Technical Committee and Subcommittee Reports

2008–2009 Report of the Technical Committee

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

Activity in 13 subcommittees was conducted by the ASBC Technical Committee and Subcommittee chairs during 2008–2009. As a result, two methods are being recommended for inclusion in the ASBC *Methods of Analysis* (MOA):

- Sulfur Dioxide in Beer by Flow Injection Analysis (FIA), chaired by Aaron Porter (Sierra Nevada Brewing Company).
- HLP Media, chaired by Tobias Fischborn (Lallemand Inc.).

Additionally, a review of the Malt Section of the MOA was led by Rebecca Jennings (Rahr Malting), and a review of the Sensory Section of the MOA was led by Sue Thompson (MillerCoors). The updated methods have been recommended for inclusion in the MOA. The Sensory Subcommittee has also recommended two updated tools for sensory analysis: a panelist performance and validation tool and a reference standards list.

Three 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).
- Accurate IBU Measurement of Dry-hopped Beers, chaired by Ruth Martin (Sierra Nevada Brewing Company).
- Wort and Beer Fermentable and Total Carbohydrates by HPLC, chaired by Mark Eurich (MillerCoors).

The review of three sections of the ASBC MOA will commence in 2009–2010. The review of the Microbiology section will be led by Chris Powell (Lallemand Inc.); the review of the Wort section will be led by Mark Eurich (MillerCoors); and the review of Filter Aids section will be led by Aaron Porter (Sierra Nevada Brewing Company).

Twelve new subcommittees will be initiated in 2009:

- ATP, chaired by Caroline Pachello (MillerCoors).
- Malt-4 Extract Mills and Mashing, chaired by Aaron Macleod (Canadian Grain Commission).
- 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).
- EMAST Standard, chaired by Jolanta Menert (Bush Agricultural Resources LLC).
- Sortimat, chaired by Paul Ritchie (Canada Malting)
- Malt 8 Protein (Barley Standard Versus EDTA), chaired by Rebecca Jennings (Rahr Malting).
- GC-FID Analysis for Beer Volatiles, chaired by Joe Palausky (Boulevard Brewing).

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As in previous years, the following seven 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 Sue Thompson (MillerCoors)
- International Hop Standards Subcommittee, chaired by Bob Foster (MillerCoors)

Jim Monroe (retired member, formerly of Anheuser-Busch) is continuing to review the Check Services Program. He is currently focused on the Malt 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!

I would like to thank the subcommittee chairs for their hard work and dedication in conducting their respective collaborative studies through 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.

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

The Soluble Starch Subcommittee 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 the ASBC. The 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 the 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.

MOA Methods Review
(Mark Eurich, Mark.Eurich@MillerCoors.com; Chris Powell, cpowell@lallemand.com; and Aaron Porter, AaronP@Sierranevada.com)

The mandate of this standing subcommittee is to review the methods published in the ASBC *Methods of Analysis*, based on criteria such as relevancy, use of hazardous chemicals, and outdated/no longer available equipment. Methods that the subcommittee identifies as being suitable for removal from the MOA will be recommended for archiving, along with a statement as to why they were archived. Methods in need of revision will be updated, keeping in mind not to change the methodology that had been collaboratively tested and approved. Notes may be included with these methods to offer guidance on alternate materials. The subcommittee will review the MOA section by section, with review of the Wort, Filter Aid, and Microbiology sections commencing this year. The chairs of the subcommittee are Mark Eurich (Wort), Aaron Porter (Filter Aids), and Chris Powell (Microbiology).

Accurate Determination of IBU Levels in Dry-hopped Beers
(Ruth Martin, ruthm@sierranevada.com)

This subcommittee was formed to evaluate a modified ASBC IAA method for the accurate determination of IBU levels in dry-hopped beers. Since this method is one that is of importance to the craft brewing membership of the ASBC (based on their style of beers), we are counting on substantial representation from this segment as collaborators in the trial.

Sensory Science
(Sue Thompson, Suzanne.Thompson@MillerCoors.com)

This is a standing subcommittee formed on recommendation from the Technical Committee to bring more focus to sensory science in the 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 from 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.

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 (SPME) 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 employing 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 (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 Fermentable 14-B and Beer 41-B to methods currently employed in the brewing industry. Polling and key findings from previous years showed great interest in these two methods. Significant technological gains would also deem 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 utilizing a common ATP bioluminescence instrument.

Malt-4 Extract Mills and Mashing
(Aaron Macleod, aaron.macleod@grainscanada.gc.ca)

Upon reviewing the Malt section of the MOA, it was determined that there are several inherent problems with the method used in Malt-4 Extract:

1. The malt used for standardizing mills needs to have ranges added to some of the parameters to account for changing crop years and varieties that are utilized.
2. The Miag-Seck mill is no longer valid. This mill was discontinued about 25 years ago and is no longer available.
3. The mashing apparatus mentioned is not common in the industry. There are several variations, and the method needs to reflect the changing world of mashing apparatus.

There are other minor things that will be tested by this subcommittee that all relate to the outcome of extract in mash.

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. Even though the reference method must be used to substantiate the validity of a new method, this does not mean a new method cannot be pursued. It has been suggested that the 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 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 from Fusarium that can lead to brewing performance issues. It has been suggested that the 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 the EZ-Tox yields results in about 5 min compared with 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).

EMAST Standard

(Jolanta Menert, Jolanta.Menert@anheuser-busch.com)

In the fall of 2007 Sigma announced that they would no longer be making the Alpha-Amylase type VIII-A (A-2771-500) that is mentioned in ASBC method Malt-7C. There has been a growing concern that there is not a standardized solution available for this method. This subcommittee aims to find a suitable replacement for this standard. EMAST, supplied by Megazyme, was developed in 2007 as a possible replacement. Once a replacement is found it may be possible to use it as a reference for the Malt-6C method as well.

Sortimat

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

Laboratories are looking for accurate methods that are easy to use and produce results in a quick and efficient manner. It has been recommended that the 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.

Malt-8 Protein (Barley Standard Versus EDTA)

(Rebecca Jennings, rjennings@rahr.com)

The levels of nitrogen in EDTA are more fitting for the meat industry than they are for the malting and barley industries. It is recommended that an alternative to EDTA be looked at to set a calibration of the combustion method that is more in line with the nitrogen levels that are present in both malt and barley. One such alternative would be the NIST-certified barley standard.

International Hop Standards Subcommittee

(Bob Foster, Robert.Foster@MillerCoors.com)

This subcommittee has existed for 13 years, previously as the International Subcommittee for Isomerized Hop α-Acids Standards (ISIHAS), and is a standing subcommittee whose goal is to produce, purify, and verify isomerized and nonisomerized 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.

Total Sulfur Dioxide Analysis Using a Segmented Flow Analyzer (International Method)

Subcommittee Members: A. Porter, *Chair*; A. Caruso; B. Els (EBC); B. Fandrey; K. Hofecker; H. Klein (EBC); M. Kucharic (EBC); T. Kunz (EBC); K. Lakenburges; Q. Le; J. Menting; J. O'Sullivan; J. Reffner; J. Scott; E. Seng; X. Sitjas (EBC); L. Young (EBC); T. M. Zarnkow (EBC); and M. Eurich (*ex officio)*.

Keywords: Automated SO_2, FIA, Flow injection analysis, SFA, SO_2

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the Beer-21 method ranged from 2.1 to 8.9% and 9.5 to 14.9%, respectively, and were judged acceptable.
2. Repeatability and reproducibility coefficients of variation for the segmented flow analysis (SFA) method ranged from 1.5 to 7.9% and 9.4 to 12.7%, respectively, and were judged acceptable.
3. Based on the paired *t* test for differences in means for SO_2 analyses, the Beer-21 and SFA methods were not significantly different at the 95% confidence level.

RECOMMENDATIONS

1. The subcommittee recommends the method for sulfur dioxide measurement by SFA be included in the ASBC *Methods of Analysis* (1).
2. Discharge the subcommittee.

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This was the second year of the subcommittee's evaluation of automated flow-injection methods for total sulfur dioxide (SO_2) determination as alternatives to Beer-21. Beer-21, which is currently the only approved method, is labor intensive and requires the use of mercuric compounds. In the first year, samples were sent to six collaborators. Only five sets of results were received, which did not meet the minimum criteria for statistical analysis. In the second year, it was recommended by the subcommittee that the collaborative study be repeated. Additions in the second year included EBC members capable of analyzing samples using Beer-21 as well as analyses of an international packaged beer sample that potentially would have an SO_2 result above the U.S. labeling limit.

PROCEDURE

Collaborators received four to six sample pairs of commercial lager beers for analyses by either the SFA or Beer-21 method. Table I shows the sample pair distribution. Sufficient numbers of sample pairs with the same production date were not available for all collaborators.

Collaborators were asked to follow their manufacturer's recommend procedures for instrument operation and analysis, analyze the sample pairs in duplicate, and report the average (1). Results were evaluated using the Youden unit block design (1) and Minitab statistical software for paired *t* test, assuming unequal variance at the 95% confidence level.

RESULTS AND DISCUSSION

Results from 12 SFA collaborators were received for the sample pair A1/A2. Results from 12 collaborators (6 SFA and 6 Beer-21) were received for the sample pair A3/A4. Results from 18 collabo-

TABLE I
SO_2 Segmented Flow Analysis and Beer-21 Method Results (ppm)

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A1**	**A2**	**A3**	**A4**	**B1**	**B2**	**C1**	**C2**
1[a]	12.3	9.9	15.8	15.5	1.8	1.9	1.5	1.4
2[a]	14.2	11.8	18.7	18.8	2.1	2.1	1.7	1.5
3[a]	14.1	11.8	17.8	17.4	2.0	1.9	1.7	1.6
4[a]	11.4[b]	9.5[b]	14.4	14.7	0.4[b]	0.2[b]	0.4[b]	0.3[b]
5[a]	13.7	10.1	17.2	16.5	2.0	2.1	1.7	1.4
6[a]	14.3	12.1	18.2	18.0	3.1[b]	2.4[b]	2.0	1.8
7[a]	16.1[b]	16.5[b]	…[c]	…[c]	2.1	2.3	1.9	1.6
8[a]	14.4	12.7	…[c]	…[c]	3.1[b]	3.1[b]	2.7[b]	2.5[b]
9[a]	13.4	10.7	…[c]	…[c]	1.6	1.6	1.4	1.6
10[a]	11.6	10.3	…[c]	…[c]	1.8	1.7	1.5	1.1
11[a]	15.5	13.3	…[c]	…[c]	1.8	1.8	1.6	1.2
12[a]	10.1	8.3	…[c]	…[c]	2.0	1.8	1.5	1.5
13[d]	…[c]	…[c]	16.8	16.7	2.1	2.0	1.8	1.7
14[d]	…[c]	…[c]	16.3	16.9	2.1	2.2	1.8	1.6
15[d]	…[c]	…[c]	15.6	16.3	1.6	1.2	1.9	1.4
16[d]	…[c]	…[c]	18.8	19.2	2.2	2.0	1.7	1.2
17[d]	…[c]	…[c]	15.2	15.6	1.8	1.9	1.3	1.2
18[d]	…[c]	…[c]	14.8	14.2	2.0	1.9	1.6	1.5
Mean	13.36	11.10	17.02[a], 16.25[d]	16.82[a], 16.48[d]	1.90[a], 1.97[d]	1.91[a], 1.87[d]	1.65[a], 1.68[d]	1.47[a], 1.43[d]
Grand mean	12.23		16.92[a], 16.37[d]		1.91[a], 1.92[d]		1.56[a], 1.56[d]	

[a] SFA method.
[b] Data excluded through Dixon's outlier test.
[c] Sample pairs not sent.
[d] Beer-21 method.

