Technical Committee and Subcommittee Reports

2007–2008 Report of the Technical Committee

Committee Members: D. Sedin, *Chair*; C. Benedict; K. Churchill; M. Eurich; R. Jennings; G. Kelly; K. Lakenburges; D. Maradyn; C. Powell; S. Thompson; and B. Foster (*Senior Advisor*).

Activity in 13 subcommittees was conducted by the ASBC Technical Committee and Subcommittee chairs during 2007–2008. As a result, three methods are being recommended for inclusion in the *Methods of Analysis* (MOA):

- Thiobarbituric Acid Index for Wort and Beer, chaired by Katie McGivney (New Belgium Brewing Company).
- Differentiation of Brewing Yeast Strains by PCR Fingerprinting, chaired by Sylvie Van Zandycke (Lallemand Inc.).
- Standard Method for Measuring Oxidative Resistance of Beer by Electron Paramagnetic Resonance (PCR), chaired by David Barr (Bruker BioSpin Corp.).

Additionally, a review of the Hops section of the MOA was led by Bob Foster (MillerCoors). The updated methods have been recommended for inclusion in the MOA.

Three methods will continue for another year of collaborative study:

- Can Packaging Methods, chaired by Scott Brendecke (Ball Corp.).
- Sulfur Dioxide in Beer by Flow Injection Analysis (FIA), chaired by Aaron Porter (Sierra Nevada Brewing Company).
- Accurate IBU Measurement of Dry-Hopped Beers, chaired by Ruth Martin (Sierra Nevada Brewing Company).

The Method for Reference Standard for Total Packaged Oxygen, chaired by Mark Eurich (MillerCoors), will not move forward with another year of collaborative testing. After multiple years of making modifications to the method, it was determined that the method could not be used to produce consistent package oxygen standards within and between laboratories. The Yeast Vitality by a Modified Method of the Intracellular pH-Measurement (ICP) Subcommittee is on hold until a chair can be identified to run the collaborative study.

Seven new subcommittees will be initiated in 2008–2009:

- Review of two sections of the MOA will commence in 2008–2009. Review of the Malt section will be led by Rebecca Jennings (Rahr Malting Co.), and review of the Sensory section will be led by Sue Thompson (MillerCoors).
- Solid-Phase Microextraction–Gas Chromatography/Mass Selective Detection (SPME-GC/MS) Fingerprint of Beer Volatiles and Semivolatiles, chaired by Roman Ortiz (MillerCoors).
- Wort and Beer Fermentable and Total Carbohydrates by HPLC, chaired by Mark Eurich (MillerCoors).
- HLP Media, chaired by Chris Powell (Lallemand Inc.).
- ATP, chaired by Gina Kelly (New Belgium Brewing Company).
- Volatile Sulfur Compounds in Beer, chaired by Jeff Cornell (MillerCoors).

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As in previous years, the following six standing subcommittees will continue activities in 2008–2009:

- Soluble Starch, chaired by Karen Churchill (Prairie Malt Co.).
- Check Services, director position to be filled.
- Coordination of New and Alternate Methods of Analysis, chaired by Jeff Cornell (MillerCoors).
- International Collaborative Methods, chaired by Dana Sedin (MillerCoors).
- Craft Brewers, chaired by Gina Kelly (New Belgium Brewing Company).
- Sensory Science, chaired by Sue Thompson (MillerCoors).

The Check Sample Service program continued in 2007–2008 without a director. Rob McCaig had completed his term at the conclusion of the ASBC Annual Meeting in Victoria, BC, Canada, and to date we have not been able to fill the position. Recently, Jim Monroe (retired member, formerly of Anheuser-Busch) has volunteered to take on the role of ASBC Check Services statistician. It is hoped that going forward, enhanced statistical reporting and analysis of the Check Service data with Jim's help will increase the inherent value of the service to current subscribers and help attract potential new subscribers. Tim Moore, 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 throughout the past year. I hope that the experience has been rewarding for them both personally and professionally. I would also like to recognize the many subcommittee members who participated during this past year.

Finally, I would like to recognize the dedication and hard work put forth by the Technical Committee. The previous chair of the Technical Committee, David Maradyn, did an excellent job over the past three years in leading the group. His hard work has been demonstrated by the number of new methods that have been added to the MOA and the strength and cohesiveness of the entire team. I look forward to working with David in transitioning to the role of Technical Committee chair and know that I will have "big shoes" to fill.

Review of Can Packaging Methods
(Scott Brendecke, Ball Corp., *Chair*)

This is a standing subcommittee charged with determining what packaging methodologies brewers currently have in use, investigating new packaging methods, and developing a technical relationship with the International Society of Beverage Technologists (ISBT), which has significant packaging expertise. The focus in 2008–2009 will be on continued collaboration with the ISBT, updating the MOA with a section on can terminology, and completion of a copper sulfate test method for exposed metal in containers with inner polymer coatings.

Coordination of New and Alternate Methods of Analysis
(Jeff Cornell, MillerCoors, *Chair*)

This is a standing subcommittee whose function is to collect, from various sources, 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, and recommendations regarding collaborative testing are made to the Technical Committee.

Soluble Starch
(Karen Churchill, Prairie Malt Co., *Interim Chair*)

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. A chair has not been selected for this subcommittee.

Craft Brewers
(Gina Kelly, New Belgium Brewing Company, *Chair*)

The mandate of the Craft Brewers Subcommittee is to connect with the craft brewing members of the ASBC and explore opportunities to make the Society more relevant to these individuals. Additionally, the subcommittee will develop and pursue strategies to bring craft brewers who are not members of the Society into the ASBC. Accomplishments and activities during the previous years include launching of the Craft Brewers Check Service; rollout of a Craft Brewers Community on ASBC*net*, with "ask the expert" and forum sections; and promotion of the ASBC to craft brewers at their events, such as the Great American Beer Festival in Denver, CO, and the Craft Brewers Conference in Austin, TX. 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.

International Collaborative Methods (ICM)
(Dana Sedin, MillerCoors, *Chair*)

This is a standing subcommittee whose function is to maintain effective communication between the ASBC and pertinent international technical societies (BCOJ, EBC, and IGB) on matters concerning the efficient, timely, and coordinated development of common methods for international use. Methods developed in this manner assume International Collaborative Method (ICM) status.

MOA Methods Review (Sensory and Malt)
(Sue Thompson, MillerCoors, *Sensory Chair*; and Rebecca Jennings, Rahr Malting Co., *Malt Chair*)

The mandate of this standing subcommittee is to review the methods published in the ASBC MOA, 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 was 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 Malt and Sensory sections commencing in 2008–2009.

Accurate Determination of IBU Levels in Dry-Hopped Beers
(Ruth Martin, Sierra Nevada Brewing Company, *Chair*)

This is the second year of this subcommittee's existence. Based on polling by the Coordination of New and Alternate Methods of Analysis Subcommittee, 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 which is important to the craft brewing members 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, MillerCoors, *Chair*)

This is the third year of the subcommittee's existence. It was formed on recommendation from the Technical Committee to bring more focus to sensory science in the ASBC, provide a forum for sensory scientists in the brewing industry to share and discuss current methodology, and propose new methodology for collaborative testing. Committee activities over the past year included 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 ICM status. Upcoming activities will include review of sensory analysis methods for the ASBC MOA.

Sulfur Dioxide in Beer by Flow-Injection Analysis (FIA)
(Aaron Porter, Sierra Nevada Brewing Company, *Chair*)

This is the second year of existence for this subcommittee. It was formed on the recommendation of the Technical Committee to evaluate the determination of sulfur dioxide in beer by flow-injection analysis (FIA). During the first year, initial collaborative testing was conducted in a limited number of laboratories. This year a full collaborative test will be conducted.

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

This is the first year of this subcommittee's existence. It was formed to evaluate the use of solid-phase microextraction as a sampling technique, coupled with gas chromatographic separation and mass selective detection, to yield volatile and semivolatile fingerprints of finished beer. Currently in the scientific literature, there are various methods described that employ this technique as a fingerprint methodology. Variations include type of SPME fiber utilized, whether the sampling of the beer is from the liquid or the headspace, and type of mass selective detection (e.g., ion trap, quadrapole, time-of-flight). Collaborative testing will not commence this year; however, the chair will be working with interested subcommittee members on choice of methodology and a list of analytes of interest.

Volatile Sulfur Compounds in Beer
(Jeff Cornell, MillerCoors, *Interim Chair*)

This is the subcommittee's first year of existence. It was formed to evaluate analytical methodology capable of measuring low-level sulfur species (parts per billion). During the first year, different methods will be evaluated in the chair's laboratory, along with identification of the volatile sulfur compounds of interest. A chair has not been selected for this subcommittee.

Wort and Beer Fermentable and Total Carbohydrates by HPLC
(Mark Eurich, MillerCoors, *Chair*)

This is the subcommittee's first year of existence. The subcommittee has been tasked with updating 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, including 2008, showed great interest in these two methods. Significant technological gains would also deem these methods be brought up to industry standards.

HLP Media
(Chris Powell, Lallemand Inc., *Interim Chair*)

This is the subcommittee's first year of existence. The subcommittee was formed to evaluate HLP media based on strong interest from the craft brewing community. A chair has not been identified for this subcommittee.

ATP
(Gina Kelly, New Belgium Brewing Company, *Chair*)

This is the subcommittee's first year of existence. Based on polling of the ASBC membership in 2008, significant interest was shown in this methodology. During the first year, the chair will determine the luminometer instrumentation to be included in the study, design the testing scheme, and determine the most appropriate method for analyzing and comparing the data.

Method for Measurement of Resistance of Free-Radical Oxidation in Beer by Electron Paramagnetic Resonance (EPR)
(David Barr, Bruker BioSpin Corp., *Chair*)

This was the third year of existence for this subcommittee, which is charged with evaluating electron paramagnetic resonance (EPR) for the determination of resistance of beer to free-radical oxidation. In the first year of the study, repeatability and reproducibility coefficients of variation for the determination of lag time and T150 in packaged beer were judged unacceptable. Improvements were made to the methodology, sample pair selection, and specifics regarding spin-trap and shipment of beer to collaborators. Analysis of the data from the second year of the study again yielded unacceptable repeatability and reproducibility coefficients of variation for the determination of lag time and T150 in packaged beer. In the third year, updates were made to the algorithms utilized for data processing, and timelines were set for when samples were to be analyzed. Analysis of the data yielded acceptable repeatability and reproducibility coefficients of variation. The method has been recommended for inclusion in the MOA.

Method for Reference Standard for Total Package Oxygen
(Mark Eurich, MillerCoors, *Chair*)

This was the sixth year of the subcommittee's existence. Based on a recommendation by the Technical Committee, this subcommittee was formed to evaluate a method to produce a reference standard for total package oxygen (TPO) analysis. In the first year, a method was determined. In the second year, different laboratories measured one single source of canned water and obtained statistically similar results from different TPO instruments. In the third year, the method using bottled beer produced unacceptable results. Based on feedback from subcommittee members, a number of modifications to the bottled beer protocol were identified to correct the deficiencies, as well as improve communication with the collaborators on how to carry out the procedure. The recommended changes to the bottled beer procedure included addition of a procedure to eliminate oxygen in the blank; standardization of the syringe type and needle size; and changes to the volume of the air spikes employed. To improve communication on how the preparation of the spiked samples should be carried out, the subcommittee recommended that a series of photos demonstrating the technique in bottled beer be included with the method. Finally, the subcommittee recommended that the collaborators be advised to practice the spiking procedure in bottled beer prior to beginning the study. In the fourth year, the minimum number of collaborators was not met to conduct a statistical analysis of the data. From the bottled beer method in the fifth year, 17 sets of data were statistically evaluated. Repeatability coefficients of variation from both the 100- and 200-µL oxygen spike levels were judged unacceptable. In the sixth year, the subcommittee opted to change the method from using bottled beer to aluminum canned beer, using hose clamps and septa and then injecting air directly into the can through the septa. The subcommittee chose the aluminum canned beer method to reduce any outside errors inherent with the bottled beer method. Air in both 100- and 200-µL volumes was injected through septa into beer in aluminum cans and analyzed for TPO. Analysis of the data yielded unacceptable repeatability and reproducibility coefficients of variation. It is recommended that the subcommittee be discharged from further study.

