64}$-in. (0.0781-in.) screens also failed method Malt-2B criteria. The screens supplied by ASBC are manufactured with a stamping accuracy of 0.003 in., with a tolerance of 0.001 in. measured with a precision gauge. A tolerance of ±0.0005 in. is not possible under these manufacturing conditions. The committee proposed a new tolerance for the slot width of ±0.005 in. for the imperial screens and ±0.05 mm for the metric screens. Even with the larger tolerances, many of the screen sets had slot widths that were either out of tolerance or at the lower end of the acceptable range.

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TABLE I
Assortment (%) for a 7/64-in. Screen for a Eureka-Niagara Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	57.3	69.7	86.5	84.1	68.0	69.7	62.8	59.1	77.6	77.8	86.8	92.6
2	47.8	66.6	83.8	82.3	67.5	64.4	59.9	53.0	71.3	72.7	84.9	90.8
3	47.9	66.0	84.2	82.3	63.6	64.8	59.3	53.4	72.9	74.2	84.5	91.8
4	52.1	70.3	87.9	84.3	68.9	66.3	64.4	56.0	77.6	78.7	94.3	89.0
5	52.2	71.3	85.2	84.2	67.4	69.0	65.8	57.0	76.3	76.0	86.5	93.8
6	56.9	71.8	86.7	84.3	69.4	69.5	65.5	57.6	75.45	75.8	88.9	92.1
Mean	52.37	69.28	85.72	83.58	67.47	67.28	62.95	56.02	75.20	75.87	87.65	91.68
Grand mean	60.83		84.65		67.38		59.48		75.53		89.67	

TABLE II
Assortment (%) for a 6/64-in. Screen for a Eureka-Niagara Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	35.6	26.6	12.0	13.8	27.6	26.4	31.0	34.8	19.7	19.4	11.5	6.7
2	39.6	28.2	13.0	14.4	26.1	28.6	31.3	36.2	24.0	23.0	12.3	7.4
3	37.6	27.9	12.7	14.2	28.9	28.2	30.9	35.4	22.4	21.1	11.8	6.8
4	36.1	24.6	9.6	12.4	25.0	24.5	27.7	33.9	18.3	17.3	3.9	8.2
5	34.7	22.9	11.6	12.5	24.8	24.5	26.1	32.1	19.4	19.5	10.2	4.9
6	34.1	23.7	11.4	12.5	25.6	25.1	27.7	34.1	21.1	20.2	9.1	7.0
Mean	36.288	25.65	11.72	13.30	26.33	26.22	29.12	34.42	20.82	20.08	9.80	6.83
Grand mean	30.97		12.51		26.28		31.77		20.45		8.32	

TABLE III
Assortment (%) for a 5/64-in. Screen for a Eureka-Niagara Sample Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	6.3	3.3	1.3	1.9	4.3	3.8	5.7	5.5	2.4	2.5	1.6	0.6
2	11.2	4.7	2.8	3.1	5.9	6.6	8.0	9.5	4.0	3.8	2.4	1.2
3	12.8	5.5	2.5	3.2	6.9	6.5	8.8	10.1	4.2	4.1	2.9	1.2
4	10.6	4.3	2.4	3.0	5.7	5.5	7.3	9.0	3.4	3.1	1.4	2.1
5	12.3	5.5	2.9	3.2	7.7	5.7	7.7	10.2	4.0	4.3	3.1	1.2
6	8.0	4.1	1.6	2.8	4.6	5.1	6.4	7.6	3.0	3.5	1.7	0.8
Mean	10.20	4.57	2.25	2.87	5.85	5.53	7.32	8.65	3.50	3.55	2.18	1.18
Grand mean	7.38		2.56		5.69		7.98		3.53		1.68	

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		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	0.8	0.4	0.3	0.3	0.3	0.2	0.6	0.7	0.4	0.5	0.3	0.1
2	1.3	0.6	0.4	0.3	0.5	0.5	0.9	1.1	0.7	0.5	0.5	0.2
3	1.6	0.6	0.6	0.4	0.6	0.6	1.0	1.4	0.6	0.5	0.6	0.2
4	1.3	0.8	0.3	0.4	0.4	0.2	0.7	1.1	0.6	0.5	0.3	0.3
5	0.9	0.4	0.4	0.3	0.3	0.3	0.6	0.9	0.4	0.4	0.4	0.1
6	1.0	0.5	0.4	0.5	0.5	0.5	0.5	0.8	0.5	0.6	0.4	0.2
Mean	1.15	0.55	0.40	0.37	0.43	0.38	0.72	1.00	0.53	0.50	0.42	0.18
Grand mean	0.85		0.38		0.41		0.86		0.52		0.30	

This condition was found more often on the imperial screens purchased for the Eureka-Niagara sample grader in recent years.