TABLE II
Statistical Summary of Results[a]

Sample Pair	No. of Labs	Grand Mean	Repeatability			Reproducibility		
			S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Segmented flow analysis								
A1/A2	10	12.23	0.45	3.6	1.25	1.55	12.7	4.33
A3/A4	6	16.92	0.26	1.5	0.73	1.59	9.4	4.46
B1/B2	9	1.91	0.09	4.7	0.25	0.18	9.7	0.52
C1/C2	10	1.56	0.12	7.9	0.34	0.19	12.6	0.54
Beer-21 method								
A3/A4	6	16.37	0.34	2.1	0.96	1.56	9.5	4.37
B1/B2	6	1.92	0.13	6.8	0.36	0.28	14.9	0.79
C1/C2	6	1.56	0.14	8.9	0.39	0.20	12.8	0.55

[a] Calculations based on Table I.

TABLE III
***t*-Test Comparison of SO_2 Measured by Segmented Flow Analysis (SFA) and ASBC Method Beer-21[a]**

	Sample A3	Sample A4	Sample B1	Sample B2	Sample C1	Sample C2
Mean over two methods	16.61	16.64	1.93	1.87	1.65	1.44
Statistical parameter						
N for SFA results	6	6	9	9	10	10
N for Beer-21 results	6	6	6	6	6	6
P value[b]	0.41	0.73	0.62	0.79	0.76	0.74
Degrees of freedom	9	9	8	7	9	10
Calculated *t*[b]	0.853	0.376	–0.597	0.281	0.452	0.289
$t_{0.05}$	2.228	2.228	2.160	2.160	2.145	2.145

[a] Calculations based on Table I; outliers were excluded.
[b] Not significant at the 95% confidence level.

rators (6 Beer-21 and 12 SFA) were received for both sample pairs B1/B2 and C1/C2. Outliers were determined using Dixon's outlier test. The results are summarized in Table I.

The statistical summary of the SO_2 data for both the SFA and Beer-21 methods are presented in Table II. Repeatability and reproducibility coefficients of variation for the SFA method ranged from 1.5 to 7.9% and 9.4 to 12.7%, respectively, and were judged acceptable. Repeatability and reproducibility coefficients of variation for the Beer-21 method ranged from 2.1 to 8.9% and 9.5 to 14.9%, respectively, and were judged acceptable.

The results of the paired *t*-test calculations comparing the SO_2 results of the SFA and Beer-21 methods are presented in Table III. The paired *t*-test results indicate the two methods were not significantly different at the 95% confidence level. Paired *t*-test calculations were not performed on samples A1 or A2 since they were only analyzed by the SFA method.

LITERATURE CITED

1. American Society of Brewing Chemist. *Methods of Analysis*, 2008 ed. Beer-10A Spectrophotometric color method, -21, Total sulfur dioxide; Statistical Analysis-4 Youden unit block collaborative testing procedure, -5 Comparison of test methods. The Society, St. Paul, MN, 2008.

HLP Medium for the Detection of Lactic Acid Bacteria (International Method)

Subcommittee Members: T. Fischborn, *Chair*; E. Belden; Y. Bergeron; W. Box; L. Castonguay; H. Kanda (BCOJ); G. Kelly; C. Pachello; J. H. Park (BCOJ); K. Suzuki (BCOJ); S. Tada (BCOJ); L. White; D. Wilson; and C. Powell (*ex officio*)

Keywords: Contaminants, *Lactobacillus*, Microbial identification, *Pediococcus*

CONCLUSIONS

1. Each collaborator could identify the growth of lactic acid bacteria on HLP medium but could not detect growth of brewing yeast or an *Enterobacter* (negative control) strain.
2. Collaborators could distinguish between *Lactobacillus* and *Pediococcus* species according to their growth morphologies.
3. Cultivation on HLP medium was demonstrated to be a suitable method for the detection and differentiation of lactic acid bacteria.

RECOMMENDATIONS

1. It is proposed that the use of HLP medium should be approved for inclusion in the ASBC *Methods of Analysis* for the detection and identification of lactic acid bacteria in process samples.
2. Discharge the subcommittee.

This was the first year of the subcommittee, which was formed to evaluate the use of Hsu's *Lactobacillus* and *Pediococcus* (HLP) medium (1) to detect and identify lactic acid bacteria (LAB). Although beer is an unfavorable habitat for many microbes, contamination by certain types of bacteria can occur during the brewing process, leading to production inconsistencies and quality defects in the final product. Currently, there are a number of media types that are recommended for the detection of LAB; however, few types of media allow for the simultaneous detection and identification of microbes. HLP medium can be prepared as a solid or semisolid medium that can be used to detect LAB. However, in semisolid form it also offers a means of identifying LAB to the genus level based on colony morphology. Because preparation of HLP medium requires only a microwave oven, it also offers a benefit to breweries that may not have the laboratory equipment necessary to perform more complex microbiological analyses.

PROCEDURE

A lager yeast (culture A), an ale strain (culture B) and three bacteria, including an *Enterobacter aerogenens* strain (culture C), *Pediococcus damnosus* strain (culture D), and *Lactobacillus brevis* strain (culture E), were sent to each collaborator, as well as a sample of HLP medium in dried form. Tubes of semisolid HLP medium were prepared by dissolving the dried powder in water before boiling in a microwave oven. Hot medium (14 mL) was aliquoted into sterile 15-mL Falcon tubes that were then cooled to 40°C and inoculated with 1 mL of each test microorganism. Microbial solutions were prepared prior to inoculation by suspending a loop full of the biomass from agar slants into 10 mL of sterile saline solution and serially diluting each organism in saline solution to a final dilution of 10^{-5}. These suspensions were pre-determined to provide approx. 10–100 cells when grown on agar. Aliquots of samples (cultures A–E) were added directly to HLP medium. In addition, mixtures of the lager yeast (culture A) and *P. damnosus* (culture D); and the ale yeast (culture B) and *L. brevis* (culture E) were prepared and inoculated to simulate mixed cultures. In conjunction, an uninoculated blank sample of HLP medium was also prepared. All tubes were sealed, placed in an incubator, and incubated aerobically at 30°C for up to 7 days. The growth and shape of colonies were recorded after 3, 5, and 7 days of incubation.

RESULTS AND DISCUSSION

Results from 11 collaborators were received for each set of strains investigated. All collaborators obtained growth for the LAB strains, while the yeast strains and negative control bacteria strain did not produce any colonies (Table I). Mixtures of yeast and bacteria produced identical results to those obtained using bacteria independently; the presence of yeast did not obscure the growth of bacteria or prevent colonies from being analyzed. All collaborators observed bacterial growth after 3 days, except for one collaborator who required 5 days to accurately ascertain growth of the strain in culture D and the culture B and D mix. Analysis of the shape and size of colonies allowed the two LAB strains to be differentiated according to their morphology, irrespective of whether brewing yeast was present (Table II). It should be noted that the definition of colony morphology varied according to each collaborator; however, a clear distinction could be observed in each instance. Typically, the *P. damnosus* strain was reported to produce smaller and longer colonies, while the *L. brevis* strain produced larger and more spherical individuals, often described as being teardrop shaped. Collaborators 6 and 8 provided images to further explain the observed morphologies (Fig. 1). The data obtained indicates that LAB species can be detected and differentiated using HLP medium, while yeast and the control bacteria strain could not be cultivated. While the collaborative did not include the analysis of an extensive range of microbes, the data obtained suggests that HLP medium could be used in breweries as a rapid means of screening production samples for LAB contaminants.

LITERATURE CITED

1. Hsu, W. P., Taparowsky, J. A., and Brenner, M. W. Two new media for culturing of brewery organisms. *Brewer's Dig.* 50:52-57, 1975.

TABLE I
Number of Collaborators Observing Growth After 3, 5, and 7 Days of Incubation[a]

Medium	3 Days	5 Days	7 Days
Culture A (lager)	0	0	0
Culture B (ale)	0	0	0
Culture C (*Enterobacter aerogenens*)	0	0	0
Culture D (*Pediococcus damnosus*)	10	11	11
Culture E (*Lactobacillus brevis*)	11	11	11
Mix of cultures B and D	10	11	11
Mix of cultures A and E	11	11	11
Blank uninoculated medium	0	0	0

[a] Values refer to the number of collaborators who obtained results (n = 11).

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TABLE II
Description of Colonies Observed After 7 Days of Growth in Hsu's *Lactobacillus* and *Pediococcus* (HLP) Medium[a]

Collaborator	Culture D	Culture E	Mix of Cultures B and D	Mix of Cultures A and E
1	Comet	Ellipsoidal	Comet	Ellipsoidal
2	Teardrop	Elongated snowball	Teardrop	Elongated snowball
3	Teardrop	Huge sesame seed	Teardrop	Huge sesame seed
4	Snowball	Teardrop	Snowball	Teardrop
5	Comet	Teardrop	Comet	Teardrop
6[b]	Streaky teardrop/meteor	Large trailing snowball	Streaky teardrop/meteor	Large trailing snowball
7	Small rain drops	Teardrop/air balloon	Small rain drops	Teardrop/air balloon
8[b]	Comet	Teardrop	Comet	Teardrop
9	Vertically long	Teardrop	Vertically long	Teardrop
10	Meteor	Teardrop	Meteor	Teardrop
11	Snowball	Teardrop	Snowball	Teardrop

[a] Cultures A–C did not produce any growth, as described in Table I.
[b] Shown in Figure 1.

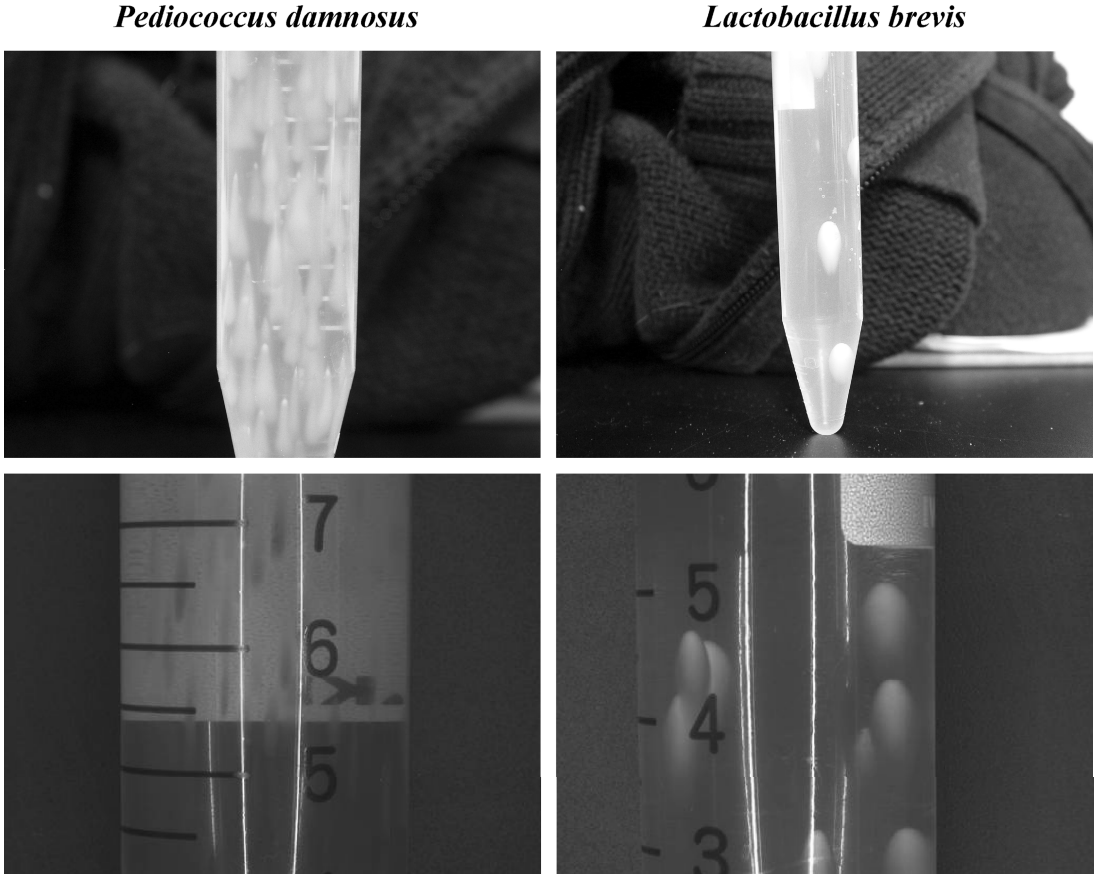

Fig. 1. Colony morphology of *Pediococcus damnosus* and *Lactobacillus brevis* species when cultivated on semisolid Hsu's *Lactobacillus* and *Pediococcus* (HLP) medium according to collaborators 6 (top) and 8 (bottom).