MOA Methods Review
(Bob Foster, MillerCoors, *Chair*)

The mandate of this standing subcommittee is to review the analytical methods published in the ASBC MOA, 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 analytical methodology that was 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 Hops commencing last year. A number of updates to the Hops section have been recommended.

TBA Test as an Indicator for Flavour Stability
(Katie McGivney, New Belgium Brewing Company, *Chair*)

This was the subcommittee's second year of existence. In its first year, discussion among the subcommittee members revealed experience with two methods—the MEBAK method and a method published in the *ASBC Journal* in 1976. The MEBAK method for the thiobarbituric acid index (TBI) was recommended for collaborative testing. The method could be used to monitor process differences for worts and/or beers. Thermal stress appears to have a connection to oxidation in the finished product, so monitoring thermal stress during start-up or throughout procedural or equipment changes could be of benefit to brewers. Analysis of the data yielded acceptable repeatability and reproducibility coefficients of variation. The method has been recommended for inclusion in the MOA.

PCR Applications to Brewing
(Sylvie Van Zandycke, Lallemand Inc., *Chair*)

This was the second year for the subcommittee, which was formed to investigate the application of PCR techniques within breweries. Currently, there are no ASBC recommended methods that utilize PCR technology, even though brewery laboratories are increasingly equipped with either standard or real-time PCR thermocyclers. Although PCR can be used as a tool to detect and identify a range of microbes associated with the brewing industry (e.g., wild yeast and bacterial contaminants), a simple application for this technology is for the analysis of production strains. The ability to identify specific strains and differentiate between production yeasts is particularly useful when a number of different yeast strains is employed within a brewery. In its first year, preliminary robustness trials were performed by the ex officio to differentiate 15 brewing yeast strains by PCR fingerprinting in conjunction with primers targeting interdelta sequences. In the second year, collaborative testing was performed to assess the intralaboratory repeatability of the method. It was determined that the use of PCR fingerprinting in conjunction with primers targeting interdelta sequences is a suitable method for the differentiation and identification of brewing yeast strains. The method was recommended for inclusion in the MOA.

Coordination of New and Alternate Methods of Analysis

Subcommittee Members: J. Cornell, *Chair*; D. Bendiak; J. Helber; G. Kelly; J. Masschelin; P. Schwarz; S. Thompson; S. Van Zandycke; and D. Maradyn (*ex officio*).

Corresponding Members: A. Mundy, European Brewery Convention (EBC), and S. Sakuma, Brewery Convention of Japan (BCOJ).

Keywords: Accelerated aging, Allergens, Arabinoxylans, ATP, Carbohydrate, Carbon dioxide, Decarbonation, Foam, Hops, Gluten, Luminescence, Malting quality, Original extract, Proteins, Rho, Tetra, Thiobarbituric acid, Total polyphenols, Volatile sulfur compounds, Yeast

RECOMMENDATIONS

1. Form a subcommittee to evaluate methodology and conduct collaborative testing on a method to quantitate volatile sulfur compounds in beer.
2. Form a subcommittee to evaluate and conduct collaborative testing on an ATP bioluminescence method for either rinse waters or surface swabs.
3. The Technical Committee will review the method, results, and report of the recent EBC collaborative study on the measurement of carbon dioxide in beer by volume expansion to evaluate potential inclusion in the ASBC MOA.
4. Archive the idea to investigate potential interferences in the existing total polyphenol method (Beer 35), due to lack of data indicating biases exist that are not well understood.
5. Archive the idea to evaluate a spectrophotometric method for the determination of protein in hopped wort, due to insufficient interest expressed by the membership.
6. Archive the idea to evaluate a methodology for the determination of the molecular weight distribution of arabinoxylans, due to the absence of a suitable reference method.
7. Archive the idea to evaluate by collaborative testing the Lg-Automatic foam tester, due to insufficient interest expressed by the membership.

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.

Where needed, the subcommittee develops polling topics and questions to more accurately determine interest in new and alternate methods from ASBC members. This subcommittee also has begun to liaise with the Emerging Issues Committee and plans to explore further work together at the annual meeting.

Accelerated Aging of Seed (Viability, Vigor testing)

There is concern over viability loss when sprouted barley is stored, particularly under higher temperatures and humidity. Sprout damage measurement is not a direct measure of loss of viability. It was proposed that accelerated aging might be incorporated into standard barley germination testing as a means of predicting viability loss (10). The third and final year of a long-term storage study has been completed at North Dakota State University, and the methodology (based on that used at the Canadian Grain Commission) appears promising. The subcommittee recommends polling the membership for interest in collaborative testing once the work at NDSU has been published.

Analytical Methodology for Allergens in Beer

The issue of potential labeling legislation and subsequent requirements from the FDA or TTB is being followed by both the Emerging Issues Committee and this subcommittee. Under the TTB's proposed regulations, producers of alcoholic beverages must declare on product labels, the use of milk, tree nuts, peanuts, fish, eggs, soybeans, wheat, and crustacean shellfish. The allergen receiving the most attention related to malt beverages is gluten, which is linked to celiac disease. The TTB has published an interim rule, effective July 26, 2006, that allows the voluntary labeling of major food allergens on the labels of wines, distilled spirits, and malt beverages. To date, there is no mandatory requirement for labeling, and it continues to be voluntary since the interim rule was published.

In January 2007, the FDA issued a proposed rule that would define the term "gluten-free" for voluntary use in food labeling (6). The comment period for the proposed rule ended April 23, 2007. This rule

- Defines the circumstances under which the term "gluten-free" can be used.
- Defines "prohibited grains" such that to use the term "gluten-free" the product ingredients must not contain any wheat, barley, rye, or hybrids thereof.
- States that whenever ingredients are used that are derived from any prohibited grain the maximum allowable concentration of gluten would be 20 ppm in the food.

ELISA methodology has been evaluated by the TTB, FDA, and BRI for the detection of gluten and does not appear to discriminate the source (i.e., wheat versus barley protein), nor is there a distinction in the proposed rule from the FDA. This subcommittee recommends continued monitoring of this developing topic and related potential analytical methodology that could lead to collaborative testing. Action by the Technical Committee in this area will be dictated through the Emerging Issues Committee.

ATP Luminometer

ATP luminescence is actively being used by several brewing companies for testing of surface swabs or rinse water for CIP (clean in place) validation. However, the methods utilized and the outputs generated can vary greatly depending on the instrument manufacturer. If a collaborative study were to be conducted, the design and data analysis would need an alternative statistical approach that falls outside the typical ASBC validation methodology. In light of this, the subcommittee performed polling of the membership and past annual meeting attendees in early 2008 to better understand the interest in this methodology. Of the 133 respondents to the survey, 26 answered questions about ATP bioluminescence. Key findings from the survey include

- Several different instruments and test kits are used, but one system was used more than the rest by the respondents.
- Of the respondents, 85% measure total ATP, as opposed to free or free and total.
- Despite the diversity in instruments and test kits, the output of nearly all instruments is in RLU (relative light units).

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- There is a range of RLU values (<2.6 to <300) that respondents and instrument manufacturers say indicate a "clean" result for a swab or rinse water.
- Most respondents do not run a standard curve.
- The most common applications for ATP technology are for checking final rinse water after a CIP and using swabs for checking tanks, lines, and other surfaces, such as packaging filler areas.

Based on the polling results, there is likely sufficient interest and the means to form a subcommittee on this topic. The subcommittee recommends the formation of a subcommittee on ATP luminescence in the fall of 2008.

Carbon Dioxide in Beer (Alternative to Temperature/Pressure Method)

Four members and two vendors showed interest in forming a collaborative study on this topic at the 2006 ASBC Annual Meeting in La Quinta, CA. Both *Analytica–EBC* and the *IOB Methods of Analysis* include methods for the determination of carbon dioxide in beer by an instrumental procedure using the Mettler Toledo (Corning) 965D carbon dioxide analyzer (5,8). This instrument is no longer manufactured and therefore not being considered for ASBC collaborative testing.

A major soft drink company communicated that they are using the Zahm and other temperature/pressure-based devices for the determination of carbon dioxide in their products. A second soft drink company communicated that they are using the CarboQC from Anton Paar, as are some breweries. The EBC has conducted collaborative testing using the CarboQC instrument for the determination of carbon dioxide in beer by volume expansion. The repeatability and reproducibility results of the study were found to be acceptable by EBC standards, and the method has been recommended for inclusion in *Analytica–EBC*. The report and method will be forwarded on to the ASBC Technical Committee for review to determine whether the method meets the requirements for adoption into the ASBC *Methods of Analysis* (MOA).

Decarbonation of Beer

A discussion of appropriate methods for decarbonating beer arose during the fall 2002 Technical Committee meeting in St. Paul, MN, and has resurfaced at most, if not all, of the subsequent ASBC Annual Meetings. The existing ASBC MOA methods for decarbonation of beer are Beer-1A and Beer-1D—decarbonation by shaking or using a rotary shaker, respectively (1). Decarbonation (or degassing) is a sample preparation technique employed to remove enough CO_2 from the sample prior to measurement to minimize sampling error, while also minimizing the impact of the procedure on the analyte(s) of interest. Therefore, the most valuable studies are those that couple various decarbonation methods with analytical methods. Several methods of decarbonation have been tested for effectiveness of carbon dioxide removal and ethanol retention (3,12). An oral presentation was given on the subject at the 2006 ASBC Annual Meeting in La Quinta by Karl Siebert. Given the current body of work and interest expressed, the subcommittee recommends the following and continues to seek someone to lead the following efforts:

- Summarize the findings from the existing work referenced above.
- Based on the summary, work with the subcommittee to pull together a recommendation for collaborative testing that would test selected degassing methods against a short list of common ASBC methods that require degassing.

Foam Tester from Lg-Automatic

Interest was expressed in evaluating the foam tester from Lg-Automatic of Frederiksvaerk, Denmark (2). A membership poll was conducted in 2004 to determine which methods for foam analysis were currently in use. The results indicated that the majority of labs were using one of two commercially available instruments: the Haffmans NIBEM-T foam stability tester (five labs) or the Lg-Automatic foam tester (two labs). Polling at the 2006 ASBC Annual Meeting in La Quinta failed to identify enough potential collaborators to commence a study on this topic. Attendees at the 2007 ASBC Annual Meeting in Victoria, BC, Canada, were polled, again seeking potential collaborators. Since sufficient interest has not been demonstrated to date, this idea will be archived.

Molecular Weight Distribution of Arabinoxylans

Interest was expressed in the analysis of the molecular weight distribution of arabinoxylans in beer. To date, a standard method has not been identified. Work presented by Paul Schwarz at the 2005 ASBC Annual Meeting in Savannah, GA, used size-exclusion chromatography for the determination of the molecular weight distribution of arabinoxylans; however, this method was deemed not to be appropriate for a routine laboratory method. The subcommittee is aware that VLB is working on a dye-based methodology for the determination of the molecular weight distribution of arabinoxylans; however, full details are not available at this time. The final report from this subcommittee last year indicated that if a method was found, polling would take place, but otherwise the idea would be archived. Since a method has not surfaced, the subcommittee recommends the idea be archived.