A larger number of collaborators are recommended for repeating this collaborative study. It is also recommended that the subcommittee test each screen in isolation and not as a set with other screens. As demonstrated by the results obtained by this subcommittee, the coefficient of variation increased with the descending slot width of the screen set. As each screen sifted out sample, it was observed that the next screen below received a smaller sample size to sift. It is recommended that the variability introduced by malt screens be estimated before we proceed further with this subcommittee.

LITERATURE CITED

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

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	55.5	72.8	87.5	85.1	72.4	70.5	66.9	57.5	77.8	79.6	89.1	94.6
2	50.3	64.5	85.6	81.6	65.8	65.2	58.8	52.9	70.4	73.3	85.2	91.4
3	57.1	72.2	88.0	85.4	71.8	71.5	65.0	64.0	80.0	81.5	89.1	94.1
4	63.2	74.2	88.0	86.0	76.7	77.1	67.6	65.0	79.5	81.2	89.9	94.2
5	51.3	69.2	85.5	84.4	70.7	66.8	63.0	57.3	74.2	76.0	86.8	92.5
6	55.7	69.0	86.7	84.4	69.4	69.4	66.1	58.7	77.6	78.1	88.4	92.9
7	58.1	74.6	87.4	86.3	70.9	73.2	66.8	61.2	79.4	80.2	87.5	92.7
Mean	55.89	70.93	86.96	84.74	71.10	70.52	64.89	59.51	78.56	76.99	88.00	93.20
Grand mean	63.41		85.85		70.81		62.20		77.77		90.60	

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

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	34.2	23.2	10.4	12.1	21.6	24.2	26.6	33.4	18.8	17.3	9.2	4.8
2	36.1	28.7	11.7	14.4	26.8	27.6	31.0	35.5	24.7	21.6	11.7	6.9
3	33.8	24.0	10.3	12.3	23.6	23.9	28.3	30.0	17.2	15.6	9.2	5.2
4	30.2	21.9	9.9	12.0	19.4	19.3	25.3	28.7	17.5	16.0	8.4	5.1
5	36.3	25.4	11.6	12.4	23.1	26.0	28.6	32.4	21.2	19.8	10.7	6.0
6	32.7	26.8	11.3	13.1	25.7	25.9	27.3	33.6	19.1	19.3	9.8	6.3
7	32.6	21.8	10.5	11.5	24.2	22.7	26.9	31.4	17.6	16.7	10.4	6.6
Mean	33.70	24.54	10.81	12.55	23.49	24.23	27.71	29.93	19.44	18.74	9.91	5.84
Grand mean	29.12		11.68		23.86		29.93		18.74		7.88	

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

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	9.5	3.7	1.9	2.5	5.7	5.2	6.1	8.1	3.1	2.9	1.6	0.5
2	12.2	6.3	2.5	3.7	7.0	6.7	9.3	10.6	4.6	4.6	2.8	1.5
3	8.3	3.5	1.5	2.0	4.5	4.3	6.0	5.6	2.6	2.4	1.4	0.7
4	5.9	3.4	1.9	1.9	3.6	3.4	6.5	5.8	2.6	2.4	1.4	0.7
5	10.8	4.8	2.6	3.1	5.7	6.5	7.9	9.1	4.1	3.8	2.2	1.2
6	10.7	3.6	1.8	2.2	4.7	4.4	6.1	7.0	3.1	2.4	1.6	0.8
7	8.4	3.3	1.5	1.7	4.7	4.1	5.8	6.8	2.8	2.7	1.7	0.7
Mean	9.40	4.09	1.96	2.44	5.13	4.94	6.81	7.57	3.27	3.03	1.81	0.87
Grand mean	6.74		2.20		5.04		7.19		3.15		1.34	