Coordination of New and Alternate Methods of Analysis

Subcommittee Members: J. Cornell, *Chair*; D. Bendiak; G. Kelly; J. Masschelin; P. Schwarz; S. Thompson; S. Van Zandycke; D. Sedin (*ex officio*) (*Please see the special note in the Membership and Meetings section*)
Corresponding Members: A. Mundy, European Brewery Convention (EBC); S. Sakuma, Brewery Convention of Japan (BCOJ)

Keywords: Accelerated aging, Allergens, Amino acids, Beer volatiles, Bitterness, Carbon dioxide, Color, Decarbonation, Flow injection analysis, Gluten, Malt sizing, Oxygen, Polyphenols, Propylene glycol, Segmented flow, Turbidity, Volatile sulfur compounds, Wort

RECOMMENDATIONS

1. Conduct online polling to obtain input on new and alternative methods.
2. Conduct online polling to obtain information on how labs are using segmented flow methods and measuring volatile esters and alcohols in beer by gas chromatography methods. Use the results to make decisions on future subcommittee formation and collaborative testing.
3. Gather additional information on methods for the determination of amino acids in wort to determine interest in the formation of a subcommittee and execution of collaborative testing.
4. Archive the topic "Portable Oxygen/CO_2 Device Evaluation" due to lack of a subcommittee chair with sufficient statistical prowess combined with a large range of devices to consider.
5. Archive the topic "Color Measurement in the Presence of Turbidity" due to lack of interest for subcommittee formation.

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

Attendance at the subcommittee meeting at WBC 2008 was very limited (5 people), but that has been typical of the WBC meetings compared with a typical ASBC Annual Meeting, which draws in excess of 30 people.

The fall report noted that additional members are being sought for the subcommittee. With recent consolidations in the industry and ever-increasing demands on time, it is becoming more difficult to find qualified members. Given the very close tie this subcommittee has with the Technical Committee, it has been decided to make the Coordination of New and Alternate Methods of Analysis Subcommittee an integral part of the Technical Committee's activities and align the membership of the two groups. Additional subject matter experts will be added to this subcommittee, or consulted with, on an as needed basis.

doi:10.1094/ASBCJ-2009-1002-01

Topics for Polling

Polling questions were developed and reviewed in the spring of 2009 for an online poll 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 survey results were presented at the annual meeting in Tucson, AZ, in June 2009. The topics in the online poll, along with background information, are described below. Summarized results from the polling 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. However, most of the input comes through the ASBC Annual Meeting. The World Brewing Congress held in 2008 led to limited input for new methods, so we are attempting to gather information by an alternative means.

Segmented Flow Analysis for Nonmalt methods (e.g., Bitterness [BU], Free Amino Nitrogen [FAN], Acetaldehyde, Diacetyl, etc.). This idea stems from six responses for the determination of sulfur dioxide (SO_2) in beer in 2007 at the annual meeting in Victoria, BC, Canada, and the resulting subcommittee formation. Polling for what analyses are being done by flow injection could provide better direction for future subcommittees and collaborative studies.

Gas Chromatography–Flame Ionization Detection of Beer Volatiles. This concept is a potentially more accessible and lower cost method than mass spectrometry that would be focused on esters and alcohols. There are likely several labs that are using a method of this sort, and polling would help determine method parameters and gauge interest for a potential collaborative (7,9).

Miniature Fermentation Assay. To assess the phenotypic characteristics of brewing yeast, small-scale fermentation vessels are often employed to replicate or mimic full-scale vessels. There are a number of different small-scale vessels that are widely used, but these are often still too large in volume for high-throughput analysis. Currently, a standard miniature-scale fermentation technique does not exist, and polling to assess interest in a potential subcommittee is warranted. One possible technique is the method described and used in studies of premature yeast flocculation (PYF), as published in the *Journal of the American Society of Brewing Chemists* by Lake et al (6).

Amino Acids in Wort by High-Performance Liquid Chromatography. This topic was not included in the poll, but will be included in the next round of polling. Six people signed up expressing interest for a potential subcommittee at the annual meeting in Victoria in 2007. Additional polling online would provide details on methods in use and potential modifications developed in light of the worldwide shortage of acetonitrile. A volunteer is needed to develop questions (3).

Researching Methods/Subcommittee Chairs Needed

Volatile Sulfur Compounds in Beer. An excellent subcommittee chair was identified, and although the person was interested, business constraints resulted in their not being able to participate. 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 (4,5,8).

Malt Sizing Method. At WBC 2008 Paul Richie (Great Western Malt) suggested that the current ASBC method Malt-2B for "assortment" determination (kernel size) should be updated (1). This method was reviewed by the Malt Methods of Analysis Subcommittee and recommended for an updated collaborative study, as referenced in their report.

Decarbonation of Beer. Several methods exist 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 necessarily 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 (1,2,10).
- Provide a recommendation to the Technical Committee for a collaborative study that tests selected decarbonation methods against common ASBC methods that require degassing.

Propylene Glycol. This method was suggested by Roman Ortiz at the fall 2008 Technical Committee meeting. Propylene glycol can be measured as a fermentation by-product or contaminant from defective heat exchangers. Method(s) are needed, and this is also a possible future polling topic and posting at the annual meeting.

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 Subcommittee. Potential labeling requirements and method selection for gluten in beer continues to be a key item to watch.

Topics to Archive

Portable Oxygen/CO_2 Device Evaluation. This topic is for the assessment of the portable or at-line measurement devices currently in use and to gauge the potential for a future collaborative. A wide range of equipment exists, and the validation of the methodology would require statistical treatments beyond the scope of a typical ASBC collaborative study. This topic will be archived.

Color Measurement in the Presence of Turbidity. This idea came about from the growth in beers and other malt beverages that contain either natural or added turbidity as part of the product design. The current beer color method is valid only for liquids that are not turbid and requires filtration. The resulting color after filtration is often not representative of the perceived color due to scattering.

Suggestions for Sign-up Sheets at the Annual Meetings

Discussion at the 2007 fall meeting yielded valuable suggestions for improvement in this area:

- Several ex officios and subcommittee chairs had difficulty reading the handwriting of people who signed up to participate. It was agreed that an envelope for business cards (and having blank write-in cards available for those without business cards) would replace sign-up sheets and could help solve this problem.
- We agreed that a short description of each method (or subcommittee purpose) should be added to the sign-up area, so interested parties would more clearly understand what they were committing to.

These ideas were successfully used at the 2009 annual meeting.

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APPENDIX

Summarized Results from 2009 Online Polling

Top Line Results from Polling

- 64 responses were received
- 39% of respondents submitted ideas for new and alternative methods
- 53% gave their contact information
- 12+ answered questions on methods for volatile esters and alcohols in beer
- 14+ answered questions on (nonmalt) segmented flow analysis

Suggestions and Input for New Methods

- 4-Ethyl phenol and 4-vinyl guaiacol
- Flow cytometry for yeast physiological determinations
- Microscale protein and FAN determinations
- Cis/trans methods of analysis applied to iso-α-acids, rho-IAA, and tetrahydro-IAA
- HPLC methods for amino acids, phenolic acids
- Total and MW distribution for arabinoxylans
- Wort viscosity methods (current MOA contains Wort-13)
- Chapon colloidal stability testing
- Lipoxygenase (LOX) activity in malted barley
- Optical methods for oxygen measurement
- Total package oxygen—headspace and dissolved components
- Malt premature flocculation testing
- HPLC and other water chemistry methods for determination of brewing water composition

- Updated diastatic power enzyme analysis for improved fermentability prediction
- Iso-α-acids by HPLC (current MOA contains several methods)
- Organic acid determination in wort, beer (2)
- Update methods for foam stability (2) and lacing
- Analytical bitterness measurement that relates better to sensory
- Analytical measurement of components related to "malty" sensory character
- Yeast vitality measured by Nalco instrument
- Shelf-life determination by chemistry and sensory measures
- Staling aldehydes in beer measured by GC/MS
- Heavy metals in raw brewing materials measured by ICP-OES or ICP/MS (current MOA contains Wort-20, Beer-45)
- Tannoids and haze-sensitive proteins in beer
- Hop volatile compounds in beer
- 3-Methyl-2-butenethiol in beer

Segmented Flow Analysis for Nonmalt Methods

- Seventeen respondents have segmented flow analyzers; fourteen of which use them for nonmalt analysis.
- A check-box matrix was presented in the survey allowing users to indicate beer process phase analyzed by method.
- Methods listed included bitterness (BU), free amino nitrogen (FAN), sulfur dioxide, polyphenols, diacetyl, acetaldehyde, color, pH, and "other" for write-in responses.
- BU, FAN, and sulfur dioxide determinations in wort, fermenter drop, and packaged beer collectively received the most responses for methods currently in use. However, the number of responses were short of the minimum collaborator number of six to form a subcommittee.
- When asked about what methods current users would like to run, but are not currently able to perform, BU, FAN, and sulfur dioxide were again listed. The potential for future collaborative studies will continue to be monitored for segmented flow analysis.

Gas Chromatography–Flame Ionization Detection for Beer Volatiles

- Eleven respondents use an in-house method that uses this technology to analyze for beer volatiles.
- Three respondents use an outside laboratory.
- Seven respondents indicated an ASBC method would be useful to their laboratory, and twelve respondents indicated they would be willing to participate in an ASBC collaborative study. Formation of a subcommittee was recommended based on these results.
- The range of compounds being measured by respondents included

  Acetaldehyde
  Ethyl acetate
  Isoamyl acetate
  n-Propanol
  Phenylethyl acetate
  Phenylethyl alcohol
  Ethyl hexanoate
  Ethyl octanoate
  2-Methyl-1-propanol
  n-Butanol
  Isobutyl alcohol
  3-Methyl-1-butanol

- Sample introduction methods were also surveyed with the following distribution of results:

  34% Static headspace
  33% Solid-phase microextraction (SPME)
  17% Other
  8% Dynamic headspace
  8% Solvent extraction

Miniature Fermentation Assay

- Ten positive responses were received indicating interest in participating in an ASBC collaborative study. A subcommittee is being formed to pursue this topic.

Soluble Starch

Subcommittee Members: R. Jennings, *Chair*; B. Amundsen; S. Bartlett; D. Christopher; M. Gastl; M. Goldsmith; S. Home; R. Joy; M. Joyce; D. Langrell; J. Menert; M. Omillian; P. Ritchie; and K. Robbins

RECOMMENDATIONS

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. 1567K was collaboratively tested and approved for sale by the Society during the summer of 2008 (1). As of May 27, 2009, 908 kg (454 units) of starch lot no. 1567K was 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 2011. The chair will monitor the soluble starch inventory and initiate the study when necessary.

LITERATURE CITED

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doi:10.1094/ASBCJ-2009-1009-01

Craft Brewers Spring Report

Subcommittee Members: Gina Kelly, *Chair*; Tom Garcia; Joe Palausky; Jeremy Roza; Fred Strachan; and David Wilson

MEMBERS

This was the first year of the formal subcommittee, which consisted of six members from various sized breweries. I am looking to invite others to grow the group this year.

ASBC CRAFT BREWER WEBPAGE

The Ask the Expert section of the ASBC Craft Brewer webpage has not been active due to time and resource availability, although I have been updating and monitoring it for any questions.

LABORATORY METHODS FOR CRAFT BREWERS CD

A total of 45 CDs have been sold since its release (in fall 2008, I reported 26 personal editions, 1 multiuser edition on a single computer, and 1 LAN edition had been sold). ASBC will have a presence at the Craft Brewers Conference in Boston, as well as the MBAA Convention in La Quinta, CA, where the CD will be offered.