Rapid Carbohydrate Determination in Beer

Interest in a new rapid method for the determination of total carbohydrate in beer was expressed by members at the 2003 ASBC Annual Meeting in Albuquerque, NM. Currently, method Beer-6D, Calculated Carbohydrate (recommended for use in label statements) (1) is available in the MOA. Method Beer-41 Total Carbohydrates (phenol-sulfuric method) states that this method is not to be used for labeling purposes as it gives lower values than Beer-6D (1). Polling conducted in 2005 and 2006 yielded limited information, except that responding labs were typically using Beer-6D; there is limited use of HPLC for profiling. The subcommittee decided that more information was needed on this topic, and polling of the membership was conducted in spring 2008. Of the 133 total respondents, 41 answered questions on carbohydrate methods. The key findings from the survey include

- Similar numbers of respondents perform some type of carbohydrate analysis in beer, wort, or both.
- The primary reasons for doing the analysis are for labeling information, to monitor or respond to changes in brewing product or process, or to evaluated sugar profiles for syrups.
- At least 73% of the respondents analyze for glucose, maltose, maltotriose, and nonfermentable dextrins.
- Of the respondents, 77% utilize HPLC ion exchange and refractive index detection.
- With regard to a rapid test, 58% (n = 22) of the respondents said they would like one, while 42% were not interested.
- For those who would like a rapid method, the most common reasons are
  - For real-time brewhouse or site lab (as opposed to central lab) analysis for quicker feedback.
  - Prediction of wort fermentability.
  - Final product carbohydrate compliance, including low-carb beers.
  - HPLC is too time consuming and/or costly.

The subcommittee will continue to monitor the literature for a rapid carbohydrate methodology in 2008–2009. If such methodology is not found in the coming year, this topic will be archived.

Spectrophotometric Analysis of Proteins in Hopped Wort

A subcommittee to evaluate an alternative to the Kjeldahl method for the analysis of proteins in hopped wort and beer using the de la Vega method was initiated in 2002 (4). In 2004–2005, collaborative testing was completed, and a method was recommended for inclusion in the MOA for finished beer and unhopped wort only. It was recommended that a subcommittee be formed to investigate the applicability of this method for hopped wort. Polling of the membership for interest in collaborative testing was conducted at the 2005 and 2006 ASBC Annual Meetings. Insufficient interest was expressed each year. The subcommittee recommended soliciting one last time for interested individuals at the 2007 ASBC Annual Meeting in Victoria. There was insufficient interest expressed in Victoria, so this topic will be archived.

Total Polyphenol Method

Interest was expressed at the 2005 ASBC Annual Meeting in Savannah in understanding observed interferences with the total polyphenol spectrophotometric method (Beer-35). No interest was expressed in the topic at the 2006 ASBC Annual Meeting in La Quinta. It is possible that this topic could have come from someone getting higher than expected results from highly hopped beers. Discussion with several of the method users, the Technical Committee, and the subcommittee in 2007 resulted in no information on interferences with this method. This idea will be archived.

UV Spectrophotometer–Colorimetric Methods

A desire was expressed at the 2005 ASBC Annual Meeting in Savannah for some simple colorimetric methods for craft brewers. This desire was reiterated at the 2006 ASBC Annual Meeting in La Quinta. It was not clear which methods were of interest, but some of the old archived ASBC methods may be useful for updating and subsequent collaborative testing. The Technical Committee has obtained a listing of the archived methods and will recommend to the Coordination of New and Alternate Methods of Analysis Subcommittee any that may be appropriate for re-introduction, revision, or updating. To start the process, selected archived ASBC MOA methods have been included in the 2008 CD update of the *Laboratory Methods for Craft Brewers*. The Technical Committee will complete its review of the archived ASBC MOA methods in 2008–2009.

Volatile Sulfur Compounds in Beer

Volatile sulfur compounds contribute in a positive manner to the flavor and aroma character of many beers. However, because of their very low sensory thresholds and powerful, often unpleasant characteristics, they are frequently the source of off-odors and -flavors. Therefore, to have an analytical method available to the brewer that can quantitate these compounds at sub–parts per billion levels is highly desirable. Over the past 10 years, methods have been published in the literature to quantitate volatile sulfur compounds in beer using sulfur-selective detectors (flame photometric detector [FPD], sulfur chemiluminescence detector [SCD], and, more recently, the pulsed-flame photometric detector [PFPD]) (7,9,11). Sulfur-selective detectors are mandatory for this type of analysis due to the low levels of these compounds within the beer matrix. In addition, various sampling techniques have been employed for volatile sulfur analyses, ranging from simple headspace to purge and trap to solid-phase microextraction. The subcommittee polled the membership in spring 2008 for information regarding sampling techniques, sulfur-selective detectors, and methodologies in use for volatile sulfur analysis. Of the 133 respondents to the survey, 32 answered the questions on this topic. The key findings from the results of this polling were

- Of the respondents, 55% (n = 17) currently use a volatile sulfur method for beer.
- Of those respondents who do not have a volatile sulfur method for beer, 100% answered that this type of analysis would be of interest.
- For the respondents who measure sulfur compounds in beer, Table I lists the compounds that are quantitated and the number of respondents who measure them.
- Static headspace combined with gas chromatography is the most commonly used analytical technique (n = 15).
- The most common detectors used are SCD (n = 5), FPD (n = 4), and PFPD (n = 2). Four respondents listed "other" as the detector used but did not specify what they use.
- Of those who currently have a volatile sulfur method, 75% said that it is used on process phases other than finished beer (e.g., wort or fermenter drop).

Based on the polling results, there was sufficient interest expressed by the membership to form a collaborative study on this topic. The subcommittee recommends initiation of this collaborative study in fall 2008.

TABLE I
Compounds Quantitated and Number of Respondents Who Measured Them

Compound	Survey Response Frequency
Dimethyl sulfide (DMS)	13
Hydrogen sulfide (H_2S)	7
Sulfur dioxide (SO_2)	6
Methanethiol	3
Ethanethiol	2
S-Methyl methionine (DMSp)	2
S-Methyl thioacetate (MTA)	1
3-Methyl-2-butenethiol (3MBT)	1
Dimethyl sulfoxide (DMSO)	1
Dimethyl disulfide (DMDS)	1
Dimethyl trisulfide (DMTS)	1

Yeast Vitality

A poster was presented at the 2005 ASBC Annual Meeting in Savannah by Boris Stambuk on a new method to evaluate yeast vitality. Unfortunately, to date the full paper has not been published in a refereed scientific journal. Once the paper is published, the Technical Committee will review the methodology and make a recommendation on whether it is appropriate for collaborative study. Last year, the subcommittee indicated that if this method was not published by the end of 2007, the idea would be archived. After discussions with the Technical Committee, it was decided that we will give the author another year to publish the full paper in a referred scientific journal. If the paper has not been published by the conclusion of the 2009 ASBC Annual Meeting in Tucson, AZ, then the idea will be archived.

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Soluble Starch

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Keywords: α-Amylase, Automated-flow analysis, Diastatic power, DP, Iodine, Potassium ferricyanide

CONCLUSIONS

Soluble starch lot 8621J was judged acceptable for analyzing brewer's-type malts for diastatic power and α-amylase activity.

RECOMMENDATIONS

1. The subcommittee recommends lot 8621J as the ASBC soluble starch standard.
2. The subcommittee recommends future evaluation of alternate sources of soluble starch.

This subcommittee is a standing subcommittee whose goal is to coordinate a testing program for 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. As of March 29, 2008, starch lot 29672A was depleted. This lot, which initially consisted of 597.875 kg (1,315.3 lb) was tested and judged acceptable in spring 2004 (2). Lot 8621J was evaluated by collaborative testing in spring 2008.

PROCEDURE

A sample of test lot 8621J was distributed to each collaborator. The collaborators were instructed to prepare test starch, as well as their current standard starch, using the methods described in Malt-6A and -7 (1). Analyses of diastatic power (DP) and α-amylase were carried out on a minimum of five brewer's-type malts in parallel. The starch, used on two consecutive days, was made from the same preparation. Collaborators utilized either of the manual standard reference methods, Malt-6A and -7, or automated flow equipment. Results were evaluated using the paired *t* test (1). The manufacturer of the test starch evaluated solubility, pH, residue upon ignition, and sensitivity according to American Chemical Society specifications as given in Table I (3).

RESULTS AND DISCUSSION

Initially, a collaborative study was performed using a modified potato starch (Paselli SA2) manufactured by Avebe/National Starch. Subcommittee members were asked to use their ASBC starch and the Paselli SA2 starch with malt samples that were supplied by the subcommittee chair. The results for the two starches were comparable for diastatic power, although the results for α-amylase, using iodine methods, were much higher for the Paselli SA2 starch than for the ASBC starch. Due to several complaints about the ability of the Paselli SA2 starch to go into solution, starches from several other suppliers were tested and identified as potential replacements for lot 29672A.

A second collaborative study was performed in spring 2008 with a modified potato starch from a different supplier (lot 8621J). To be accepted as the ASBC soluble-starch standard, a lot of starch must meet all specifications listed in the Certificate of Analysis given in Table I. Results from tests performed by the starch supplier indicated that starch lot 8621J met the specifications for solubility, pH, and residue upon ignition. DP and α-amylase were evaluated by 11 collaborators. Only 1 collaborator used the standard reference method for DP determination, and 10 used automated flow analysis. For α-amylase, five collaborators used the manual standard reference method, and six used automated flow analysis. Of the six who used automated flow analysis, three used potassium ferricyanide, and three used iodine for the determination. Each sample pair was compared for differences in means for DP and α-amylase for the two starch lots. All methods were combined for the statistical analysis. The results for α-amylase can be found in Table II, and those for DP can be found in Table III.

The results for the two starches were comparable for both DP and α-amylase. Paired *t* tests were used to correlate the data from both analyses, resulting in no significant differences for the two starches at the 95% confidence level. There was an exception for DP for the first two samples when the two days were combined as the other samples were. When the two days were left separate, there was no significant difference. These results indicate that the test starch is acceptable.

Starch lot 8621J was determined to meet all specifications and was judged to acceptable. As of this time, 1,000 kg (2,200 lb) of soluble starch is available for sale.

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TABLE I
Certificate of Analysis for Starch Lot 8621J

Test	ACS Specification	Result
Solubility	Pass test	Passed
pH	5.0–7.0%	6.50%
Residue after ignition	≤0.4%	0.17%
Sensitivity	Pass test	Passed

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TABLE II
Comparison of α-Amylase Results Using a Paired Two-Sample *t* Test for Differences in Means[a]

Statistical Parameter	Var. 1-a	Var. 2-a	Var. 1-b	Var. 2-b	Var. 1-c	Var. 2-c	Var. 1-d	Var. 2-d	Var. 1-e	Var. 2-e
Mean	35.10	36.00	38.57	40.34	50.76	52.03	47.43	48.3	54.69	55.88
Variance	31.10	30.25	30.75	26.42	28.68	18.60	38.65	18.281	20.52	14.56
Observations	22	22	22	22	22	22	21	21	22	22
Pearson correlation	0.66		0.67		0.71		0.60		0.73	
Hypothesized mean difference	0		0		0		0		0	
df	21		21		21		20		21	
t Stat	–0.94		–1.90		–1.57		–0.79		–1.77	
P (*T* ≤ *t*) one-tail	0.18		0.04		0.07		0.22		0.05	
t Critical one-tail	1.72		1.72		1.72		1.72		1.72	
***P* (*T* ≤ *t*) two-tail**	**0.36**		**0.07**		**0.13**		**0.43**		**0.09**	
t Critical two-tail	2.10		2.08		2.08		2.09		2.08	
	Var. 1-f	**Var. 2-f**	**Var. 1-g**	**Var. 2-g**	**Var. 1-h**	**Var. 2-h**	**Var. 1-i**	**Var. 2-i**	**Var. 1-j**	**Var. 2-j**
Mean	56.57	57.35	53.79	55.07	59.08	59.69	61.28	61.82	75.74	75.18
Variance	20.98	11.65	28.57	22.18	22.28	10.85	25.29	12.57	148.28	144.01
Observations	22	22	22	22	20	20	19	19	19	19
Pearson correlation	0.64		0.73		0.73		0.76		0.92	
Hypothesized mean difference	0		0		0		0		0	
df	21		21		19		18		18	
t Stat	–1.03		–1.59		–0.85		–0.71		0.50	
P (*T* ≤ *t*) one-tail	0.158		0.063		0.20		0.24		0.31	
t Critical one-tail	1.72		1.72		1.73		1.73		1.73	
***P* (*T* ≤ *t*) two-tail**	**0.32**		**0.13**		**0.40**		**0.49**		**0.62**	
t Critical two-tail	2.08		2.08		2.09		2.10		2.10	

[a] All calculations were based on ASBC methods (1).