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

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	0.9	0.4	0.2	0.3	0.3	0.2	0.4	1.1	0.4	0.3	0.3	0.1
2	1.4	0.6	0.3	0.5	0.6	0.6	1.1	1.1	0.4	0.6	0.5	0.2
3	0.9	0.3	0.2	0.3	0.2	0.3	0.8	0.5	0.3	0.5	0.3	0.1
4	0.5	0.4	0.2	0.2	0.3	0.2	0.6	0.6	0.4	0.3	0.2	0.1
5	1.3	0.6	0.4	0.3	0.5	0.5	0.8	1.1	0.7	0.5	0.4	0.1
6	1.0	0.7	0.3	0.4	0.3	0.4	0.6	0.8	0.3	0.4	0.4	0.1
7	1.0	0.4	0.3	0.3	0.3	0.4	0.6	0.7	0.4	0.5	0.4	0.1
Mean	1.00	0.49	0.27	0.33	0.36	0.37	0.70	0.84	0.41	0.44	0.36	0.11
Grand mean	0.74		0.30		0.36		0.77		0.43		0.24	

TABLE IX
Assortment (%) for a 2.80-mm Screen for a Sortimat Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	59.4	76.0	91.1	85.7	75.5	74.7	70.0	59.9	81.2	81.0	90.0	94.0
2	53.4	68.0	86.4	83.7	67.0	66.8	62.0	57.6	73.3	74.1	92.1	85.8
3	55.8	71.6	88.1	85.7	69.8	67.0	67.8	58.3	77.4	77.9	87.0	93.3
4	50.2	67.3	86.5	84.1	68.5	66.3	62.2	52.4	75.1	74.0	88.0	92.9
5	50.6	69.3	84.7	83.2	68.5	70.3	65.9	60.5	77.1	76.8	88.2	91.7
6	52.6	71.2	84.8	84.4	67.7	69.1	66.2	58.2	76.5	76.0	86.3	92.9
7	55.6	69.9	84.6	85.4	71.1	70.9	67.5	62.1	76.8	78.4	88.5	92.2
Mean	53.94	70.47	86.6	84.6	69.73	69.30	65.94	58.43	76.77	76.46	88.59	91.83
Grand mean	62.21		85.60		69.51		62.19		76.61		90.21	

TABLE X
Assortment (%) for a 2.50-mm Screen for a Sortimat Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	26.1	17.9	6.9	10.2	17.3	18.4	20.9	27.1	14.9	14.2	7.8	5.0
2	28.6	23.2	9.4	11.1	21.9	22.5	24.1	27.0	19.5	18.1	6.1	10.0
3	28.4	22.1	9.3	11.1	21.9	24.5	22.8	28.2	17.8	17.0	10.4	5.5
4	32.9	24.0	10.7	11.2	22.0	23.2	24.8	31.6	18.5	18.9	8.3	5.7
5	31.5	23.3	11.5	12.2	22.2	21.2	23.8	26.9	17.1	17.6	9.3	7.0
6	31.3	21.9	12.2	11.6	22.7	22.6	23.9	28.7	18.4	18.6	10.1	5.6
7	29.2	22.2	12.2	10.9	21.3	21.1	22.5	25.5	17.7	16.4	8.0	6.3
Mean	29.71	22.09	10.31	11.19	21.33	21.93	23.26	27.86	17.70	17.26	8.57	6.44
Grand mean	25.88		10.74		21.62		25.53		17.46		7.50	

TABLE XI
Assortment (%) for a 2.20-mm Screen for a Sortimat Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	11.7	5.2	1.9	3.2	6.3	6.1	8.1	11.0	3.3	4.0	1.8	0.8
2	15.0	7.9	3.6	4.5	9.5	9.6	11.7	13.1	5.7	6.5	1.5	3.4
3	13.3	5.4	2.1	2.9	7.1	7.5	7.9	11.2	4.1	4.5	2.5	1.2
4	14.4	7.2	2.4	4.1	8.2	10.0	11.0	13.3	5.5	5.8	3.1	1.2
5	14.6	6.4	3.1	3.8	8.3	7.4	8.8	10.6	4.9	4.6	2.2	1.2
6	13.1	5.5	2.6	3.4	8.3	6.9	8.4	10.7	4.2	4.5	2.8	1.2
7	12.7	6.7	2.5	2.9	6.6	6.8	8.7	10.4	4.6	4.4	2.7	1.2
Mean	13.54	6.33	2.60	3.54	7.76	7.76	9.23	11.47	4.61	4.90	2.37	1.46
Grand mean	9.94		3.07		7.76		10.35		4.76		1.91	