UPCOMING COLLABORATIVES RELEVANT TO THE CRAFT BREWER

A number of subcommittees are in process or scheduled for the fall of 2009 that are relevant to craft brewers. Continued input and recommendations for new methods from the craft brewing community are welcome.

ASBC LOCAL SECTIONS

The ASBC Local Sections are a vital link to the craft brewing community. I am looking at ways to increase membership through the various sections and find ways to attract more individuals to these meetings. For the last Wild West Section meeting in November, I e-mailed local homebrew shops, as well as specific departments (i.e., Food Science) at Colorado State to pass on to students. I had a larger number of respondents who were interested in attending the local meeting, which was held at MillerCoors in Golden, CO. I believe that getting the word out to universities and the brewing community will help attendance numbers at local meetings. A poll/questionnaire will be sent to section chairs this fall to determine attendance and the frequency of their meetings and will also look at working more closely with sections to determine their challenges in keeping their sections active, including ways to improve these meetings.

doi:10.1094/ASBCJ-2009-1005-01

Determination of Bitterness Units and Iso-α-acid Levels in Dry-hopped Beers Using the Archived 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; K. McGivney; S. Mulqueen; J. Palausky; A. Porter; J. Rick; J. Roza; R. Smith; G. Spedding; S. Steele; P. Takacs; C. Taylor; S. Taylor; J. Trujillo; and R. Foster (*ex officio*).

Keywords: BU, HPLC, IAA

RECOMMENDATION

1. The subcommittee recommends repeating this collaborative with sample pairs, using high and low dry-hopped beers from two different production dates.

This is the second year of the subcommittee. The subcommittee was formed to evaluate the archived iso-α-acids spectrophotometric method for the analysis of bitterness in dry-hopped beers. Twenty-six collaborators were sent samples of normally and high dry-hopped beer for analysis. The data were not analyzed using the Youden unit block approach because sample pairs were not submitted to each collaborator for analysis.

doi:10.1094/ASBCJ-2009-1002-02

PROCEDURE

Two samples of normally dry-hopped beer (sample A1/A2) and two samples of high dry-hopped beer (sample B1/B2) were sent to each collaborator. Each sample pair was of the same brand and production dates. Collaborators were asked to follow the methods as closely as possible and document any adjustments made in the methods (1). Collaborators were asked to analyze the samples in duplicate and report the results.

RESULTS AND DISCUSSION

Results from 25 collaborators were received for the sample pair A1/A2 and B1/B2 for both the bitterness (BU) and iso-α-acids (IAA) methods. The results are summarized in Tables I and II, respectively. High-pressure liquid chromatography (HPLC) results were received from four of the collaborators and used as reference values for beers A and B (Table III). Statistical measures and variances are reported in Table IV. A large range of variances was observed using the archived IAA spectrophotometric method, (60.2, 70.1, 167.2, and 181.0). The spectrophotometric IAA grand mean

TABLE I
Archived Iso-α-acids Spectrophotometric Method

	Sample Pair		Sample Pair	
Collaborator	A1	A2	B1	B2
1	41.5	39.5	72.0	76.0
2	43.0	52.5	87.0	79.0
3	42.5	44.0	79.5	79.0
4	41.0[a]	41.5[a]	73.5[a]	76.0[a]
5	34.5	38.5	47.0	45.0
6	25.5	25.0	46.0	44.0
7	48.5	48.5	78.5	81.5
8	34.0	34.0	66.5	64.5
9	33.5	38.0	72.0	75.0
10	45.5	43.5	78.5	78.0
12	36.5	39.5	68.0	68.0
13	39.5	41.0	70.0	72.0
14	36.5	39.5	71.5	72.0
15	12.0[b]	11.5[b]	26.5[b]	25.0[b]
16	43.0	43.5	76.0	59.0
17	34.5	34.5	59.0	59.0
18	39.0	40.0	68.0	68.5
19	40.0	40.0	72.0	73.5
20	16.0	42.5	73.0	79.5
21	32.5	36.5	74.5	72.5
22	37.5	37.0	53.5	56.5
23	36.5	39.0	74.5	73.0
24	31.5	31.0	62.0	62.0
25	43.0[a]	42.5[a]	75.0[a]	77.0[a]
26	40.5	40.0	70.5	73.5
Mean	37.30	39.60	69.50	69.30
Grand mean	38.45		69.40	

[a] Different dilution used.
[b] Data not used.

TABLE II
Bitterness Units Spectrophotometric Method

	Sample Pair		Sample Pair	
Collaborator	A1	A2	B1	B2
1	43.0	43.0	76.0	76.0
2	43.5	45.0	76.5	75.5
3	45.5	46.0	76.0	73.5
4	48.0[a]	52.0[a]	87.0[a]	89.5[a]
5	41.0	37.5	68.5	70.0
6	41.5	42.0	73.5	75.5
7	52.0	51.5	80.5	76.0
8	42.5	44.0	76.5	77.0
9	38.0	39.5	68.5	70.5
10	44.5	43.5	75.0	74.5
12	47.0	48.5	80.5	77.0
13	48.5	50.0	78.5	79.0
14	48.0[a]	45.5[a]	69.5[a]	85.0[a]
15	41.5	40.5	73.5	74.0
16	49.0	44.0	83.0	82.0
17	42.5	43.0	74.0	75.5
18	48.5	47.5	82.5	82.5
19	49.0	49.0	82.5	82.5
20	46.0	46.0	80.5	80.5
21	47.0	47.0	82.5	82.5
22	51.0	48.0	75.5	82.0
23	46.5	46.0	79.5	81.0
24	45.5	45.5	78.5	77.0
25	52.0[a]	51.5[a]	88.5[a]	90.5[a]
26	47.5	47.0	76.5	77.0
Mean	46.00	45.70	77.70	78.60
Grand mean	45.85		78.20	

[a] Different dilution used.

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

	Sample	
Collaborator	**A**	**B**
4	38.0	70.9
11	39.0	73.0
14	38.0	70.0
25	38.0	69.9
Mean	38.25	70.95

was 38.45 for sample A and 69.40 for sample B. The HPLC IAA mean was 38.25 and 70.95, respectively. These initial results indicate the archived IAA method should show accurate results.

TABLE IV
Statistical Comparison of Bitterness (BU) and Iso-α-acids (IAA) in Normally and High Dry-hopped Beer Samples

	A1 (BU)	**A1 (IAA)**	**A2 (BU)**	**A2 (IAA)**	**B1 (BU)**	**B1 (IAA)**	**B2 (BU)**	**B2 (IAA)**
Mean	46.0	37.3	45.7	39.6	77.7	69.5	78.6	69.3
Variance	13.0	70.1	13.7	60.2	26.5	167.2	26.5	181.0

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*, 7th ed. Beer-23A Beer bitterness (BU), -23B, Iso-α-acids (IAA) (archived); Beer-23C Iso-α-acids by solid phase extraction and HPLC. The Society, St. Paul, MN, 1976.

Sensory Report

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

SUMMARY

EBC Collaboration

The EBC has published the revised Triangle Test Method, which the ASBC participated in reviewing. The revised method has been forwarded by Katja Tiitinen, EBC Sensory Analysis Committee chair, and will be included in the next ASBC *Methods of Analysis* (MOA) update. The EBC has also forwarded Method 13.2: Test Room, Equipment, Conduct of Test, for review and approval by the ASBC. The EBC Sensory Analysis Committee will be collaborating with the ASBC on review of Flavor Terminology and the Flavor Wheel.

Panelist Performance and Validation

The panelist performance-monitoring Excel pivot chart tool has undergone some minor revisions after review by the committee. This will be released next year in the MOA with the updated sensory methods.

Reference Standards

The list of reference standards has been finalized. Calculations were reviewed by several members of the committee. The updated list will also be released next year in the MOA with the updated sensory methods.

doi:10.1094/ASBCJ-2009-1008-01

MOA Sensory Methods Review

The MOA Sensory Methods Review Subcommittee is in the process of reviewing the following MOA sensory methods:

1. Terms and definitions
2. Test room, equipment, conduct of test
3. Choice of method
4. Selection and training of assessors
5. Author guidelines for reporting
6. Paired comparison test
7. Duo-trio test
8. Threshold of added substances—Ascending method of limits test
9. Descriptive analysis
10. Ranking test
11. Flavor terminology and reference standards
12. Difference-from-control

Flavor Wheel/Flavor Terminology

The group has begun to discuss revamping the Flavor Wheel. The group is currently putting together a list of terms that committee members utilize, as well as gathering terms used in the industry. The EBC Sensory Analysis Committee will be collaborating with the ASBC on review of flavor terminology and the Flavor Wheel.

Anyone interested in joining this subcommittee should contact Sue Thompson at thompson.suzanne@millercoors.com or +1.414.931.2863.

Measurement of Volatile Aldehydes in Beer by Solid-Phase Microextraction–Gas Chromatography/Mass Spectrometry[1]

Subcommittee Members: R. Ortiz, *Chair*; J. Cornell (*ex officio*)

Keywords: Beer staling, Benzaldehyde, Carbonyl, Flavor stability, Furfural, GC/MS, *cis*-11-Hexadecenal, 2-Methylbutanal, 3-Methylbutanal, 2-Methylpropanal, *trans*-2-Nonenal, Phenyl acetaldehyde, SPME

This is the first full year of the subcommittee's existence. Based on membership polling, the Technical Committee recommended formation of this subcommittee in 2008 to evaluate measurement methods for beer volatiles by solid-phase microextraction (SPME) combined with gas chromatography/mass spectrometry (GC/MS).

PRELIMINARY CONSIDERATION OF THE METHODOLOGY

As described in the 2008 fall report, the effort began with reviewing published methodologies, along with methods currently in use and validated on a single-lab basis. Two general categories of compounds that fell into distinctly different method conditions were considered: one includes a wide range of volatile esters and alcohols typically considered positive for beer flavor, and the other method measures volatile aldehydes typically associated with stale flavors in aged beer (2,4,5). The committee decided to focus on the aldehyde method—both because of the lack of such a method in the ASBC *Methods of Analysis* (1) and the keen interest in beer flavor stability expressed by brewers.

Building on published work utilizing SPME-GC/MS to detect volatile aldehydes in beer or water, this procedure has been structured in a manner that is ideal for breweries due to its simplicity, enhanced detection, reliability, and selectivity toward volatile aldehydes in beer. The standard additions method is utilized to quantify the compounds of interest because it is essential the calibration standards matrix equals that of the beer sample matrix. The selected ion monitoring (SIM) approach is utilized because the volatile aldehydes readily react with the on-fiber derivatizing agent *o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBOA) and subsequently fragment characteristically in the mass spectrometer detector with a base peak at *m/z* 181 for all aldehydes.

The aldehydes chosen for quantification in this method are only a fraction of the aldehydes and carbonyl compounds that are present in beer. However, these compounds have been observed to have characteristic trends that are indicative of changes in stability and, hence, changes in the flavor and aroma of packaged beer, making it a useful method for brewery quality control/assurance laboratories. In addition to acting as flavor stability markers from an analytical perspective, these compounds can impart characteristic sensory properties to aged beer. For example, isobutyraldehyde imparts a grainy character, with a flavor threshold of approx. 10 ppb. Benzaldehyde imparts an almond character, with a flavor threshold of approx. 1,000 ppb. *trans*-2-Nonenal imparts a papery character, with a flavor threshold of 0.050–0.100 ppb (3). This method is also capable of measuring furfural, which is a reliable marker for heat exposure to packaged beer.