TABLE III
Comparison of Diastatic Power Results Using a Paired Two-Sample *t* Test for Differences in Means[a,b]

Statistical Parameter	Var. 1-a	Var. 2-a	Var. 1-b	Var. 2-b	Var. 1-c	Var. 2-c	Var. 1-d	Var. 2-d	Var. 1-e	Var. 2-e
Mean	118.83	118.61	118.08	121.89	119.05	120.78	122.12	123.06	136.65	138.06
Variance	61.96	83.90	32.43	101.69	50.653	48.26	33.90	63.96	47.68	87.82
Observations	11	11	11	11	22	22	22	22	22	22
Pearson correlation	0.84		0.56		0.37		0.56		0.59	
Hypothesized mean difference	0		0		0		0		0	
df	10		10		21		21		21	
t Stat	0.15		–1.51		–1.03		–0.65		–0.86	
P (*T* ≤ *t*) one-tail	0.44		0.08		0.16		0.26		0.20	
t Critical one-tail	1.812		1.81		1.72		1.72		1.72	
***P* (*T* ≤ *t*) two-tail**	**0.88**		**0.16**		**0.32**		**0.52**		**0.40**	
t Critical two-tail	2.23		2.23		2.087		2.08		2.08	
	Var. 1-f	**Var. 2-f**	**Var. 1-g**	**Var. 2-g**	**Var. 1-h**	**Var. 2-h**	**Var. 1-i**	**Var. 2-i**	**Var. 1-j**	**Var. 2-j**
Mean	134.51	136.03	157.64	158.44	180.9	180.26	213.60	207.69	257.25	259.43
Variance	38.71	145.63	35.17	120.78	36.58	71.73	355.15	68.67	509.9	768.65
Observations	22	22	22	22	20	20	20	20	16	16
Pearson correlation	0.70		0.76		0.71		0.04		0.86	
Hypothesized mean difference	0		0		0		0		0	
df	21		21		19		19		15	
t Stat	–0.80		–0.50		0.48		1.30		–0.61	
P (*T* ≤ *t*) one-tail	0.22		0.31		0.32		0.10		0.28	
t Critical one-tail	1.72		1.72		1.73		1.73		1.75	
***P* (*T* ≤ *t*) two-tail**	**0.43**		**0.62**		**0.64**		**0.21**		**0.55**	
t Critical two-tail	2.08		2.08		2.09		2.09		2.13	

[a] All calculations were based on ASBC methods (1).
[b] The *t* test was run on each day individually. Days were not combined for A and B.

Standard Method for Measurement of Oxidative Resistance of Beer by Electron Paramagnetic Resonance

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Keywords: E_A, EPR, ESR, Free radicals, Lag time, PBN, T150, Tempol

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the determination of lag time ranged from 8.15 to 14.68% and from 12.62 to 25.47%, respectively, and were judged acceptable.
2. The repeatability and reproducibility coefficients of variation for the determination of T150 ranged from 4.25 to 10.43% and from 18.06 to 23.30%, respectively, and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends that the method be included in the *Methods of Analysis.*
2. Discharge the subcommittee.

Based on polling by the subcommittee for the Coordination of New and Alternate Methods of Analysis, this subcommittee was charged with evaluating a standard method for determining the oxidative resistance of beer by electron paramagnetic resonance (EPR) (2). This technique is also referred to as electron spin resonance (ESR). In the first year, 7 of 12 participating laboratories returned results (3). It was noted that there were some potentially unique issues with running a collaborative using the EPR oxidation assay. Specifically, we learned that the EPR oxidation profiles of the samples were quite sensitive to shipping and storage conditions. We also learned that the spin trap reagent (PBN) should be sent in powder form and reconstituted by the collaborators, as reagent leaked from some of the bottles during shipping to the collaborators. In the second year, 10 participants returned results (4). The quality of the data returned was improved after implementation of changes recommended after the first year; however, acceptable reproducibility and repeatability coefficients of variation for lag time and T150 were not realized. The main issue identified in the second year was the high variability in the time of analysis after collaborators received their samples.

PROCEDURE

Four sample pairs of commercial beer (A/B, C/D, E/F, and G/H) were sent to each collaborator. Sample pair A/B was 100% light lager beer, sample pair C/D was a mixture of 75% light lager and 25% amber lager beer, sample pair E/F was a mixture of 25% light lager beer and 75% amber lager beer, and sample pair G/H was 100% amber lager beer. EPR oxidation values for the beer samples were expressed in terms of either lag times or percent intensity relative to a 5 μ*M* Tempol reference standard at the T150 time point (5,6). Results were evaluated using the Youden unit block design (1).

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RESULTS AND DISCUSSION

Results from eight collaborators were received for the four sample pairs. Collaborators were allowed to perform the EPR analyses using one of two types of instruments: an EMX research-grade EPR spectrometer or an e-scan bench-top EPR spectrometer. Data for lag time and T150 are presented in Tables I and II, respectively. Outliers were identified using Dixon's ratio test (1).

The statistical summary of the lag time and T150 data is shown in Table III. Repeatability and reproducibility coefficients of variation for the determination of lag time ranged from 8.15 to 14.68% and from 12.62 to 25.47%, respectively, and were judged acceptable. The repeatability and reproducibility coefficients of variation for the determination of T150 ranged from 4.25 to 10.43% and from 18.06 to 23.30%, respectively, and were judged acceptable.

Sample pairs were created from two different production dates of the same light and amber beers, as were the blends of each sample pair. Thus, the grand means represent two different production dates. The data for lag time and T150 were much improved this year compared to previous years. This was quite an accomplishment considering that this metric is very sensitive to shipping and storage conditions; variables that can be difficult to control in an interlaboratory collaborative study. The Tempol normalized T150 values were also much improved from the previous years. It is believed that the improved statistics in the third year were due to the following reasons: samples were all run within a 2- to 3-week period after being received in a frozen or cold state; sample pairs were created from beers of the same type or brand but from different production dates; the two linear regression fit method gave a more accurate lag-time value than the sigmoidal curve estimate.

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TABLE I
Lag Time (min) in Beer Measured by Electron Paramagnetic Resonance

Collaborator (Instrument)	Sample Pair A	Sample Pair B	Sample Pair C	Sample Pair D	Sample Pair E	Sample Pair F	Sample Pair G	Sample Pair H
1 (EMX)	84.9	72.8	55.0	26.4	25.3	36.9	25.2	23.2
2 (e-scan)	82.4	69.0	51.7	33.8	18.9	26.7	16.9	17.8
3 (e-scan)	80.7	88.4	56.7	44.5	16.5	33.9	20.4	19.6
4 (e-scan)	63.6	77.1	46.9	42.2	24.2	31.1	22.3	25.4
5 (EMX)	79.5	81.2	61.1	53.0	26.5	37.9	27.9	30.1
6 (EMX)	81.4	63.3	40.5	30.8	24.4	28.9	28.2	34.8
7 (EMX)	73.3	50.9	40.8	40.6	21.4	27.2	17.8	18.9
8 (e-scan)	75.4	69.1	38.3	34.2	21.9	26.7	15.1	17.1
Mean	77.65	71.48	48.88	38.19	22.39	31.16	21.73	23.36
Grand mean	74.57		43.54		26.78		22.55	

TABLE II
Normalized T150 (%) in Beer Measured by Electron Paramagnetic Resonance

Collaborator (Instrument)	Sample Pair A	Sample Pair B	Sample Pair C	Sample Pair D	Sample Pair E	Sample Pair F	Sample Pair G	Sample Pair H
1 (EMX)	20.4	29.2	46.0	41.1	52.7	43.7	44.7	42.1
2 (e-scan)	23.3	26.6	38.9	50.4	57.3	57.4	59.3	64.3
3 (e-scan)	…[a]	…[a]	24.9	33.6	40.4	36.8	43.5	34.9
4 (e-scan)	30.0	29.2	47.5	55.7	68.2	67.8	73.1	63.4
5 (EMX)	14.9	18.2	33.1	36.8	51.3	47.4	53.9	55.4
6 (EMX)	22.3	23.2	44.0	49.5	70.0	64.2	78.2	71.3
7 (EMX)	…[a]	…[a]	54.0	50.6	61.5	54.9	52.3	46.6
8 (e-scan)	25.0	24.7	42.2	44.2	55.5	55.1	45.0	50.0
Mean[b]	22.65	25.18	41.33	45.24	57.11	53.41	56.25	53.50
Grand mean[b]	23.92		43.29		55.26		54.88	

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

TABLE III
Statistical Summary of Results[a]

Sample Pair	No. of Labs	Grand Mean	Repeatability S_r	Repeatability cv_r	Repeatability r_{95}	Reproducibility S_R	Reproducibility cv_R	Reproducibility R_{95}
Lag time (min)								
A/B	8	74.57	9.00	12.07	25.21	9.41	12.62	26.35
C/D	8	43.54	6.39	14.68	17.90	8.52	19.58	23.87
E/F	8	26.78	3.12	11.67	8.75	4.03	15.05	11.28
G/H	8	22.55	1.84	8.15	5.14	5.74	25.47	16.08
Normalized T150 (%)								
A/B	6	23.92	0.03	10.43	0.08	0.05	19.27	0.13
C/D	8	43.29	0.04	9.49	0.12	0.08	19.33	0.23
E/F	8	55.26	0.02	4.25	0.07	0.10	18.06	0.28
G/H	8	54.88	0.04	7.65	0.19	0.13	23.30	0.36

[a] All calculations were made based on ASBC methods (1).

Method for Reference Standard for Total Package Oxygen

Subcommittee Members: M. Eurich, *Chair*; J. Angres; D. Bendiak; M. Hamilton; K. Malphrus; K. McGivney; A. Porter; V. Weiner; T. Gojanovic (*statistician*); and C. Benedict (*ex officio*).

Keywords: Dissolved oxygen, Oxygen, TPO

CONCLUSIONS

1. Repeatability coefficients of variation for the determination of the 100-μL oxygen spike recovery ranged from 3.8 to 16.2% and were judged unacceptable.
2. Repeatability coefficients of variation for the determination of the 200-μL oxygen spike recovery ranged from 2.7 to 27.4% and were judged unacceptable.

RECOMMENDATIONS

1. Terminate the subcommittee.

This is the sixth year of the subcommittee's existence. Based on a recommendation by the ASBC Technical Committee, this subcommittee was formed to evaluate a method to produce a reference standard for total package oxygen (TPO) analysis. In the first year, a method to produce a reference standard was devised. In the second year, different laboratories measured a single source of canned water and obtained statistically similar results from different TPO instruments. In the third year, the method used with bottled beer produced unacceptable results (1). Based on feedback from members of the subcommittee, a number of modifications to the bottled beer protocol were identified to correct the deficiencies, as well as to improve communication with the collaborators on how to carry out the procedure. The recommended changes to the bottled beer procedure included addition of a procedure to eliminate oxygen in the blank; standardization of the syringe type and needle size; and changes to the volume of the air spikes employed. To improve communication on how the preparation of the spiked samples should be carried out, the subcommittee recommended that a series of photos demonstrating the technique in bottled beer be included with the method. Finally, the committee recommended that the collaborators be advised to practice the spiking procedure in bottled beer prior to beginning the study. In the fourth year, the minimum number of collaborators was not met to perform a statistical analysis of the data.