TABLE XII
Assortment (%) for Sample Through a 2.20-mm Screen for a Sortimat Grader

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	2.6	1.0	0.5	0.9	1.0	0.8	1.1	2.1	0.7	0.8	0.6	0.2
2	3.0	1.2	0.7	0.8	1.6	1.3	2.3	2.5	1.4	1.3	0.4	1.0
3	2.7	1.1	0.6	0.5	1.3	1.2	1.7	2.5	0.9	0.8	0.5	0.1
4	2.7	1.6	0.4	0.7	1.3	1.1	2.0	2.8	1.0	1.4	0.7	0.3
5	3.1	1.1	0.6	0.5	1.0	1.0	1.4	1.9	1.0	1.0	0.5	0.2
6	3.2	1.6	0.5	0.7	1.5	1.4	1.6	2.6	1.0	1.1	0.9	0.3
7	2.6	1.3	0.8	0.8	1.0	1.3	1.4	2.1	1.0	0.8	0.9	0.4
Mean	2.84	1.27	0.59	0.70	1.24	1.16	1.64	2.36	1.00	1.03	0.64	0.36
Grand mean	2.06		0.64		1.20		2.00		1.01		0.50	

TABLE XIII
Statistical Summary of Results for 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	6	60.83	1.89	3.11	5.30	3.40	5.59	9.51
C/D	6	84.65	0.64	0.75	1.78	1.33	1.57	3.71
E/F	6	67.38	1.52	2.25	4.25	2.24	3.33	6.27
G/H	6	59.48	1.35	2.26	3.77	2.61	4.39	7.32
I/J	6	75.53	0.49	0.65	1.38	2.41	3.19	6.74
K/L	6	89.67	3.40	3.79	9.53	2.81	3.13	7.86
6/64 in.								
A/B	6	30.97	0.79	2.56	2.22	2.13	6.88	5.97
C/D	6	12.51	0.48	3.81	1.33	1.08	8.63	3.02
E/F	6	26.28	0.93	3.55	2.61	1.72	6.56	4.83
G/H	6	31.77	0.75	2.35	2.09	1.86	5.86	5.21
I/J	6	20.45	0.37	1.81	1.04	2.01	9.83	5.63
K/L	6	8.32	2.65	31.87	7.42	2.34	28.09	6.54
5/64 in.								
A/B	6	7.38	1.24	16.74	3.46	1.90	25.71	5.32
C/D	6	2.56	0.23	9.15	0.66	0.58	22.69	1.63
E/F	6	5.69	0.68	11.90	1.90	1.18	20.65	3.29
G/H	6	7.98	0.62	7.82	1.75	1.51	18.85	4.21
I/J	6	3.53	0.22	6.18	0.61	0.69	19.44	1.92
K/L	6	1.68	0.65	38.68	1.82	0.63	37.18	1.75
Through 5/64 in.								
A/B	6	0.85	0.16	18.23	0.43	0.24	28.09	0.67
C/D	6	0.38	0.09	22.34	0.24	0.10	25.20	0.27
E/F	6	0.41	0.06	14.49	0.17	0.15	36.46	0.42
G/H	6	0.86	0.08	9.63	0.23	0.23	26.27	0.63
I/J	6	0.52	0.09	16.57	0.24	0.10	18.70	0.27
K/L	6	0.30	0.10	32.20	0.27	0.10	32.77	0.28

[a] Calculations were made based on Youden unit block design analysis (1).