[1] Listed manufacturers and catalog numbers are for reference only, are subject to change without notice, and do not constitute an endorsement by the American Society of Brewing Chemists.

doi:10.1094/ASBCJ-2009-1009-02

Apparatus

a. Four-place analytical balance
b. Glass pipettes, 10 mL, graduated
c. Volumetric flasks: 1–1,000, 1–200, 1–100, 7–50, and 1–25 mL
d. Baffled flask, 500 mL
e. Mechanical platform orbital shaker capable of 175 rpm
f. Vials, clear 20-mL screw thread (MicroLiter Analytical Supplies, Inc., cat. no. 16-2000), or equivalent
g. Caps, S/T headspace cap PTFE/silicone (MicroLiter Analytical Supplies, cat. no.16-0050M), or equivalent
h. Pasteur glass pipettes
i. Hamilton glass syringes (25-, 500-, and 1,000-µL vol, graduated)
j. Gilson or Rainin single-channel manual or electronic pipette (100-µL vol)
k. SPME fiber, 65-µm PDMS-DVB, 23-gauge needle (Supelco cat. no. 57345-U)
l. GC analytical column, J&W 122-503E DB-5 or equivalent
m. Gas chromatograph with a split/splitless heated injector equipped with the above specified column interfaced to a mass spectrometer and data acquisition system capable of SIM, such as an Agilent 6890 GC and 5973 MSD or equivalent
n. SPME programmable automated sampler, such as Gerstel MPS or other apparatus capable of achieving the extraction and derivatization parameters described in the next section

Reagents

a. Ethanol, 200 proof absolute anhydrous
b. 2-Methylpropanal (isobutyraldehyde), density (*d*) = 0.79 g/mL (Sigma-Aldrich cat. no. 240788)
c. 2-Methylbutanal (2-methylbutyraldehyde), *d* = 0.82 g/mL (Sigma-Aldrich cat. no. m33476)
d. 3-Methylbutanal (isovaleraldehyde), *d* = 0.797 g/mL (Sigma-Aldrich cat. no. 59820)
e. 2-Furaldehyde (furfural), *d* = 1.159 g/mL (Sigma-Aldrich cat. no. 319910)
f. Benzaldehyde, *d* = 1.05 g/mL (Sigma-Aldrich cat. no. 418099)
g. Phenyl acetaldehyde, *d* = 1.075 g/mL (Sigma-Aldrich cat. no. 107395)
h. *trans*-2-Nonenal, *d* = 0.846 g/mL (Sigma-Aldrich cat. no. 255653)
i. *cis*-11-Hexadecenal, *d* = 0.963 g/mL (Sigma-Aldrich cat. no. 249084-100 mg)
j. PFBOA (Sigma-Aldrich cat. no. 76735)
k. Sodium chloride (Sigma-Aldrich cat. no. S7653-1kg)
l. Deionized water, 18 MΩ resistivity

Stock Standard Solution (SSS) Preparation by Weight

To a 200-mL volumetric flask half filled with ethanol, add the following approximate amounts of analytes using a glass Pasteur pipette, record the actual weight of each analyte, and then bring to volume with ethanol:

0.300 g of 2-methylpropanal
0.300 g of 2-methylbutanal
0.300 g of 3-methybutanal
2.000 g of furaldehyde
0.300 g of benzaldehyde
0.600 g of phenyl acetaldehyde
0.015 g of *trans*-2-nonenal (1 drop from Pasteur pipette)

SSS Alternative preparation by Volume

To a 100-mL volumetric flask half filled with ethanol, add the following amounts of analytes using syringes and bring to volume with ethanol:

200 µL of 2-methylpropanal
200 µL of 2-methylbutanal
200 µL of 3-methylbutanal
600 µL of 2-furaldehyde
150 µL of benzaldehyde
300 µL of phenylacetaldehyde
10 µL of *trans*-2-nonenal

Primary Dilution Standard Solution preparation

Transfer 0.2 mL of the SSS to a 100-mL volumetric flask containing ethanol and bring to volume with ethanol.

Stock Internal Standard Solution Preparation (SISS)

To a 50-mL volumetric flask half filled with ethanol, add 30 µL of *cis*-11-hexadecenal and bring to volume with ethanol.

Primary Dilution Internal Standard Solution Preparation (PDISS)

Transfer 4 mL of the SISS solution to a 50-mL volumetric flask half filled with ethanol and bring to volume with ethanol. Use this solution as the daily internal standard added to calibration and sample vials. It is recommended that a 10-mL vial be filled with the PDISS, capped with septum, and kept refrigerated during use. Prepare a fresh 10-mL vial daily. Store the remaining PDISS and SISS in the freezer.

Derivatization Solution Preparation Stock Solution

To a 25-mL volumetric flask accurately add 0.150 g of PFBOA, bring to volume with deionized water, and invert several times to dissolve. It is recommended that the 25-mL supply be divided into three vials. Keep the vial in use refrigerated, but freeze the other two vials until ready to use.

Instrument Parameters

Gas Chromatograph

Oven Conditions

Initial temp.: 40°C
Initial time: 0.00 min
Ramp 1:
Rate: 10.00 degrees Celsius/min
Final temp.: 140°C
Final time: 0.00 min
Ramp 2:
Rate: 7.00 degrees Celsius/min
Final temp.: 250°C
Final time: 14.00 min
Run time: 39.71 min

Inlet Conditions

Mode: Splitless
Temp.: 250°C
Pressure: 8.32 psi
Purge flow: 53.0 mL/min
Purge time: 0.10 min
Total flow: 57.7 mL/min
Gas type: Helium, 99.999% minimum purity, 99.9999% recommended

Column

Model no.: J&W 122-503E DB-5
Max temp.: 325°C
Length: 30 m
Inner diameter: 0.25 mm
Film thickness: 0.5 µm
Mode: Constant pressure
Pressure: 8.32 psi
Initial flow: 1.1 mL/min
Average velocity: 38 cm/sec
Inlet: Front inlet
Outlet: MSD
Outlet pressure: Vacuum
MSD transfer line temp.: 260°C

Mass Spectrometer

Acquisition mode: SIM
Plot 1 ion: 181.00
MS quad temp.: 150°C
MS source: 230°C

Gerstel MPS SPME Injection

Sample Preparation

Incubation temp.: 50°C
Incubation time: 5.00 min
Agitator speed: 250 rpm
Agitator on time: 5 sec
Agitator off time: 30 sec

Sample Parameters

Vial penetration: 24.00 mm
Extraction time: 60.00 min
Injection penetration: 54.00 mm
Desorption time: 180 sec

Derivatization

Derivatization mode: Pre-extraction
Derivatization time: 10.00 min
Derivatization penetration: 24.00 mm

Cycle Settings

Cycle time: 30.0 min

Calibration

Calibration Standards Preparation for Calibration Curve. Prepare a vial of derivatization solution by adding to a 20-mL vial 10 mL of deionized water and, using a single-channel pipette, add 100 µL of PFBOA solution. Cap and swirl for 15 sec using an automated vortex mixer. This vial is used to introduce the PFBOA onto the fiber prior to sample extraction (pre-extraction mode). The fiber will then be introduced into the sample vial for on-fiber derivatization to occur. Note, a fresh vial of derivatization solution is used for each run.

Obtain a baffled flask and beer sample at 0°C. Pour 100 mL of the beer sample into the flask and swirl it around by hand to wet and cool the inner surface. Discard this beer, and then gently pour down the side of the flask the beer sample to the 100-mL graduated mark. Place the flask containing the beer sample onto the orbital shaker platform and set the timer for 5 min at 175 rpm.

Note, the amount of time the beer shakes directly impacts the stability of the aldehydes—the longer the shaking, the more degradation of aldehydes occurs. Ensure consistency using a 5-min shake time and prepare the standard or sample as soon as the 5 min has elapsed.

Note, do not prepare all calibration standards at the same time. Due to the long instrument analysis time and the relative instability of aldehydes, prepare each standard vial only after the preceding standard has completed its GC run time.

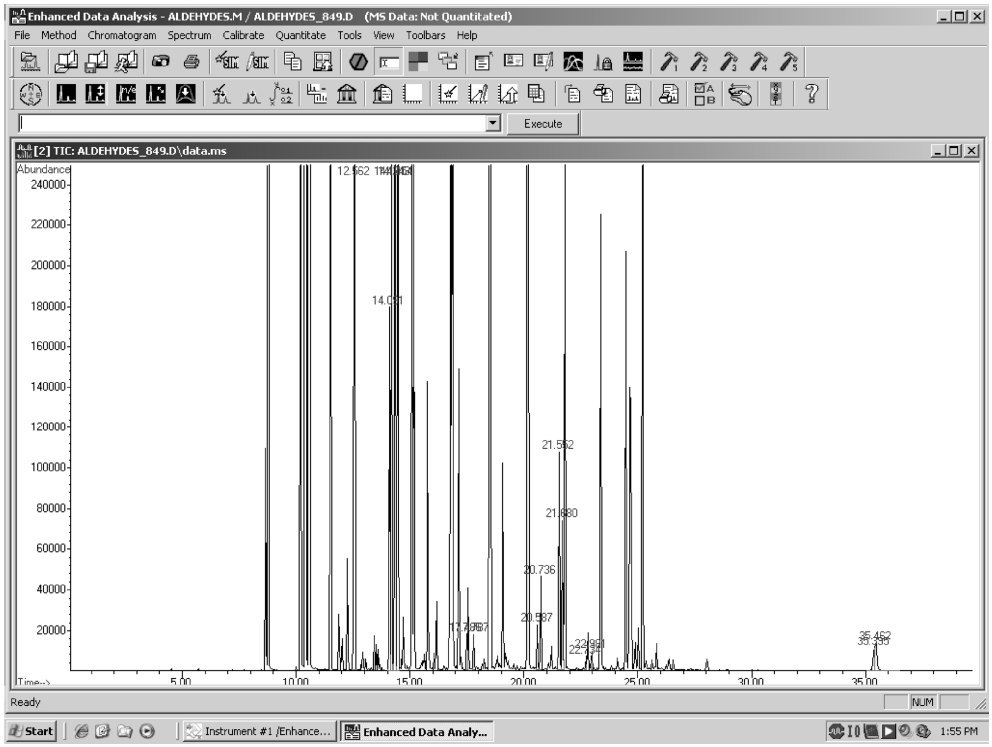

Fig. 1. Example of a chromatogram of over-add in beer.

For each level of standard, add to a 20-mL vial in the following order:

10 mL of beer using a glass pipette
10 µL of PDISS
0 (for blank), 2, 4, 6, 16, and 40-µL of PDS
3.5 g of sodium chloride

Quickly cap the vial and swirl using an automated vortex mixer for 1 min. Immediately perform instrument analysis.

Generate Calibration Curve. Prepare each level of standard as mentioned above, along with a vial of derivatization solution. Analyze the calibration standards and blank using the instrumental method parameters described. Generate a calibration curve and correct for standard additions for each analyte.

Approximate Retention Times of Aldehyde Geometric Isomers

2-Methylpropanal (isobutyraldehyde): 12.53 and 12.56 min
2-Methylbutanal (2-methylbutyraldehyde): 14.09 and 14.18 min
3-Methylbutanal (isovaleraldehyde): 14.34 and 14.45 min
2-Furaldehyde (furfural): 17.48 and 17.78 min
Benzaldehyde: 20.58 and 20.73 min
Phenylacetaldehyde: 21.55 and 21.68 min
trans-2-Nonenal: 22.73 and 22.98 min
cis-11-Hexadecenal (internal standard): 35.37 and 35.46 min

Method

Sample Preparation and Analysis. Follow precisely the same preparation as the calibration standards, except of course there is no addition of aldehyde standards to the beer. To a 20-mL vial, add in the following order:

10 mL of beer sample using a glass pipette
10 µL of PDISS
3.5 g of sodium chloride

Quickly cap the vial and swirl using an automated vortex for 1 min. Immediately perform instrument analysis.

Calculations

Calculate the concentrations of samples using the equation generated by linear regression. The method of standard additions is utilized in this procedure, so correct each equation based on the response (area) from the blank. (Author's note: example calculations will be added prior to the fall 2009 collaborative study.)

Recommendations

SPME fibers have a limited useful lifetime and must be monitored for their performance. The criteria for replacing the SPME fiber is when the area of the internal standard is <50% of the area of the internal standard when the fiber was initially installed. Sometimes, but not often, after about 30 runs the bottom end of the fiber will wear off, resulting in decreased peak areas. Inspect the fiber daily to verify whether the coating has maintained its integrity or lost coating. A new calibration curve must be established after installing a new fiber. Figure 1 shows an example of a chromatogram of over-add in beer.