From the bottled beer method in the fifth year, 17 sets of data were evaluated statistically. Repeatability coefficients of variation from both the 100- and 200-μL oxygen spike levels were judged unacceptable. In the sixth year, the subcommittee opted to change the method from using bottled beer to aluminum canned beer, utilizing hose clamps and septa and then injecting air directly into the can through the septa. The subcommittee chose the aluminum canned beer method to reduce any outside errors inherent with the bottled beer method. Air in both 100- and 200-μL volumes was injected through septa into beer in aluminum cans and analyzed for TPO.

PROCEDURE

Each collaborator was sent a case containing 24, 12-oz aluminum canned beers. The beer was unpasteurized and was required to be aged a minimum of 1 month to ensure all oxygen in the can was converted. Each collaborator was also sent six septa (Restek 11-mm IceBlue) and two hose clamps (2.75-in. size 36). Collaborators were required to procure their own gastight syringe (Hamilton 1700) along with a minimum of six removable needles (Hamilton 0.5 in., 26 gauge, #2 point style). Collaborators were instructed to practice the technique for injecting the can samples with air as outlined in the procedure provided. Following this test method, samples were prepared at the 0, 100-μL spike, and 200-μL spike levels. The prepared samples then were measured for TPO following the manufacturer's instructions for calibration and operation of each collaborator's specific instrument. Results were evaluated using SPSS Inc. software and mixed-effects model ANOVA test criteria.

RESULTS AND DISCUSSION

Results were received from seven collaborators. All collaborators provided results obtained using only the method as described. Results were measured on each collaborator's individual instruments and sampling devices of various models. Data for the mass of oxygen measured and percent recovery at the 0, 100-μL spike, and 200-μL spike levels by each collaborator are presented in Table I.

The statistical summary of the 100- and 200-μL spike level data are shown in Tables II and III, respectively. Percent recovery values were calculated using the calculated mean for the blank results for each collaborator. Repeatability coefficients of variation for the determination of the percent recovery of oxygen addition at the 100-μL injection level ranged from 3.8 to 16.2% and were judged unacceptable. Repeatability coefficients of variation for the percent recovery of oxygen addition at the 200-μL injection level ranged from 2.7 to 27.4% and were judged unacceptable. Based on the reproducibility coefficients of variation shown in Table IV, 17.3 and 17.0% for each collaborator and between collaborators, respectively, would be expected at the 0.05 confidence level. When combined, a reproducibility coefficient of variation of 24.2% would be expected for individual collaborators and between collaborators.

Prior to statistical analysis, data were examined to assess integrity. Two techniques were used during the examination. The first was based on knowledge of the measurement system and physical properties. The second was used to eliminate specific outliers within individual data sets based on comparisons of the average and the median. Because subsequent techniques to find estimated variances for reproducibility and repeatability are based on averages, proper assessment of the data was crucial to assure the data provided representative estimates. Because the average was an estimator of the mean, it was highly unstable in the presence of outliers, even one. The median, in contrast, provided a more robust estimator of location when up to 50% of values that were discrepant before the median were affected. Closeness of agreement between the estimated mean within a lab and within a spike level, and the median integrated with expert knowledge, revealed one outlier, which was removed from the data set.

Estimated marginal means recovery rates refer to the average of multiple readings for each of the collaborators at each of the two levels tested. The term marginal refers to the partition (averages) of the data by collaborator and level. The averages were plotted to graphically depict the measure and magnitude of difference between collaborators based on injection level. The greater the dispersion between collaborators shown in the graph, the greater the collaborator variability. Looking at Figure 1, it can be seen that collaborator four could consistently read between the 100- and 200-μL levels, whereas collaborator six had level-dependent disparate readings. The value of Figure 1 is that it shows precisely which collaborators

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TABLE I
Mass of Oxygen Measured (mg) and Percent Recovery

Collaborator	Blank (mg)	100 µL (mg)	Percent Recovery, 100 µL	200 µL (mg)	Percent Recovery, 200 µL
1	0.001	0.029	103.0	0.057	101.5
1	0.000	0.026	92.3	0.059	105.1
1	0.000	0.027	95.8	0.055	97.9
1	0.000	0.028	99.4	0.057	101.5
1	0.000	0.028	99.4	0.059	105.1
1	0.000	0.029	103.0	0.058	103.3
2	0.003	0.032	109.3	0.056	99.1
2	0.003	0.032	109.3	0.052	91.7
2	0.003	0.031	105.6	0.054	95.4
2	0.002	0.034	116.7	0.049	86.1
2	0.002	0.032	109.3	0.044	76.9
2	0.002	0.035	120.4	0.032[a]	54.6[a]
3	0.000	0.023	104.5	0.042	93.3
3	0.000	0.022	100.0	0.044	97.8
3	0.000	0.024	109.1	0.046	102.2
3	0.000	0.024	109.1	0.046	102.2
3	0.000	0.023	104.5	0.047	104.4
3	0.000	0.022	100.0	0.045	100.0
4	0.004	0.019[a]	60.7[a]	0.043	74.8
4	0.003	0.024	79.3	0.047	82.2
4	0.003	0.026	86.7	0.050	87.8
4	0.002	0.027	90.4	…[b]	…[b]
4	0.001	0.025	83.0	…[b]	…[b]
4	…[b]	0.024	79.3	…[b]	…[b]
5	0.002	0.020	82.4	0.055	116.5
5	0.003	0.021	87.4	0.051	109.0
5	0.002	0.025	105.6	0.053	112.1
5	0.002	0.028	115.6	0.055	117.4
5	0.003	0.028	116.1	0.050	106.3
5	0.002	…[b]	…[b]	0.052	110.7
6	0.000	0.011[a]	39.3[a]	0.036	65.5
6	0.000	0.011[a]	39.3[a]	0.040	72.7
6	0.000	0.008[a]	28.6[a]	0.056	101.8
6	0.000	0.017[a]	60.7[a]	0.064	116.4
6	0.000	0.014[a]	50.0[a]	0.047	85.5
6	0.000	0.008[a]	28.6[a]	0.031	56.4
7	0.012	0.046	125.6	0.050[a]	68.7[a]
7	0.011	0.041	107.7	0.047[a]	63.5[a]
7	0.011	0.039	100.6	0.053[a]	74.0[a]
7	0.011	0.037	93.5	0.052[a]	72.2[a]
7	0.011	0.036	89.9	0.048[a]	65.2[a]
7	0.009	0.033	79.2	0.048[a]	65.2[a]

[a] Data not used.
[b] Data not provided.

were farthest from the others and which ones could determine recovery rates accurately at either the 100- or 200-µL level. The graph provides immediate and clear details on the magnitude of the variability. Figure 1 also shows precisely which collaborators fell outside the generally accepted values, which were 0.80 to 1.20.

The following sources most likely caused the results of this method to be unacceptable: instrument-to-instrument variability, including calibration and/or maintenance requirements; destruction of needles due to gauge size; and puncturing of the sidewall of the can as part of the technique.

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TABLE II
Statistical Summary of 100-µL Injection Results

Collaborator	Valid *N*	Mean	Median	Min	Max	*S*	*cv*
1	6	0.99	0.99	0.92	1.03	0.04	4.0
2	6	1.12	1.07	1.06	1.20	0.06	5.4
3	6	1.05	1.05	1.00	1.09	0.04	3.8
4	5	0.84	0.83	0.79	0.90	0.05	6.0
5	5	1.01	1.06	0.82	1.16	0.16	15.8
6[a]	6	0.41	0.39	0.29	0.61	0.13	31.7
7	6	0.99	0.97	0.79	1.26	0.16	16.2

[a] Data not used.

TABLE III
Statistical Summary of 200-µL Injection Results

Collaborator	Valid *N*	Mean	Median	Min	Max	*S*	*cv*
1	6	1.02	1.02	0.98	1.05	0.03	2.7
2	5	0.90	0.93	0.77	0.99	0.09	10.0
3	6	1.00	1.01	0.93	1.04	0.04	4.0
4	3	0.82	0.82	0.75	0.88	0.07	8.0
5	6	1.12	1.11	1.06	1.17	0.04	3.9
6	6	0.83	0.79	0.56	1.16	0.23	27.4
7[a]	6	0.68	0.67	0.63	0.74	0.04	6.2

[a] Data not used.

TABLE IV
Precision Within and Between Collaborators[a]

Term	Variance S^2	cv_r (%)
S_r^2	0.025	17.3
S_b^2	0.024	17.0
$S_R^2 = (S_r^2 + S_b^2)$	0.049	24.2
Grand mean = 0.912		
$n = 79$		

[a] Calculations based on Tables II and III.

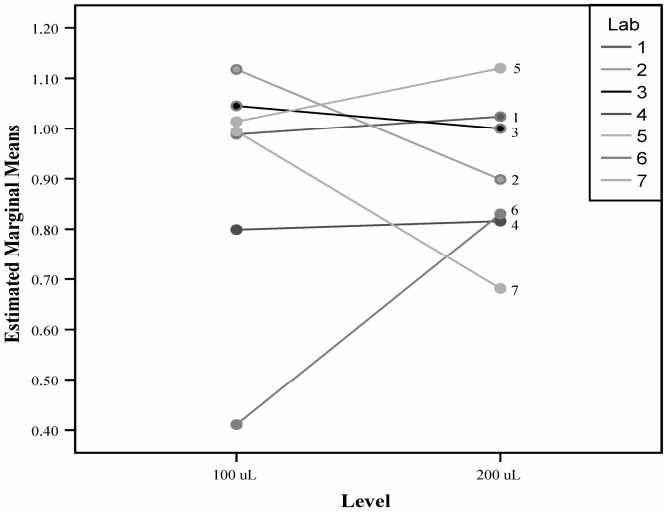

Fig. 1. Estimated marginal mean of recovery for each collaborator. No outliers were statistically identified.

TBA Test as an Indicator for Flavour Stability: Thiobarbituric Acid Index for Wort and Beer

Subcommittee Members: K. McGivney, *Chair*; S. Abishek; J. Mellem; R. Ortiz; J. Palausky; and K. Lakenburges (*ex officio*).

Keywords: TBI, TBZ, Thermal stress

CONCLUSIONS

1. Repeatability coefficients of variation for the determination of the thiobarbituric acid index (TBI) in wort and beer samples ranged from 3.5 to 6.7% and were judged acceptable.
2. Reproducibility coefficients of variation for the determination of the TBI in wort and beer samples ranged from 5.5 to 10.5% and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends that the method be included in the *Methods of Analysis*.
2. Discharge the subcommittee.

This is the subcommittee's second year of existence. In its first year, discussion among the subcommittee members revealed experience with two methods—the MEBAK method and a method published in the *ASBC Journal* in 1976 (2–4). The MEBAK method for TBI was recommended for collaborative testing. The method could be used to monitor the effects of process differences on worts and/or beers. Thermal stress during the brewing process has been linked in the brewing literature to oxidation in the finished product. Therefore, monitoring of thermal stress during start-up or throughout procedural or equipment changes could be of benefit to brewers.

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PROCEDURE

Six sample pairs (three sample pairs of wort and three sample pairs of finished beer) were sent to each collaborator. Each sample pair was of the same brand but from different production times. They were selected to cover a wide range of TBI values and represented the following product types: light, amber, and dark beers and worts. Results were evaluated using the Youden unit block design (1).

RESULTS AND DISCUSSION

Results from six collaborators were received for the six sample pairs. TBI data received are presented in Table I. Sample pairs A/B, C/D, and E/F were dark, amber, and light colored worts, respectively. Sample pairs G/H, I/J, and K/L were dark, amber, and light colored beers, respectively. All beers were made from their corresponding worts (e.g., dark colored beer was made from dark colored wort) after dilution.

The statistical summary of the TBI data is presented in Table II. Repeatability coefficients of variation for the determination of TBI in wort and beer samples ranged from 3.4 to 6.6% and were judged acceptable. Reproducibility coefficients of variation for the determination of TBI in wort and beer samples ranged from 5.5 to 10.5% and were judged acceptable.