TABLE XIV
Statistical Summary of Results for Sortimat Grader Imperial 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	7	63.41	1.72	2.71	4.81	3.98	6.27	11.13
C/D	7	85.85	0.70	0.82	1.97	1.34	1.56	3.74
E/F	7	70.81	1.67	1.93	3.82	3.65	5.15	10.21
G/H	7	62.20	1.99	3.20	5.58	3.69	5.94	10.34
I/J	7	77.77	0.55	0.71	1.54	3.26	4.19	9.12
K/L	7	90.60	0.47	0.52	1.32	1.40	1.55	3.92
6/64 in.								
A/B	7	29.12	1.41	4.85	3.96	2.36	8.11	6.61
C/D	7	11.68	0.46	3.97	1.30	0.84	7.16	2.34
E/F	7	23.86	1.09	4.58	3.06	2.60	10.88	7.27
G/H	7	29.93	1.23	4.10	3.43	2.08	6.95	5.82
I/J	7	18.74	0.69	3.70	1.94	2.47	13.18	6.92
K/L	7	7.88	0.41	5.23	1.15	0.97	12.34	2.72
5/64 in.								
A/B	7	6.74	1.02	15.12	2.85	1.66	24.66	4.66
C/D	7	2.20	0.27	12.09	0.75	0.60	27.13	1.67
E/F	7	5.04	0.33	6.46	0.91	1.18	23.38	3.30
G/H	7	7.19	0.68	9.48	1.91	1.58	21.95	4.42
I/J	7	3.15	0.16	5.00	0.44	0.82	25.93	2.29
K/L	7	1.34	0.16	11.72	0.44	0.44	32.62	1.23
Through 5/64 in.								
A/B	7	0.74	0.17	22.94	0.48	0.23	31.30	0.65
C/D	7	0.30	0.07	23.00	0.19	0.09	28.64	0.24
E/F	7	0.36	0.06	17.47	0.18	0.15	39.74	0.41
G/H	7	0.77	0.22	28.43	0.61	0.24	31.25	0.68
I/J	7	0.44	0.11	26.46	0.32	0.12	29.03	0.35
K/L	7	0.24	0.06	23.60	0.16	0.07	31.39	0.21

[a] Calculations were made based on Youden unit block design analysis (1).

TABLE XV
Statistical Summary of Results for Sortimat Grader Metric Screens[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	7	63.21	1.24	2.00	3.48	3.08	4.94	8.61
C/D	7	85.60	1.39	1.62	3.88	1.82	2.12	5.08
E/F	7	69.51	1.20	1.73	3.36	2.93	4.22	8.21
G/H	7	62.19	1.70	2.74	4.76	3.01	4.85	8.44
I/J	7	76.61	1.17	1.52	3.27	2.66	3.48	7.46
K/L	7	90.21	3.10	3.44	8.68	2.39	2.64	6.68
2.5 mm								
A/B	7	25.90	1.02	3.95	2.86	2.16	8.35	6.05
C/D	7	10.75	1.10	10.22	3.08	1.43	13.28	4.00
E/F	7	21.63	0.83	3.85	2.33	1.89	8.72	5.28
G/H	7	25.56	1.15	4.49	3.21	1.65	6.46	4.63
I/J	7	17.48	0.57	3.24	1.59	1.53	8.75	4.28
K/L	7	7.51	2.05	27.34	5.75	1.60	21.25	4.47
2.2 mm								
A/B	7	9.94	0.55	5.50	1.53	1.10	11.08	3.08
C/D	7	3.07	0.30	9.85	0.85	0.60	19.48	1.68
E/F	7	7.76	0.72	9.31	2.02	1.31	16.95	3.68
G/H	7	10.35	0.48	4.63	1.34	1.36	13.15	3.81
I/J	7	4.76	0.29	6.15	0.82	0.87	18.25	2.43
K/L	7	1.91	0.91	47.37	2.54	0.74	38.42	2.06
Through 2.2 mm								
A/B	7	2.06	0.21	10.26	0.59	0.25	12.00	0.69
C/D	7	0.64	0.14	21.47	0.39	0.14	22.39	0.40
E/F	7	1.20	0.14	11.50	0.39	0.23	19.16	0.64
G/H	7	2.00	0.20	10.09	0.57	0.37	18.34	1.03
I/J	7	1.01	0.14	13.78	0.39	0.23	22.67	0.64
K/L	7	0.50	0.28	56.82	0.80	0.25	50.80	0.71

[a] Calculations were made based on Youden unit block design analysis (1).