LITERATURE CITED

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Wort and Beer Fermentable and Total Carbohydrates Measured by High-Performance Liquid Chromatography

Subcommittee Members: M. Eurich, *Chair*; L. Barber; K. Butterfield; X. Castañé (EBC); L. Lacke; A. Porter; J. Reffner; A. Ugajin (BCOJ); and K. Lakenburges (*ex officio*).

Keywords: Fructose, Glucose, HPLC, Maltose, Maltotetraose, Maltotriose, Sugars

CONCLUSIONS

1. Three different high pressure liquid chromotography (HPLC) methods for determining fermentable carbohydrates were used to evaluate four different samples provided to collaborators. The methods evaluated were Wort-14B and Beer-41B, as listed in the ASBC *Methods of Analyses* (1). Based on the results provided, statistics were not performed due to an insufficient number of results for each of the three methods.
2. Based on the results provided, overall the three methods directionally showed similar values for fructose, glucose, maltose, and maltotriose. An insufficient number of results were provided for maltotetraose.

RECOMMENDATIONS

1. The subcommittee recommends repeating the collaborative study with a revised procedure to update Wort-14B and Beer-41B in the ASBC *Methods of Analyses* (1). The updated procedure would compare the approved method to those incorporating newer columns, differing eluents, and differing fermentable carbohydrate detection. Collaborators would also be provided a calibration standard incorporating the fermentable carbohydrates. Directions would be provided to account for the different concentration levels of fermentable carbohydrates in the samples provided. Standard ASBC protocol would be followed, using sample pairs representing wort through packaged beer. A model solution of 5% (vol/vol) ethanol in distilled water with known fermentable carbohydrate concentrations would also be included.
2. Repeat the collaborative study using HPLC methods with evaporative light scattering detection (ELSD) and pulsed ampermetric detection (PAD) for comparison with Wort-14B and Beer-41B. Standard ASBC Youden statistics would be performed provided a sufficient number of results are provided for either method.
3. Discontinue analyses for maltotetraose in future collaborative studies.
4. Repeat the collaborative study using ethanol as an eluent in place of acetonitrile as HPLC methods are made available. Due to the worldwide shortage of acetonitrile, HPLC method developers are evaluating alternatives to this eluent, with ethanol as a potential replacement.

Various HPLC methods for fermentable and total carbohydrate measurements exist in the brewing industry. Due to technological advances, methods other than Wort-14B and Beer-41B are commonly used. This subcommittee is charged with investigating HPLC methods currently used in the industry for fermentable and total carbohydrate determinations. In this first year, subcommittee members were asked to provide general details about their HPLC instrumentation and methodology. Two samples of wort, a packaged beer, and a model solution were sent to collaborators for analysis using their in-house methods. The fermentable carbohydrates analyzed included glucose, fructose, maltose, maltotriose, and maltotetraose. Based on the results and findings from these initial tests, the subcommittee will determine a standard method or methods for collaborative testing. Due to the worldwide shortage of acetonitrile, methods incorporating solvents other than acetonitrile may be evaluated in future collaborative studies if they become available.

PROCEDURE

A total of four samples was sent to collaborators to be analyzed using their typical in-house HPLC method for measuring fermentable carbohydrates. The general outlines of the three methods used are listed below. All results were evaluated using Microsoft Excel software and applying basic statistical calculations.

Two samples of wort with very different fermentable carbohydrate profiles were collected, clarified, and pasteurized for shipping purposes. One single case of packaged beer was collected. A model solution was prepared with exact amounts of glucose, fructose, maltose, maltotriose, and maltotetraose added. These fermentable carbohydrate weights were approximated to be those of a typical lager fermentation at the midpoint.

HPLC Method 1. This method was the same or very similar to the methods published in the ASBC *Methods of Analyses* for Wort-14B and Beer-41B using refractive index (RI) detection (1).

HPLC Method 2. This method used typical HPLC instrumentation. Columns used were one Alltech Prevail Carbohydrate ES guard column and one Alltech Prevail Carbohydrate ES column. Eluents were degassed HPLC-grade acetonitrile and distilled, deionized, and degassed water. Component detection was by ELSD.

HPLC Method 3. This method used typical HPLC instrumentation. Columns used were one Dionex Carbopac PA1 guard column and one Dionex Carbopac PA1 column. Eluent one for this method was a 150 m*M* HPLC-grade sodium hydroxide solution. Eluent two was a combined solution of 150 m*M* HPLC-grade anhydrous sodium acetate and 150 m*M* HPLC-grade sodium hydroxide. Components were detected by PAD.

RESULTS AND DISCUSSION

Results were received from seven collaborators. These included results for two wort samples, one model solution sample, and one packaged beer sample. All seven collaborators provided results using their typical HPLC method of measuring fermentable carbohydrate and instrumentation, as briefly described in one of the three methods listed above. Data for all four samples from each collaborator are presented in Tables I–IV.

The data summaries for both wort samples are presented in Tables I and II. The mean was calculated for glucose, fructose, maltose, and maltotriose for each of the two wort samples for HPLC method 1 only. The mean was not calculated for maltotetraose due to an insufficient number of results.

The data and statistical summary for the packaged beer sample is presented in Table III. The mean was calculated for fructose, maltose, and maltotriose for HPLC method 1 only. The mean was not

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TABLE I
Wort Sample 1: Percent (wt/vol) Fermentable Carbohydrates

Collaborator	Method	Glucose	Fructose	Maltose	Maltotriose	Maltotetraose
1	1	9.79	0.49	3.44	0.85	–[a]
2	1	10.80	0.33	3.30	0.34	–[a]
3	1	10.32	0.22	2.44	0.61	–[a]
4	1	11.58	0.30	3.44	0.39	0.50
Mean		10.62	0.34	3.16	0.55	–[b]
5	2	9.86	0.25	2.98	0.65	–[a]
6	2	9.76	0.20	2.68	0.63	0.28
7	3	9.63	0.29	3.03	0.64	–[a]

[a] Data not provided.
[b] Mean not calculated.

TABLE II
Wort Sample 2: Percent (wt/vol) Fermentable Carbohydrates

Collaborator	Method	Glucose	Fructose	Maltose	Maltotriose	Maltotetraose
1	1	2.39	0.37	5.29	1.33	–[a]
2	1	2.50	0.26	5.10	1.50	–[a]
3	1	2.39	0.18	4.05	1.37	–[a]
4	1	3.15	0.25	5.61	1.78	0.98
Mean		2.61	0.27	5.01	1.50	–[b]
5	2	2.86	0.25	4.93	1.26	–[a]
6	2	1.92	0.15	4.79	1.47	0.46
7	3	2.49	0.22	5.01	1.51	–[a]

[a] Data not provided
[b] Mean not calculated

TABLE III
Packaged Beer: Percent (wt/vol) Fermentable Carbohydrates

Collaborator	Method	Glucose	Fructose	Maltose	Maltotriose	Maltotetraose
1	1	–[a]	–[a]	0.68	0.44	–[b]
2	1	0.02	0.06	0.54	0.33	–[b]
3	1	–[a]	0.02	0.44	0.24	–[b]
4	1	0.01	0.03	0.67	0.48	0.98
Mean		–[c]	0.04	0.58	0.37	–[c]
5	2	0.04	0.03	0.49	0.32	–[b]
6	2	–[a]	–[a]	0.85	0.53	0.53
7	3	0.04	0.02	0.48	0.31	0.27

[a] Below collaborator method detection.
[b] Data not provided.
[c] Mean not calculated.

TABLE IV
Model Solution: Percent (wt/vol) Fermentable Carbohydrates

Collaborator	Method	Glucose	Fructose	Maltose	Maltotriose	Maltotetraose
1	1	1.62	–[a]	1.70	0.34	–[a]
2	1	1.80	0.09	1.70	0.36	–[a]
3	1	1.64	0.08	1.29	0.29	–[a]
4	1	1.95	0.10	1.81	0.39	0.15
5	2	1.46	0.08	1.40	0.29	–[a]
6	2	1.42	0.07	1.58	0.29	0.08
7	3	1.64	0.10	1.47	0.40	0.08
Known concentration		1.75	0.09	1.65	0.34	0.08

[a] Data not provided.

calculated for glucose or maltotetraose due to an insufficient number of results.

The data for the model solution sample is presented in Table IV. The true percent weight to volume values for glucose, fructose, maltose, maltotriose, and maltotetraose are also presented in the "Known Concentration" line in Table IV for comparison.

Collaborators were not provided with a specific procedure and HPLC method for component determination. Collaborators used in-house methods that may include differences between sample preparation and calibration, including varying standards and concentrations, as well as differences between manufacturers of columns and instrumentation.

Differences in results for wort and packaged samples were likely due to the different method calibration ranges and sample dilutions used for the analyses. Other differences were most likely due to differences in component calibration standard purities and inherent differences between HPLC instrumentation and methods.

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1. American Society of Brewing Chemists. *Methods of Analysis*, 2008 ed. Beer-41B Total carbohydrate by HPLC; Wort-14B Fermentable saccharides by HPLC. The Society, St. Paul, MN, 2008.

Methods of Analysis Malt Review

Subcommittee Members: J. Barr; S. Bartlett; A. Budde; K. Churchill; S. Home (EBC); J. Jewett; R. Joy; Y. Li; J. Lowe; A. Macleod; M. Maurice; J. McCann; J. Menert; A. Mundy (EBC); P. Ritchie; K. Robbins; S. Roumeliotis; P. Schwarz; M. Sheehy (EBC); J. Vogel; J. Zinati; and R. Jennings (*chair/ex officio*)

CONCLUSIONS

1. The subcommittee reviewed the Malt section of the ASBC *Methods of Analysis* (MOA). Several of the methods have alternatives that are used in the industry, and many need modification. The methods were reviewed for accuracy of citations and availability of described supplies.
2. Several of the malt methods in the MOA require additional work, i.e., collaborative study or determination of alternative methods. These methods cannot be modified completely until all other work has been completed.

RECOMMENDATIONS

1. Adoption of the revisions to the malt methods for inclusion in the 2010 ASBC MOA is recommended.
2. Maintenance of the subcommittee for testing new and/or updated methods is recommended.
3. The following are recommended for the subcommittee as new or alternative methods to the MOA:
   a. Automated potassium ferricyanide method for Malt-7 (α-amylase)
   b. Near infrared method for Malt-8 (protein)
   c. New method for Malt-4 (extract)
   d. Elisa/EZ-Tox method for Malt-13 (deoxynivalenol)
   e. Sortimat for Malt-2B (assortment)
4. The following are recommended for collaborative studies:
   a. α-Amylase and diastatic power standard (EMAST) in Malt-6 and Malt-7 (diastatic power and α-amylase)
   b. National Institute of Standards and Technology (NIST) barley protein standard in Malt-8 (protein)
   c. Partly unmodified malt in Malt-12 (malt modification by friability)
5. Removal of Malt-5 Wort Analysis from the MOA is recommended.
6. No methods are recommended for archival.

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 Malt section of the ASBC *Methods of Analysis*. This subcommittee is 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 Malt Methods Review Subcommittee. Subcommittee members were provided with each malt method and asked to review it for accuracy (i.e., spelling, calculations, conformity to laboratory practices, etc.). Subcommittee members were asked to participate in two conference calls for open discussion of methods. Subcommittee members were asked to comment on a set of questions generated by the ex officio on each method (a poll).

Following the review process all minor revisions/updates were made to the methods. Significant changes to the methods are listed in the results and discussion section with explanation of why the change is required. An additional list of new methods was generated based on new technology and/or methods currently used in the industry.

RESULTS AND DISCUSSION

The results of the MOA Malt Methods Review Subcommittee are listed by method.

Malt-4

The cone and roller mill from Miag-Seck, as listed in the method, is no longer available. The DFLU disk mill is the standard laboratory mill used in the industry. This is a significant change to the method and will require a collaborative study.