TABLE I
Thiobarbituric Acid Index Values for Wort and Beer

	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	164.3	156	110	111	54	53	87	96	80	79	26	32
2	167	191	129	119	62	54	91	97	80	81	27	34
3	172	183	113	115	56	57	120	116	86	85	26	30
4	163	168	118	115	54	54	96	104	76	78	23	30
5	174	167	121	104	57	51	110	110	81	80	27	30
6	146	176	116	123	49	53	110	112	69	83	26	34
Mean	164.3	173.5	117.8	114.5	55.3	53.7	102.3	105.8	78.7	81.0	25.8	31.7
Grand mean	168.9		116.2		54.5		104.1		79.8		28.8	

TABLE II
Statistical Summary of Results[a]

		Repeatability			Reproducibility		
Sample Pair	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Wort							
A/B	168.9	11.3	6.7	31.7	11.4	6.8	32.0
C/D	116.2	5.9	5.1	16.7	6.4	5.5	18.0
E/F	54.5	3.0	5.5	8.5	3.2	5.9	9.0
Beer							
G/H	104.1	3.6	3.5	10.0	10.9	10.5	30.6
I/J	79.8	4.1	5.2	11.6	4.4	5.6	12.4
K/L	28.8	1.4	4.8	3.8	1.7	6.0	4.9

[a] All calculations were made based on ASBC methods (1). Six labs provided results.

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PCR Applications to Brewing: Differentiation of Brewing Yeast Strains by PCR Fingerprinting

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Keywords: Interdelta sequences, PCR, Polymerase chain reaction, Yeast identification

CONCLUSIONS

1. Four brewing yeast strains (two ales and two lagers) were analyzed as blind samples by polymerase chain reaction (PCR) fingerprinting using primers targeting interdelta regions of the genome. Each strain was identified by comparison with a corresponding control yeast.
2. In each instance, the PCR fingerprints obtained for identical strains within each laboratory were the same, indicating suitable repeatability of the method.
3. It is well known that due to variations in PCR reagents and laboratory equipment, some PCR fingerprinting methods do not produce identical results when performed in different laboratories. Consequently, interlaboratory reproducibility was not determined.

RECOMMENDATIONS

1. The use of PCR fingerprinting in conjunction with primers targeting interdelta sequences was demonstrated to be a suitable method for the differentiation and identification of brewing yeast strains.
2. Despite the fact that PCR fingerprinting methods are known to produce variable results between laboratories, it is proposed that the method described here should be approved for inclusion in the *Methods of Analysis* for the analysis and identification of brewing strains within a laboratory.
3. Discharge the subcommittee.

This was the second year for the subcommittee, which was formed to investigate the application of PCR techniques within breweries. Currently, there are no ASBC recommended methods that utilize PCR technology, even though brewery laboratories are increasingly equipped with either standard or real-time PCR thermocyclers. Although PCR can be used as a tool to detect and identify a range of microbes associated with the brewing industry (e.g., wild yeast and bacterial contaminants), a simple application for this technology is for the analysis of production strains. The ability to identify and differentiate between production yeasts is particularly useful when a number of different strains is employed within a brewery. In the subcommittee's first year, preliminary robustness trials were performed by the ex officio to differentiate 15 brewing yeast strains by PCR fingerprinting in conjunction with primers targeting interdelta sequences (1–3). In the second year of the subcommittee, collaborative testing was performed to assess the intralaboratory repeatability of the method.

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PROCEDURE

Eleven laboratories, with a total of twelve individual collaborators, participated in the subcommittee collaborative study. Four yeast strains (two ales designated A and B and two lagers designated C and D) were sent to each collaborator as reference samples alongside a set of blind samples designated 1, 2, 3, and 4. DNA from each sample was extracted and subjected to PCR using primers corresponding to sequences within interdelta regions of the genome (1–3). The resulting DNA amplicons were separated by gel electrophoresis to produce a DNA fingerprint. Each PCR reaction was obtained in duplicate unless otherwise stated. The size of each amplicon was determined by referring to a DNA ladder that was included on each gel. The ladder utilized was composed of DNA fragments at 100-bp increments, which were well separated up to 1,500 bp under the electrophoresis conditions specified. This particular ladder was employed because it previously was demonstrated that the majority of fragments obtained using interdelta primers are within this size range (2). Only amplicons between 100 and 1,500 bp, with a 25-bp increment, were considered to facilitate data interpretation.

RESULTS AND DISCUSSION

Results from 12 collaborators were received for each set of strains investigated. Each strain was analyzed in duplicate unless otherwise stated. Duplicate samples were composed of DNA taken from a single extraction and were subsequently processed at the same time in the PCR machine but in two separate reaction tubes. All collaborators obtained clear genetic profiles that varied between strains in terms of the number and size of the amplicons (Tables I–IV; Figures 1–12). Each collaborator successfully matched the four blind samples with the four reference strains as A-4, B-3, C-2, and D-1. One exception was observed for collaborator 11, who paired strain B with strain 2 and strain C with strain 3. The reason for this discrepancy is not known; however, since each of the four yeasts was clearly differentiated, albeit paired wrongly, it is suggested that it may have been an issue with the labeling of strains either at the source or within the collaborator's laboratory. Each collaborator reported that no amplification was observed in negative controls (blank samples), indicating that the amplified DNA fragments observed in the samples originated from yeast DNA and not from a foreign source.

Although collaborators often reported that the size of certain DNA fragments were common to the four strains examined, the presence of unique amplicons frequently allowed direct differentiation of each yeast (highlighted in bold in Tables I–IV). Strains analyzed by collaborators 1, 2, 3, 4, 5, 6, 7, 10, 11, and 12 displayed unique amplicons (Table I–IV) and, therefore, were readily differentiated. For collaborator 8, strains D-1 and A-4 did not show specific amplicons that were unique to a particular strain; however, the yeasts could be differentiated based on the analysis of each fingerprint as a whole. For example, strain D-1 displayed a band at 550 bp that was not present in A-4 (Table III). Similarly, for collaborator 9, strains B-3 and D-1 could be differentiated by two amplicons at 1,100 and 1,300 bp that were present in the former but not in the latter (Table III).

A comparison of the profiles obtained for blind and control yeasts indicated that fingerprints were identical in terms of the number and size of amplicons for collaborators 1, 3, 7, 8, 9, 10, 11, and 12

(Figs. 1, 3, 7, 8, 9, 10, 11, and 12, respectively). An exception was observed for collaborator 2, who did not obtain a complete profile for sample D; bands at 1,200 and 1,300 bp were missing when compared with blind sample 1 (Fig. 2). Given that the PCR reagents were identical for strain D and strain 1 duplicates and that the only difference between each set was the DNA extraction, it is suggested that this may have been the cause of the error, particularly because both strain D profiles were derived from identical DNA. Because fragments of high molecular weight require DNA polymerase to work for a longer time during synthesis, it is possible that this activity may have been influenced by the purity and quality of the extracted DNA. In this instance, the DNA extraction of sample D may not have been optimal and may have resulted in the differences observed. Collaborator 4 obtained a paler profile for one sample of strain 1 (Fig. 4), possibly due to an inaccurate volume of DNA being loaded into the agarose gel. This highlights the requirement for precision, especially when pipetting, to achieve reproducibility during PCR and subsequent processing. Collaborator 5 reported that strain A exhibited a slightly different profile than strain 4, with a missing amplicon at 1,000 bp (Fig. 5). Given that duplicate samples were not produced, the precise reasons for these differences are not known; however, all of the genetic profiles produced from this collaborator had only small numbers of amplicons, suggesting that in this instance the PCR reaction may need to be optimized for the particular instrument and working conditions. Finally, collaborator 6 did not obtain a profile for reference sample C (Fig. 6). It is possible that this occurred due to operator error and that the DNA had not been added to the PCR sample.

It should be noted that although data were comparable within each laboratory the profiles of each strain were typically observed to differ between collaborators. This was not unexpected, because the efficiency of the PCR annealing and extension processes are known to depend on the thermocycler employed (e.g., ramping time) and laboratory environment (e.g., temperature and humidity) (4). In addition, although specific PCR reagents were recommended some collaborators used components from their usual supplier rather than those suggested by the subcommittee chair due to lack of availability in certain regions. Variation between different manufacturers and

TABLE I
Size and Number of Amplicons (bp) for Strains A-4, B-3, C-2, and D-1 for Collaborators 1, 2, and 3[a]

Collaborator 1				Collaborator 2				Collaborator 3			
A-4	**B-3**	**C-2**	**D-1**	**A-4**	**B-3**	**C-2**	**D-1**	**A-4**	**B-3**	**C-2**	**D-1**
100	200	200	200	**100**	200	**150**	200	125	100	100	125
125	**225**	350	250	**125**	350	200	250	150	150	150	150
200	350	**375**	300	200	500	350	300	275	200	**175**	200
250	475	475	450	250	**525**	**375**	450	300	**225**	200	275
300	500	500	475	300	600	450	500	350	275	300	300
450	600	**675**	500	450	700	500	600	475	350	350	350
600	700	700	600	**625**	**725**	700	1,000	525	475	**375**	475
700	**725**	900	700	700	1,000	**900**	1,100	600	525	475	500
925	950	975	975	950	1,100	950	1,250	725	600	525	525
950	1,075	1,000	1,000	1,000	1,200	1,000	1,300	800	**775**	600	725
1,000	1,250	**1,125**	1,075	1,100	1,300	1,200		950	800	725	800
1,075	1,300	**1,150**	1,250	1,200	1,400	1,300		1,025	875	800	950
1,250	1,400	1,300	1,300	1,250	1,500	1,400		1,100	950	875	1,025
1,300	1,500	**1,350**				1,500		1,200	1,100	**925**	1,100
		1,400						1,300	1,200	**1,050**	1,200
								1,400	1,300	**1,150**	1,300
									1,400	1,300	1,400
									1,500	1,400	

[a] If the number of amplicons differed between replicates of the same strain, the fragments reported reflect those from the most complete genetic profile obtained.

TABLE II
Size and Number of Amplicons (bp) for Strains A-4, B-3, C-2, and D-1 for Collaborators 4, 5, and 6[a]

Collaborator 4				Collaborator 5				Collaborator 6			
A-4	**B-3**	**C-2**	**D-1**	**A-4**	**B-3**	**C-2**	**D-1**	**A-4**	**B-3**	**C-2**	**D-1**
125	150	150	125	175	225	175	**150**	150	150	150	150
150	200	200	150	250	**350**	225	250	275	200	200	200
275	350	**375**	200	**325**	500	**375**	475	350	375	**400**	275
300	450	**400**	**225**	475	**650**	475	**1,000**	375	425	425	**325**
350	475	475	300		1,300	500	1,300	425	450	450	350
450	525	500	**325**		1,400	**900**		450	500	**475**	375
475	600	600	450		1,500	1,300		500	**600**	**700**	425
525	725	725	475			1,400		625	725	725	450
725	800	800	500					725	800	800	500
800	850	850	525					800	825	850	625
850	1,000	1,000	725					825	975	975	725
975	1,125	1,200	800					950	1,100	**1,125**	800
1,050	1,200	1,350	975					1,000	1,300	1,300	850
1,125	1,350	1,450	1,050					1,100			950
1,200	1,450		1,125					1,250			1,000
1,350	1,500		1,350					1,300			1,100
1,450			1,450					1,400			**1,150**
											1,300
											1,400

[a] If the number of amplicons differed between replicates of the same strain, the fragments reported reflect those from the most complete genetic profile obtained.