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

Subcommittee Members: R. Ortiz, *Chair*; T. Ishihara (BCOJ); V. Kellner (EBC); 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, Phenylacetaldehyde, SPME, *trans*-2-Nonenal

CONCLUSIONS

1. Standard deviations for the measurement of seven compounds by solid-phase microextraction–gas chromatography/mass spectrometry (SPME GC/MS) in packaged American lager beer were determined. With limited results, the range of standard deviations and relative standard deviations for the seven compounds studied for fresh beer sample pair A/C was 0.05008–25.24 µg/L and 8.845–112.1%, respectively, and was judged unacceptable.
2. With limited results, the range of standard deviations and relative standard deviations for the seven compounds studied for aged beer sample pair B/D was 0.07548–89.99 µg/L and 0.7463–77.28%, respectively, and was judged unacceptable.

RECOMMENDATION

1. The subcommittee chair recommends the collaborative study be repeated with the existing subcommittee members and additional collaborators to achieve enough data sets for standard statistical treatment. Additional efforts to minimize sample and analytical variability will also be applied to the next study.

This is the first 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 measurement methods for beer volatiles using SPME GC/MS (1). The method used for the collaborative was based on several published methods (3,4,5), as well as the method used by the subcommittee chair's laboratory.

The aldehydes selected to be quantified by 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 the changes in flavor stability and the resulting changes in taste and aroma of packaged beers (2). The aldehydes measured by this method were 2-methyl-propanal (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: samples A, B, C, and D. Samples A and C were the same brand but were from different packaging runs; they were stored at 4°C until shipping. Samples B and D were created by subsampling from the A and C cartons (respectively) and were force aged at 50°C for 7 days. This created sample pairs A/C (fresh) and B/D (aged). The elevated temperature samples B and D were subjected to for 7 days force aged the beer samples to increase the levels of staling aldehydes studied. In contrast, beer samples A and C were kept at 4°C to maintain fresh beer flavor and, likely, correspondingly lower levels of aldehydes.

The samples were packed with cold packs in thermally protected containers and shipped to the collaborators cold. The collaborators were instructed to place the samples in a refrigerator upon receipt and store them there until ready to analyze to minimize any changes in aldehyde concentrations prior to analysis.

The collaborators were instructed to analyze samples within two weeks of receipt and report results using a data sheet provided by the subcommittee chair.

RESULTS AND DISCUSSION

Results were received from only three of the seven collaborators. The results for each of the measured aldehydes for sample pairs A/C and B/D are listed in Tables I–VII. With limited data available, Youden Block statistical analysis could not be performed. Instead, standard deviations and percent relative standard deviations were determined for the results. The range of standard deviations and relative standard deviations for the seven compounds studied for fresh beer sample pair A/C was 0.05008–25.24 µg/L and 8.845–112.1%, respectively. The range of standard deviations and relative standard deviations for the seven compounds studied for aged beer sample pair B/D was 0.07548–89.99 µg/L and 0.7463–77.28%, respectively. These results confirmed there was high variation in the reported concentrations for most compounds in both sample pairs. The subcommittee chair expected the possibility of higher results reported compared with his own, particularly from labs outside the United States due to possible temperature increase during shipping, which would drive increases in staling aldehyde concentrations in those samples. However, the data did not indicate this occurred. Another potential variable was the relative lack of experience some collaborators had with this SPME-GC/MS method and the limited time allowed to work on the project. Follow-up with collaborators from this round will be valuable in obtaining feedback to improve the method and study instructions.

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

	Sample Pair		Sample Pair	
Collaborator	**A**	**C**	**B**	**D**
1	4.04	2.80	10.8	6.30
2	4.83	4.21	30.0	22.7
3	3.51	3.29	32.0	32.2
Mean	4.127	3.433	24.27	20.40
Grand mean	3.780		22.33	
SD	0.664	0.7158	11.71	13.10
%RSD	16.10	20.85	48.24	64.23

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

	Sample Pair		Sample Pair	
Collaborator	**A**	**C**	**B**	**D**
1	1.26	0.918	5.84	3.25
2	1.00	0.893	4.67	3.07
3	1.18	1.05	6.04	5.68
Mean	1.147	0.9537	5.517	4.000
Grand mean	1.0502		4.758	
SD	0.1332	0.0844	0.7400	1.458
%RSD	11.61	8.845	13.41	36.44