Malt-5

This method is recommended for removal from the Malt section of the MOA because it only incorporates two methods of the several available for wort.

Malt-6

It is recommended that the note for chromic acid be deleted. Cleaning is good laboratory practice and does not need to be included in this method. Chromic acid is hazardous, and there are many alternative cleaning methods in place.

Malt-7

Malt-7C reagent section (h): α-amylase type VIII-A is no longer available. EMAST from Megazyme is the preferred standard used in the industry. This is a significant change to the method and will require a collaborative study. A potassium ferricyanide method for α-amylase detection is recommended for the New and Alternative Methods Subcommittee.

Malt-8

Malt-8B uses EDTA. This is a very high standard. An alternative to EDTA is the NIST-certified barley standard. It is recommended that a collaborative study be done on the NIST-certified barley standard since it is more in the range of malt. An NIR method is recommended for the New and Alternative Methods Subcommittee.

Malt-12

It is recommended that a collaborative study be done for partly unmodified malt.

Malt-13

An Elisa/EZ-Tox method is recommended for the New and Alternative Methods Subcommittee.

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1. American Society of Brewing Chemists. *Methods of Analysis,* 2008 ed. Malt-1 through Malt-15. The Society, St. Paul, MN, 2008.

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International Hop Standards Committee (IHSC)[1] (EBC/ASBC/BCOJ/IBD)

Subcommittee Members: B. Foster, *Co-chair*, North America (ASBC); M. Biendl, *Co-chair*, Rest of the World (EBC); J. P. Maye, Secretary of the Americas (ASBC); L. Burroughs (ASBC); K. Butterfield (ASBC); J. Carr (ASBC); S. Garden (ASBC); P. Hughes (IBD); M. Raver (ASBC); S. Sakuma (BCOJ); R. Smith (ASBC); J. Snyder (ASBC); S. B. Sorensen (EBC); K. Suzuki (BCOJ); P. Ting (ASBC); and R. Wilson (IBD)

RECOMMENDATIONS

1. The subcommittee recommends continuing to provide purified hop acids standards to be sold to the brewing, hops, and related industries by the American Society of Brewing Chemists and Labor Veritas organizations.

This subcommittee has existed for 13 years, previously as the International Subcommittee for Isomerized Hop α-Acids Standards (ISIHAS), and is a standing subcommittee whose goal is to produce, purify, and verify isomerized and nonisomerized hop standards for the brewing, hops, and related industries. This first report in the ASBC Technical Committee format is a summary of the efforts and minutes of our meeting at the 2009 ASBC Annual Meeting in Tucson, AZ.

REPORT SUMMARY

Status of Isomerized Standards

Vials (1,790) of the new iso-α-acid dicyclohexylamine standard (ICS-I3) were produced by Lou Burroughs (Kalsec). Of these, 720 vials were sent to Labor Veritas and 432 vials to the ASBC. It is recommended that the remaining 638 vials be shipped to these organizations between December 2009 and February 2010. Revenue from the sales of these hop acids standards will be used to pay for the preparation, packaging, and shipping of new standards.

Supplies of the following isomerized α-acid standards are expected to run out:

ICS-I3: 6.2 years
ICS-R2: 6.4 years
ICS-T2: 4.2 years
ICS-H2: 4.9 years

Bob Smith performed an accelerated stability test on ICS-I2, in which this standard was exposed to a temperature of 60°C for 4 weeks. The standard showed little to no degradation under these conditions. It is recommended that the new ICS-I3 standard also undergo this 4-week 60°C stability test. Tracking the status of these standards is an important function of this committee and has been done by Richard Wilson. We will ask Richard if he would consider remaining on the subcommittee and if he would still be interested in performing this task.

Organizational Changes

The International Subcommittee for Isomerized α-Acid Standards was approached by both EBC and ASBC Technical Committee members to become a formal branch of the EBC Hop Subcommittee and the ASBC Technical Subcommittees. Currently, our group is an international subcommittee recognized by the ASBC but is not formally an ASBC Subcommittee. Both the EBC and ASBC have recognized the significant importance of our group and feel we would be better recognized, mentored, and supported if we achieve official subcommittee status. After much discussion on this topic, it was agreed that our group would still be an international committee and function the same way we always have with two co-chairs, one from the EBC (Europe and Rest of the World) and one from the ASBC (United States and the Americas). A summary of meeting minutes, hop bitters standards stocks, and information will be reported to the appropriate EBC and ASBC subcommittee venues and newsletters for publication.

ICE-3 Recommendation

The ASBC also recommended that our group support the efforts of the EBC with regard to the new α-acid and β-acid standard, ICE-3. Our committee agreed to take on that responsibility and support for ICE-3, hence the need to expand our scope for nonisomerized standards. This prompted our desire to change our name from the International Subcommittee for Isomerized Hop α-Acids Standards to the International Hop Standards Committee (EBC/ASBC/BCOJ/IBD).

Status of α-Acid and β-Acid Standard ICE-2

The current supply of α-acid and β-acid standard ICE-2 is expected to run out in late 2010. The EBC has performed stability testing on several extracts of the same de-oiled hop variety (Hallertau Perle) as ICE-2 and has chosen the most stable extract to be the next standard, ICE-3. The German Hop Research Institute at Huell will produce a primary calibration standard, pure α-acids, which will be measured by LCV. The new extract, ICE-3, will be calibrated based on a pure α-acids/LCV method and, thus, the same protocol as that used for ICE-2. Alternative methods of calibration will support this protocol and results. A collaborative analysis of the new extract will start this fall, and it is expected that the new ICE-3 standard will be commercially available by the middle of 2010.

Next Iso-DCHA Standard update

Patrick Ting produced approx. 100 g of iso-DCHA and offered it as a supplement for the next time we produce this standard (ICS-I4). This material will be shipped to Bob Smith for storage.

Other Standards: Xanthohumol, Iso-xanthohumol, and Resveratrol

The committee agreed and discussed the idea of producing xanthohumol, iso-xanthohumol, and resveratrol standards in the future for use in brewing research studies.

[1] Proposed and agreed upon name change from the International Subcommittee for Isomerized Hop α-Acids Standards (ISIHAS).

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Report of 2008 BCOJ Collaborative Work

Determination of Real Extract Using the Alcolyzer Method

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Subcommittee Members 2005: T. Kaneko (Sapporo Breweries Ltd.), *Chair* (*April 2005 – March 2006*); K. Ito (Sapporo Breweries Ltd.); T. Izumi (Suntory Ltd.); A. Matsuyama (Kirin Brewery Co. Ltd.); N. Mukai (National Research Institute of Brewing); T. Ohuchi (Sapporo Breweries Ltd.); M. Takeuchi (Asahi Breweries, Ltd.); S. Tatematsu (Asahi Breweries, Ltd.); and T. Teramoto (Sapporo Breweries Ltd.)
Keywords: Alcohol, Alcolyzer, Real extract

CONCLUSIONS

1. Results were received from eight collaborators who analyzed a total of six sample pairs. The data (sample specific gravity, alcohol, and real extract) were obtained using the Anton PAAR Alcolyzer method. No outliers were identified using Dixon's ratio test, and all results were used.
2. Based on the paired *t* test for differences in the means for real extract, no statistically significant difference was found between the Alcolyzer and calculation methods.
3. Reproducibility errors for the two methods were also measured using an *F* test. No statistically significant difference was found between the Alcolyzer (pooled variance = 0.00034) and calculation (pooled variance = 0.0041) methods.

RECOMMENDATIONS

1. No statistically significant difference was found between the Alcolyzer and calculation methods for the means for real extract in beer, *happo-shu*, and nonalcoholic beer.
2. No statistically significant difference was found between the Alcolyzer and calculation methods for the reproducibility errors for real extract in beer, *happo-shu*, and nonalcoholic beer.
3. The 2008 subcommittee recommends that the Anton PAAR Alcolyzer method for the determination of real extract in beer, *happo-shu*, and nonalcoholic beer should be included in the *Methods of Analysis of BCOJ*.
4. Discharge the 2008 subcommittee.

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The Anton PAAR Alcolyzer is based on a near infrared spectrometer, which measures the alcohol content of beer and other beverages. In combination with a density meter, the Alcolyzer automatically determines real extract and original gravity. The 2005 subcommittee was charged with evaluating the reproducibility of the Alcolyzer method for the determination of alcohol and real extract in beer, *happo-shu*, and nonalcoholic beer as an alternative to the SCABA method.

For determination of alcohol, the repeatability and reproducibility of the Alcolyzer method were acceptable. In addition, no significant difference was found between the means for the Alcolyzer and SCABA methods (official method of BCOJ). The 2005 subcommittee recommended that the Alcolyzer method for the determination of alcohol in beer, *happo-shu*, and nonalcoholic beer should be included in the *Methods of Analysis of BCOJ*. For the determination of real extract, the repeatability and reproducibility of the Alcolyzer method were acceptable, but there was a significant difference between the means for the Alcolyzer and SCABA methods. Consequently, the 2005 subcommittee recommended that the Alcolyzer method for the determination of real extract in beer, *happo-shu*, and nonalcoholic beer should not be included in the *Methods of Analysis of BCOJ* (2,4).

The 2005 subcommittee considered that the difference in the values for real extract concentration between the Alcolyzer and SCABA methods was caused by differences in the calculation processes of the instruments.

The 2008 subcommittee defined the real value of extract as the data from the calculation method using Tabarie's formula and the formula based on the table of Goldiner, Klemann, and Kämpf (3). To verify the practical use of the Alcolyzer, the 2008 subcommittee reevaluated its applicability to real extract using the 2005 subcommittee data to compare the Alcolyzer method and Calculation methods.

PROCEDURE

Six sample pairs (A/B, C/D, E/F, G/H, I/J, and K/L) of packaged beer, *happo-shu*, and nonalcoholic beer were sent to each collaborator. They were selected as follows: nonalcoholic beer (A/B), beer and *happo-shu* (C/D–G/H), and dark beer (I/J, K/H). The instruments were calibrated according to the manufacturer's instruction manual. Samples were degassed at 20–22°C and filtrated by filter paper. After placement in autosampler vials, samples were measured automatically by each instrument.

TABLE I
Specific Gravity Determined by the Alcolyzer Method

	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	1.01010	1.01044	1.00024	1.00017	1.00529	1.00547	1.00729	1.00732	1.00957	1.00959	1.01243	1.01245
2	1.01002	1.01031	1.00012	1.00004	1.00514	1.00538	1.00716	1.00720	1.00947	1.00941	1.01231	1.01232
3	1.01014	1.01049	1.00027	1.00022	1.00536	1.00559	1.00730	1.00733	1.00960	1.00962	1.01250	1.01248
4	1.01009	1.01038	1.00022	1.00011	1.00529	1.00548	1.00724	1.00728	1.00956	1.00954	1.01243	1.01244
5	1.01010	1.01039	1.00019	1.00013	1.00532	1.00553	1.00732	1.00736	1.00960	1.00958	1.01250	1.01250
6	1.01011	1.01044	1.00022	1.00014	1.00531	1.00549	1.00726	1.00730	1.00958	1.00956	1.01244	1.01245
7	1.01008	1.01047	1.00031	1.00029	1.00543	1.00563	1.00742	1.00742	1.00968	1.00971	1.01260	1.01263
8	1.01020	1.01047	1.00021	1.00018	1.00540	1.00552	1.00733	1.00730	1.00960	1.00957	1.01245	1.01251
Mean	1.010105	1.010424	1.000223	1.000160	1.005318	1.005511	1.007290	1.007314	1.009583	1.009573	1.012458	1.012473
Grand mean	1.010264		1.000191		1.005414		1.007302		1.009578		1.012465	