TABLE III
Size and Number of Amplicons (bp) for Strains A-4, B-3, C-2, and D-1 for Collaborators 7, 8, and 9[a]

Collaborator 7				Collaborator 8				Collaborator 9			
A-4	**B-3**	**C-2**	**D-1**	**A-4**	**B-3**	**C-2**	**D-1**	**A-4**	**B-3**	**C-2**	**D-1**
150	200	225	200	100	**250**	100	100	110	100	125	110
275	225	**375**	225	300	400	**275**	300	125	150	150	125
300	350	**400**	275	500	550	400	500	150	175	175	150
350	425	450	300	750	**650**	500	550	250	200	215	200
425	450	475	**325**	800	800	**700**	750	300	215	**275**	215
450	475	500	450	1,000	1,300	**900**	800	325	300	300	250
500	500	600	475	1,300	**1,500**	1,300	1,000	350	325	325	300
750	600	750	500			**1,400**	1,300	425	350	350	325
825	750	825	750					450	425	**375**	350
950	**800**	900	825					500	450	425	425
1,000	825	1,100	950					700	475	450	450
1,050	900	1,300	1,000					775	500	475	475
1,100	1,000	1,450	1,050					900	575	**485**	500
1,300	1,050	1,500	1,100					950	700	500	700
	1,100		1,300					**1,050**	725	575	775
	1,300							1,200	775	700	900
	1,450								800	725	950
	1,500								850	775	1,200
									1,100	800	
									1,200	850	
									1,300	1,100	
										1,200	
										1,300	

[a] If the number of amplicons differed between replicates of the same strain, the fragments reported reflect those from the most complete genetic profile obtained.

TABLE IV
Size and Number of Amplicons (bp) for Strains A-4, B-3, C-2, and D-1 for Collaborators 10, 11, and 12[a]

Collaborator 10				Collaborator 11				Collaborator 12			
A-4	**B-3**	**C-2**	**D-1**	**A-4**	**B-2**	**C-3**	**D-1**	**A-4**	**B-3**	**C-2**	**D-1**
100	100	100	100	450	**400**	**300**	450	100	300	100	100
175	150	150	**125**	600	**550**	**500**	600	450	**325**	350	300
275	200	225	150	1,000	**650**	700	700	500	350	700	450
300	375	**400**	200	1,350	**850**	1,200	**800**	600	**625**	**750**	500
375	425	425	300		1,000	1,350	**900**	800	**650**	900	600
425	475	475	375		1,250		1,000	900	800	1,100	900
475	550	550	425				**1,200**	1,200	900	1,200	1,100
550	**600**	750	475				1,350	**1,300**	**1,000**		1,200
800	800	800	**500**						1,100		
825	825	850	550						1,200		
850	850	1,300	750						**1,500**		
950	1,300	**1,400**	800								
1,000			825								
1,300			950								
			1,000								
			1,300								

[a] If the number of amplicons differed between replicates of the same strain, the fragments reported reflect those from the most complete genetic profile obtained.

batch variation for identical products may also have led to differences in PCR fingerprinting. Such variation can be observed by analyzing each set of profiles. For example, the profiles obtained using strain A-4 varied from 4 to 17 amplicons, strain B-3 from 6 to 21 amplicons, strain C-2 from 5 to 23 amplicons, and strain D-1 from 5 to 19 amplicons. Collaborators 5, 8, and 11 consistently observed a lower number of amplicons than the other collaborators (Table II, III, and IV, respectively), indicating that PCR or process optimization may need to be performed according to the respective equipment and reagents used. However, it should be stressed that even when few amplicons were observed, differentiation of strains was still achievable. Interestingly, collaborator 9 observed the highest number of fragments (Table III), suggesting either lower primer specificity or conditions favoring primer binding and extension in this laboratory.

Despite the discrepancies between blind and reference samples reported above, as stated previously, each blind sample was correctly identified within the participating laboratories. It should also be noted that each collaborator performed the analysis of reference and blind samples in duplicate, except for collaborators 3 and 5, who provided one set of profiles only. In each instance, genetic profiles were identical between duplicates, further indicating the repeatability of the PCR method within laboratories.

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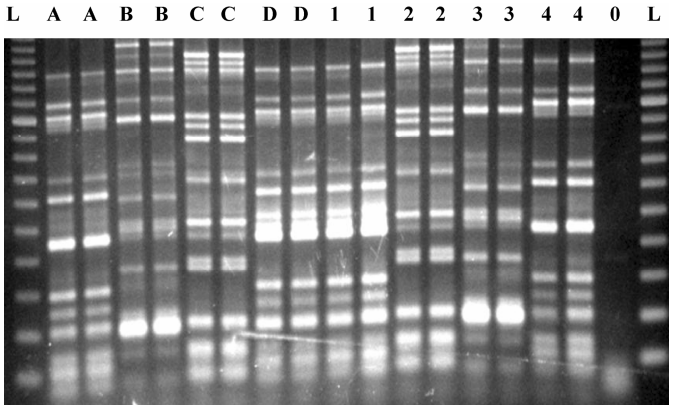

Fig. 1. PCR analysis of strains by collaborator 1. DNA ladders (L), with 100-bp increments, were used to estimate the size of the amplicons. Reference samples (designated A–D) were used to determine the identity of four blind samples (designated 1–4). A PCR blank (0) was also produced as a negative control.

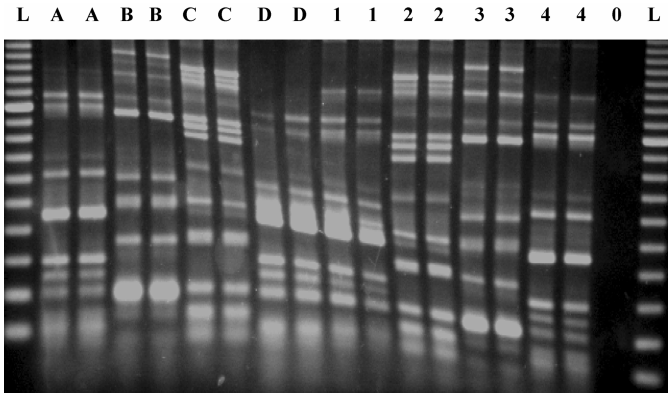

Fig. 2. PCR analysis of strains by collaborator 2. DNA ladders (L), with 100-bp increments, were used to estimate the size of the amplicons. Reference samples (designated A–D) were used to determine the identity of four blind samples (designated 1–4). A PCR blank (0) was also produced as a negative control.

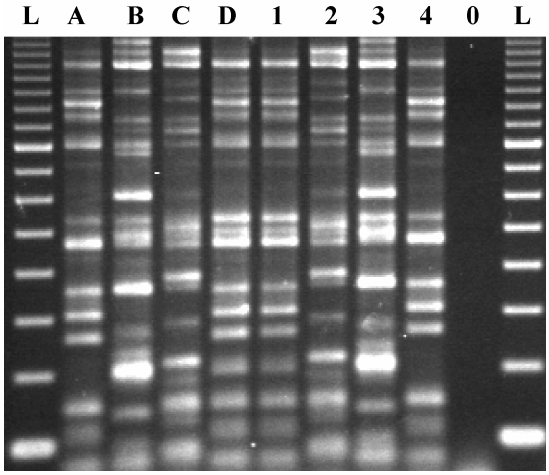

Fig. 3. PCR analysis of strains by collaborator 3. DNA ladders (L), with 100-bp increments, were used to estimate the size of the amplicons. Reference samples (designated A–D) were used to determine the identity of four blind samples (designated 1–4). A PCR blank (0) was also produced as a negative control.

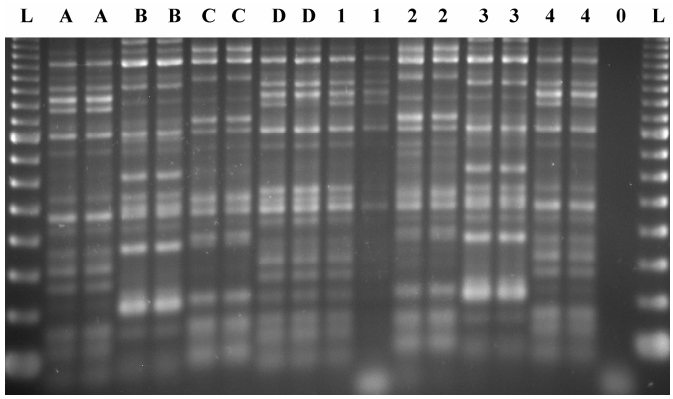

Fig. 4. PCR analysis of strains by collaborator 4. DNA ladders (L), with 100-bp increments, were used to estimate the size of the amplicons. Reference samples (designated A–D) were used to determine the identity of four blind samples (designated 1–4). A PCR blank (0) was also produced as a negative control.

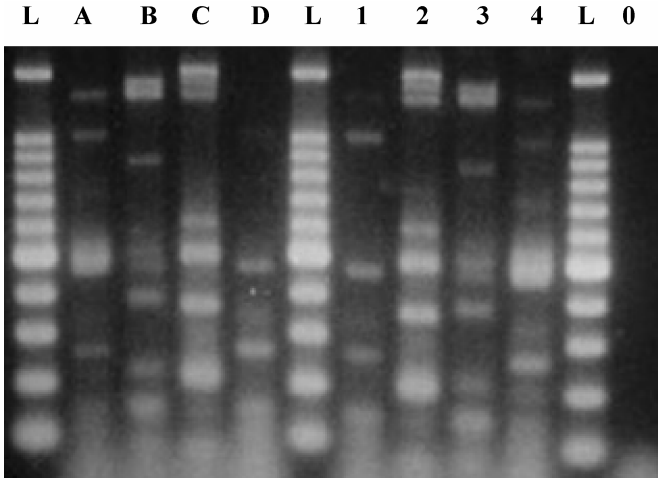

Fig. 5. PCR analysis of strains by collaborator 5. DNA ladders (L), with 100-bp increments, were used to estimate the size of the amplicons. Reference samples (designated A–D) were used to determine the identity of four blind samples (designated 1–4). A PCR blank (0) was also produced as a negative control.

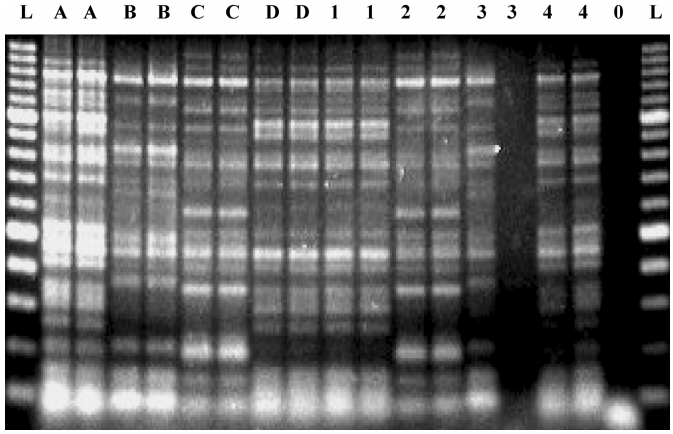

Fig. 6. PCR analysis of strains by collaborator 6. DNA ladders (L), with 100-bp increments, were used to estimate the size of the amplicons. Reference samples (designated A–D) were used to determine the identity of four blind samples (designated 1–4). A PCR blank (0) was also produced as a negative control.

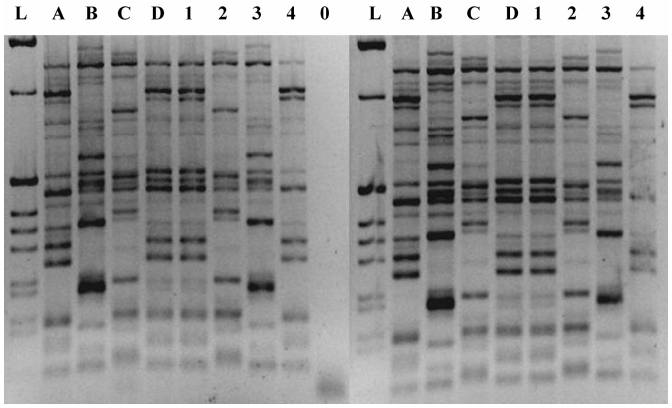

Fig. 7. PCR analysis of strains by collaborator 7. DNA ladders (L) were used to estimate the size of amplicons. The ladders were composed of 10 bands of 135, 155, 200, 220, 300, 350, 400, 500, 1,000, and 1,600 bp. Reference samples (designated A–D) were used to determine the identity of four blind samples (designated 1–4). A PCR blank (0) was also produced as a negative control.