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

	Sample Pair		Sample Pair	
Collaborator	**A**	**C**	**B**	**D**
1	4.83	3.49	13.4	7.82
2	6.68	5.49	13.5	10.3
3	4.89	4.71	13.3	13.0
Mean	5.467	4.563	13.40	10.37
Grand mean	5.015		11.89	
SD	1.051	1.008	0.1000	2.591
%RSD	19.23	22.09	0.7463	24.97

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

	Sample Pair		Sample Pair	
Collaborator	**A**	**C**	**B**	**D**
1	8.64	7.30	209	123
2	54.0	47.6	338	301
3	12.1	11.5	251	235
Mean	24.92	22.13	266.0	219.7
Grand mean	23.53		242.8	
SD	25.24	22.15	65.79	89.98
%RSD	101.3	100.1	24.73	40.96

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

	Sample Pair		Sample Pair	
Collaborator	**A**	**C**	**B**	**D**
1	0.961	0.719	0.981	0.764
2	3.25	1.48	2.08	1.53
3	0.571	0.589	0.682	0.656
Mean	1.594	0.9293	1.248	0.9833
Grand mean	1.262		1.115	
SD	1.447	0.4813	0.7362	0.4765
%RSD	90.80	51.79	59.00	48.46

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

	Sample Pair		Sample Pair	
Collaborator	**A**	**C**	**B**	**D**
1	5.67	7.89	2.95	2.05
2	16.2	14.4	28.5	26.3
3	6.37	5.85	21.8	21.5
Mean	9.413	9.380	17.75	16.62
Grand mean	9.397		17.18	
SD	5.888	4.465	13.25	12.84
%RSD	62.55	47.61	74.63	77.28

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

	Sample Pair		Sample Pair	
Collaborator	**A**	**C**	**B**	**D**
1	0.0257	0.0201	0.0792	0.496
2	0.225	0.117	0.225	0.154
3	0.0440	0.0466	0.186	0.173
Mean	0.0982	0.0612	0.1634	0.2743
Grand mean	0.07973		0.2189	
SD	0.1102	0.05008	0.07548	0.1922
%RSD	112.1	81.78	46.19	70.06

Soluble Starch

Subcommittee Members: R. Jennings, *Chair/Ex officio*; B. Amundsen; S. Bartlett; M. Gastl; M. Goldsmith; R. Joy; M. Joyce; A. MacLeod; J. Menert; A. Mundy; M. Omillian; and P. Ritchie

RECOMMENDATION

1. The subcommittee recommends continuing to provide this lot of modified potato starch for the upcoming year.

This subcommittee is a standing subcommittee whose goal is to coordinate a testing program for modified potato soluble starch that will ensure a consistent supply of high-quality soluble starch for the Society. 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. Starch lot no. 8621J was collaboratively tested and approved for sale by the Society during the summer of 2008 (1). As of February 17, 2010, 858 kg (429 units) of starch lot no. 8621J were available for sale.

The subcommittee recommends evaluation of a new lot of starch when current supplies fall below 350 kg. It is anticipated that at the current rate of depletion a new lot will not need to be evaluated prior to 2012. The chair will monitor the soluble starch inventory and initiate the study when necessary.

doi:10.1094/ASBCJ-2010-0928-01

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Craft Brewers Annual Meeting Report

Subcommittee Members: G. Kelly, *Chair*; J. Palausky; F. Strachan; and D. Wilson

COMMITTEE UPDATE

- There are now questions put in the monthly *ASBC Buzz* in lieu of polling members about their ASBC Local Sections in order to informally gather demographics about ASBC membership and to gather feedback.
- A suggestion was made to create an "Add a Local Section" link on the ASBC website so individuals who have an interest in starting a Local Section can contact ASBC headquarters directly.
- Local Section officers have been contacted to find out how their sections are doing and to find out if there is anything either myself or ASBC can do to help their sections out more. These were the questions sent out to the section officers listed on the ASBC website:
  1. How often does your Section meet a year?
  2. How many attendees do you see at your meetings? What percentage of those attendees are from small breweries/ brewpubs?
  3. What would you say is the biggest challenge for your Section?
  4. What could ASBC do better to support your Section?
  5. Other needs or suggestions for ASBC either at the national or local level?

  There was low response to these questions.

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