TABLE II
Alcohol (%, vol/vol) Determined by the Alcolyzer Method

	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	0.43	0.44	3.33	3.34	5.17	5.16	5.94	5.99	5.34	5.36	8.12	8.13
2	0.44	0.43	3.31	3.33	5.14	5.15	5.92	5.98	5.32	5.32	8.09	8.12
3	0.40	0.42	3.33	3.36	5.20	5.19	5.99	6.03	5.37	5.38	8.19	8.19
4	0.41	0.42	3.29	3.29	5.14	5.12	5.92	5.94	5.30	5.30	8.08	8.08
5	0.38	0.38	3.28	3.28	5.09	5.15	5.89	5.99	5.28	5.31	8.05	8.09
6	0.42	0.43	3.30	3.32	5.14	5.13	5.92	5.97	5.31	5.31	8.10	8.10
7	0.41	0.43	3.29	3.31	5.13	5.12	5.91	5.95	5.30	5.30	8.05	8.06
8	0.41	0.42	3.29	3.31	5.15	5.14	5.93	5.97	5.33	5.34	8.13	8.12
Mean	0.413	0.421	3.303	3.318	5.145	5.145	5.928	5.978	5.319	5.328	8.101	8.111
Grand mean	0.417		3.310		5.145		5.953		5.323		8.106	

TABLE III
Real Extract (%, wt/wt) Determined by the Alcolyzer Method

	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	2.76	2.85	1.31	1.30	3.24	3.28	4.01	4.03	4.38	4.39	5.99	6.00
2	2.74	2.81	1.27	1.26	3.19	3.26	3.97	4.00	4.35	4.33	5.95	5.96
3	2.75	2.85	1.31	1.31	3.25	3.30	4.00	4.02	4.37	4.38	5.97	5.97
4	2.75	2.82	1.29	1.26	3.23	3.27	3.99	4.00	4.36	4.36	5.98	5.98
5	2.74	2.81	1.28	1.26	3.22	3.30	4.00	4.04	4.37	4.37	5.98	6.00
6	2.76	2.84	1.30	1.29	3.24	3.28	4.00	4.01	4.37	4.37	5.99	5.99
7	2.75	2.85	1.33	1.31	3.26	3.32	4.02	4.04	4.39	4.40	6.01	6.03
8	2.77	2.85	1.29	1.29	3.26	3.29	4.01	4.02	4.38	4.38	5.98	6.01
Mean	2.753	2.835	1.298	1.285	3.236	3.288	4.000	4.020	4.371	4.373	5.981	5.993
Grand mean	2.794		1.291		3.262		4.010		4.372		5.987	

TABLE IV
Real Extract (%, wt/wt) Determined by the Calculation Method

	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	2.75	2.84	1.31	1.30	3.24	3.29	4.01	4.03	4.38	4.39	5.98	5.99
2	2.73	2.80	1.28	1.26	3.19	3.26	3.97	4.00	4.35	4.33	5.95	5.96
3	2.75	2.85	1.32	1.32	3.27	3.32	4.03	4.05	4.40	4.40	6.02	6.02
4	2.74	2.82	1.29	1.27	3.23	3.28	3.99	4.00	4.36	4.36	5.97	5.98
5	2.73	2.80	1.28	1.27	3.22	3.30	4.00	4.04	4.37	4.37	5.98	5.99
6	2.75	2.84	1.30	1.29	3.24	3.28	3.99	4.02	4.37	4.37	5.98	5.98
7	2.74	2.84	1.32	1.32	3.27	3.31	4.03	4.04	4.39	4.40	6.01	6.02
8	2.77	2.84	1.29	1.29	3.26	3.29	4.01	4.02	4.38	4.38	5.99	6.00
Mean	2.745	2.829	1.299	1.290	3.240	3.291	4.004	4.025	4.375	4.375	5.985	5.993
Grand mean	2.787		1.294		3.266		4.014		4.375		5.989	

TABLE V
Statistical Summary of Results of the Alcolyzer Method for Real Extract (N = 8)

		Repeatability			Reproducibility		
Sample Pair	**Grand Mean**	S_r	**RSD**$_r$	r_{95}	S_R	**RSD**$_R$	R_{95}
A/B	2.794	0.009	0.3	0.025	0.015	0.5	0.042
C/D	1.291	0.007	0.6	0.020	0.021	1.6	0.058
E/F	3.262	0.012	0.4	0.034	0.021	0.7	0.060
G/H	4.010	0.008	0.2	0.021	0.016	0.4	0.044
I/J	4.372	0.007	0.2	0.020	0.017	0.4	0.049
K/L	5.987	0.008	0.1	0.022	0.020	0.3	0.056

TABLE VI
Statistical Summary of Results of the Calculation Method for Real Extract (N = 8)

		Repeatability			Reproducibility		
Sample Pair	**Grand Mean**	S_r	**RSD**$_r$	r_{95}	S_R	**RSD**$_R$	R_{95}
A/B	2.787	0.009	0.3	0.026	0.017	0.6	0.047
C/D	1.294	0.006	0.5	0.017	0.020	1.5	0.055
E/F	3.266	0.012	0.4	0.033	0.023	0.7	0.066
G/H	4.014	0.008	0.2	0.022	0.020	0.5	0.055
I/J	4.375	0.007	0.1	0.018	0.020	0.5	0.056
K/L	5.989	0.003	0.1	0.009	0.021	0.4	0.060

The procedure for calculating the real extract using the calculation method is as follows:

1. Convert the alcohol content (%, vol/vol) to the specific gravity of alcohol (SG_A) based on the ASBC alcohol tables (1).
2. From the specific gravity of water (SG_W), specific gravity of sample (SG_S), and SG_A, calculate the specific gravity of real

TABLE VII
Comparison of Means of the Alcolyzer and Calculation Methods for Determination of Real Extract Using the Paired *t* Test[a]

Parameter	Real Extract (%, wt/wt)
No. of paired results (*N*)	96
Mean of differences (*D*)	–0.002
Standard Error of Differences (*SD*)	0.011
Calculated *t*	–1.36[b]
$t_{0.05}$	1.99

[a] All calculations were made based on Statistical Analysis-5 (1).
[b] Not significant at the 95% confidence level.

TABLE IX
Precision Comparison of the Alcolyzer and Calculation Methods (*N* = 8)[a]

Method	Sample Pair	Reproducibility Error	Pooled Variance
Alcolyzer	A/B	0.015	
	C/D	0.021	
	E/F	0.021	
	G/H	0.016	
	I/J	0.017	
	K/L	0.020	0.00034
Calculation	A/B	0.017	
	C/D	0.020	
	E/F	0.023	
	G/H	0.020	
	I/J	0.020	
	K/L	0.021	0.00041
Calculated *F*		1.198[b]	
$F_{0.025;42,\ 42}$		1.846	

[a] All calculations were made based on Statistical Analysis-5 (1). df = 42.
[b] Not significant at the 95% confidence level.

TABLE VIII
Comparison of Means for the Alcolyzer and Calculation Methods

Set and Sample	Collaborator	Alcolyzer Method	Calculation Method	Difference	Set and Sample	Collaborator	Alcolyzer Method	Calculation Method	Difference
I – A	1	2.76	2.75	0.01	IV – G	1	4.01	4.01	0.00
	2	2.74	2.73	0.01		2	3.97	3.97	0.00
	3	2.75	2.75	0.00		3	4.00	4.03	–0.03
	4	2.75	2.74	0.01		4	3.99	3.99	0.00
	5	2.74	2.73	0.01		5	4.00	4.00	0.00
	6	2.76	2.75	0.01		6	4.00	3.99	0.01
	7	2.75	2.74	0.01		7	4.02	4.03	–0.01
	8	2.77	2.77	0.00		8	4.01	4.01	0.00
I – B	1	2.85	2.84	0.01	IV – H	1	4.03	4.03	0.00
	2	2.81	2.80	0.01		2	4.00	4.00	0.00
	3	2.85	2.85	0.00		3	4.02	4.05	–0.03
	4	2.82	2.82	0.00		4	4.00	4.00	0.00
	5	2.81	2.80	0.01		5	4.04	4.04	0.00
	6	2.84	2.84	0.00		6	4.01	4.02	–0.01
	7	2.85	2.84	0.01		7	4.04	4.04	0.00
	8	2.85	2.84	0.01		8	4.02	4.02	0.00
II – C	1	1.31	1.31	0.00	V – I	1	4.38	4.38	0.00
	2	1.27	1.28	–0.01		2	4.35	4.35	0.00
	3	1.31	1.32	–0.01		3	4.37	4.40	–0.03
	4	1.29	1.29	0.00		4	4.36	4.36	0.00
	5	1.28	1.28	0.00		5	4.37	4.37	0.00
	6	1.30	1.30	0.00		6	4.37	4.37	0.00
	7	1.33	1.32	0.01		7	4.39	4.39	0.00
	8	1.29	1.29	0.00		8	4.38	4.38	0.00
II – D	1	1.30	1.30	0.00	V – J	1	4.39	4.39	0.00
	2	1.26	1.26	0.00		2	4.33	4.33	0.00
	3	1.31	1.32	–0.01		3	4.38	4.40	–0.02
	4	1.26	1.27	–0.01		4	4.36	4.36	0.00
	5	1.26	1.27	–0.01		5	4.37	4.37	0.00
	6	1.29	1.29	0.00		6	4.37	4.37	0.00
	7	1.31	1.32	–0.01		7	4.40	4.40	0.00
	8	1.29	1.29	0.00		8	4.38	4.38	0.00
III – E	1	3.24	3.24	0.00	VI – K	1	5.99	5.98	0.01
	2	3.19	3.19	0.00		2	5.95	5.95	0.00
	3	3.25	3.27	–0.02		3	5.97	6.02	–0.05
	4	3.23	3.23	0.00		4	5.98	5.97	0.01
	5	3.22	3.22	0.00		5	5.98	5.98	0.00
	6	3.24	3.24	0.00		6	5.99	5.98	0.01
	7	3.26	3.27	–0.01		7	6.01	6.01	0.00
	8	3.26	3.26	0.00		8	5.98	5.99	–0.01
III – F	1	3.28	3.29	–0.01	VI – L	1	6.00	5.99	0.01
	2	3.26	3.26	0.00		2	5.96	5.96	0.00
	3	3.30	3.32	–0.02		3	5.97	6.02	–0.05
	4	3.27	3.28	–0.01		4	5.98	5.98	0.00
	5	3.30	3.30	0.00		5	6.00	5.99	0.01
	6	3.28	3.28	0.00		6	5.99	5.98	0.01
	7	3.32	3.31	0.01		7	6.03	6.02	0.01
	8	3.29	3.29	0.00		8	6.01	6.00	0.01

extract (SG_{ER}) using Tabarie's formula (5), where a is SG_W at 20C°/20°C = 1:

$$SG_{ER} = SG_W{}^a + SG_S - SG_A$$

3. Convert SG_{ER} to the real extract concentration (%, wt/wt) using the formula based on the table of Goldiner, Klemann, and Kämpf (5):

$$E_R = -460.234 + 662.649 \times SG_{ER} - 202.414 \times SG_{ER}{}^2$$

The results were evaluated using the Youden unit block experimental design and comparison of test methods (1).

RESULTS AND DISCUSSION

A total of eight collaborators participated in the study. The results for sample specific gravity, alcohol, and real extract from the Alcolyzer method are shown in Tables I–III. No outliers were identified using Dixon's ratio test, and all of the data were adopted for the calculation method. The real extract values from the calculation method are shown in Table IV. The repeatability and reproducibility coefficients of variation for the determination of real extract by the Alcolyzer and calculation methods were judged acceptable (Tables V and VI).

A comparison of the Alcolyzer and calculation methods, using the paired t test for comparison of means, is shown in Tables VII and VIII. The means of the Alcolyzer and calculation methods were not significantly different for real extract at a 95% confidence level based on the paired t test. Reproducibility errors of the two methods were also measured using an F test. No statistically significant difference was found between the Alcolyzer (pooled variance = 0.00034) and calculation methods (pooled variance = 0.0041) at a 95% confidence level (Table IX).

The 2008 subcommittee recommends that the Alcolyzer method for the determination of real extract in beer, *happo-shu*, and nonalcoholic beer should be included in the *Methods of Analysis of BCOJ*.

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