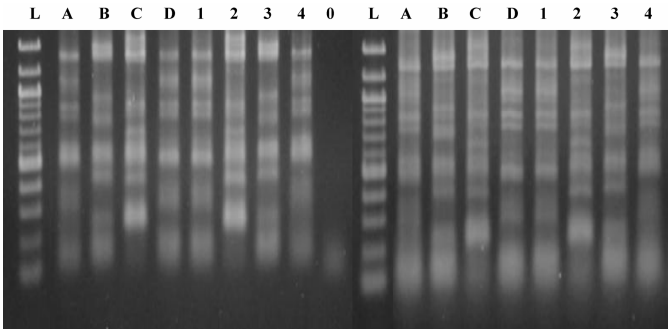

Fig. 8. PCR analysis of strains by collaborator 8. DNA ladders (L), with 100-bp increments, were used to estimate the size of the amplicons. Reference samples (designated A–D) were used to determine the identity of four blind samples (designated 1–4). A PCR blank (0) was also produced as a negative control.

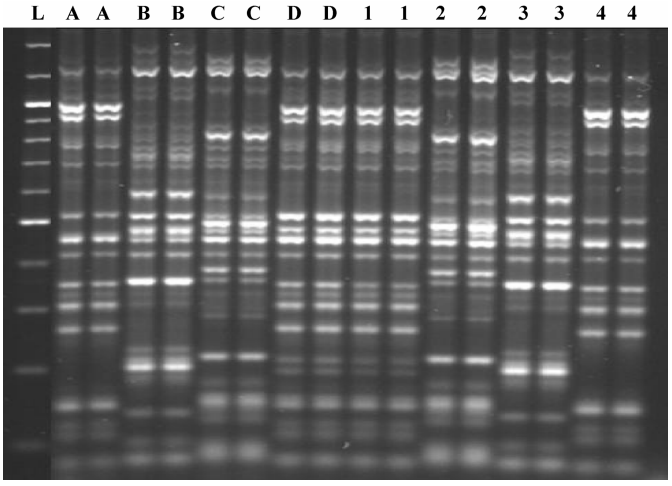

Fig. 9. PCR analysis of strains by collaborator 9. A DNA ladder (L), with 100-bp increments, was used to estimate the size of the amplicons. Reference samples (designated A–D) were used to determine the identity of four blind samples (designated 1–4).

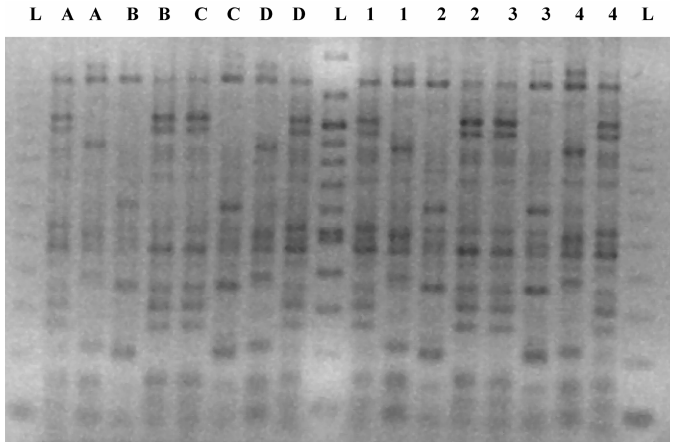

Fig. 10. PCR analysis of strains by collaborator 10. DNA ladders (L), with 100-bp increments, were used to estimate the size of the amplicons. Reference samples (designated A–D) were used to determine the identity of four blind samples (designated 1–4).

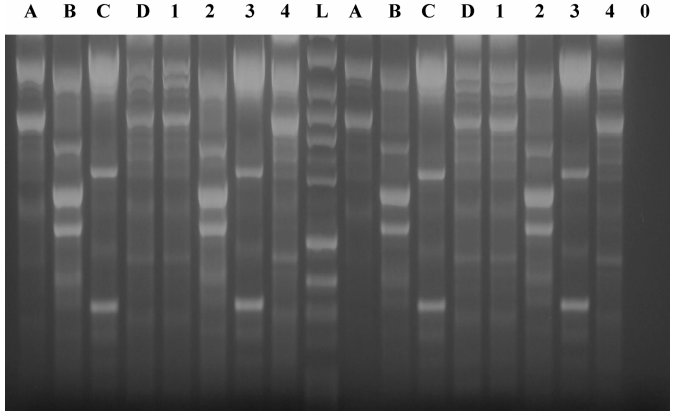

Fig. 11. PCR analysis of strains by collaborator 11. A DNA ladder (L), with 100-bp increments, was used to estimate the size of the amplicons. Reference samples (designated A–D) were used to determine the identity of four blind samples (designated 1–4). A PCR blank (0) was also produced as a negative control.

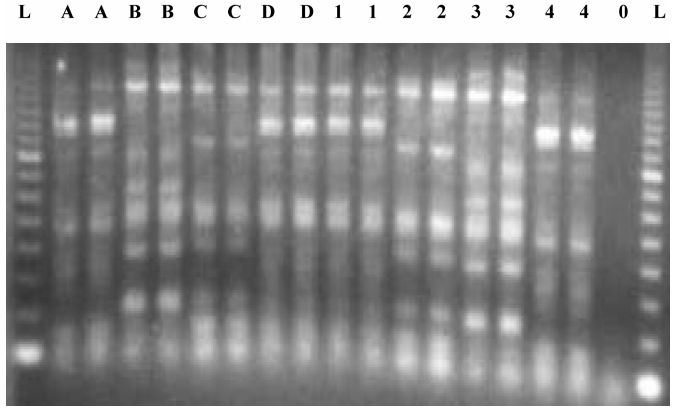

Fig. 12. PCR analysis of strains by collaborator 12. DNA ladders (L), with 100-bp increments, were used to estimate the size of the amplicons. Reference samples (designated A–D) were used to determine the identity of four blind samples (designated 1–4). A PCR blank (0) was also produced as a negative control.

Report of 2007 BCOJ Collaborative Work

Foam Stability of Beer Using the NIBEM-T Meter

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Keywords: Foam stability, NIBEM-T

CONCLUSIONS

1. The repeatability relative standard deviation (RSD_r) and repeatability (r_{95}) for the determination of foam stability by the NIBEM-T meter ranged from 1.05 to 1.91% and from 7.48 to 12.58%, respectively, and were judged acceptable.
2. The reproducibility relative standard deviation (RSD_R) and reproducibility (R_{95}) for the determination of foam stability by the NIBEM-T meter ranged from 1.79 to 4.13% and from 12.23 to 27.18%, respectively, and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends that the method for the determination of the foam stability of beer, *happoshu*, and the third category beer by the NIBEM-T meter should be included in the *Methods of Analysis of the BCOJ*.
2. Discharge the subcommittee.

The NIBEM-T meter (Haffmans B.V., Holland) is an apparatus with which the stability of beer foam is evaluated by measuring the time intervals in which the foam surface collapses over determined distances. The NIBEM-T meter measures the average temperature of the foam and compensates for the effect of temperature to exclude this external factor that affects beer foam stability.

In 1997, the EBC Analysis Committee conducted trials on the NIBEM method, which is the previous model, but the method was not accepted due to the low precision figures obtained. A new collaborative test was carried out to compare the new NIBEM-T meter with the old NIBEM meter in 2002, and the EBC Analysis Committee included the determination of the foam stability of beer using the NIBEM-T meter in *Analytica–EBC* because repeatability and reproducibility for the NIBEM-T method were judged acceptable (1).

In this trial, repeatability and reproducibility were calculated after temperature compensation only when the temperature did not fall within the specified range (20.0 ± 0.5°C). This procedure was carried out based on self-constructed calibration curves, without using the automatic compensation functions of the NIBEM-T meter.

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PROCEDURE

Two samples of five different types of beer, *happoshu*, and the third category beer, packaged without nitrogen, with CO_2 levels greater than 3.4 g/L, were sent to each collaborator. The samples were selected as follows: sparkling alcoholic beverage brewed without malt (the third category beer) (A), *happoshu* (B), pilsner beer with adjunct (C), all malt beer (D), and dark beer (E).

The NIBEM-T meter was calibrated, and the standard glass was cleaned according to the manufacturer's instruction manual. After the samples were adjusted to a temperature of 20.0 ± 0.5°C, the foam stability was analyzed by the NIBEM-T method at a temperature of 20.0 ± 0.5°C.

The five types of beer samples were measured at temperatures of 15, 20, and 25°C, and the calibration curves for temperature compensation were prepared. If the sample temperature did not fall within the specified range, those data were compensated using the calibration curves.

The results were evaluated according to the collaborative tests for the determination of repeatability and reproducibility in the *Methods of Analysis of the BCOJ* (2).

RESULTS AND DISCUSSION

Beer foam stability (seconds) was evaluated by measuring the collapse time over a distance of 30 mm from a set position. The data obtained outside the specified temperature range (20.0 ± 0.5°C) were compensated using the calibration curves and adjusted to the values corresponding to those acquired at 20°C (Table I). Graphic consistency testing using Mandel's *h* and *k* statistics was used in the statistical evaluation of the data to identify outliers. No outlier was identified using the Cochran's test and Grubbs test. The precision data are summarized in Table II. The repeatability relative

TABLE I
Beer Foam Stability Measured by the NIBEM-T Method (sec)

Collaborator	Sample A		Sample B		Sample C		Sample D		Sample E	
1	177	183	239	237	267	255	265	267	275	273
2	195	195	249	251	279	280	288	292	301	291
3	188	199	240	246	272	276	282	281	286	283
4	190[a]	190[a]	250[a]	250[a]	273[a]	278[a]	278[a]	280[a]	285[a]	281[a]
5	172[a]	165[a]	249[a]	241	275[a]	271	276[a]	276[a]	276[a]	274
6	183	190	243	240	270	282	270	279	291	286
7	184	187	241	244	283	277	287	287	292	292
8	183	183	242	247	265	268	267	266	277	274
9	186	185	246	247	286	292	288	291	299	300
10	179	182	238	239	284	283	287	279	299	292
11	185	184	243	245	286	282	291	288	299	293

[a] The sample temperature did not fall within the specified range, and the data were compensated using self-constructed calibration curves.

TABLE II
Summary of Precision Data

	Sample A	Sample B	Sample C	Sample D	Sample E
No. of labs	11	11	11	11	11
Mean	185	244	277	280	287
s_r	3.54	2.67	4.49	2.93	3.39
RSD_r (%)	1.91	1.10	1.62	1.05	1.18
r_{95}	9.90	7.48	12.58	8.21	9.50
s_r	7.64	4.37	8.62	8.99	9.71
RSD_R (%)	4.13	1.79	3.12	3.21	3.38
R_{95}	21.39	12.23	24.15	25.18	27.18

standard deviation (RSD_r) and repeatability (r_{95}) for the determination of foam stability by the NIBEM-T meter ranged from 1.05 to 1.91% and from 7.48 to 12.58%, respectively, and the reproducibility was comparable to that of the collaborative trial conducted by the EBC Analysis Committee (r_{95} = 11.17) (1). The reproducibility relative standard deviation (RSD_R) and reproducibility (R_{95}) for the determination of foam stability by the NIBEM-T meter ranged from 1.79 to 4.13% and from 12.23 to 27.18%, respectively, and the reproducibility was comparable to that of the collaborative trial conducted by the EBC Analysis Committee (R_{95} = 22.18) (1).

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