Technical Committee and Subcommittee Reports

2006–2007 Report of the Technical Committee

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

Activity in 12 subcommittees was conducted by the Technical Committee and Subcommittee chairs during 2006–2007. As a result, one method is being recommended for inclusion in the *Methods of Analysis* (MOA): Determination of Alpha-Amylase by Automated Flow Analysis, chaired by Jolanta Menert (Anheuser-Busch). Four methods will continue for another year of collaborative study: Can Packaging Methods, chaired by Scott Brendecke (Ball Corp.); Method for Measurement of Resistance of Oxidation in Beer by Electron Paramagnetic Resonance, chaired by David Barr (Bruker BioSpin); Method for Reference Standard for Total Packaged Oxygen, chaired by Mark Eurich (Coors Brewing Company); and TBA Test as an Indicator for Flavor Stability, chaired by Karl Lakenburges (Anheuser-Busch). In 2007–2008, Katie McGivney (New Belgium Brewing Company) will assume the role of chair for this subcommittee, with Karl Lakenburges remaining as *ex-officio*.

The collaborative study PCR Applications to Brewing, chaired by Kelly Tretter (Coors Brewing Company), was not initiated as planned in 2006–2007, and thus, a final report could not be written. The chair has since stepped down, and we are in the process of seeking a replacement.

Two new standing subcommittees were initiated in 2006–2007: MOA Methods Review, chaired by Bob Foster (Coors Brewing Company); and Sensory Science, chaired by Suzanne Thompson (Miller Brewing Company). Bob and his subcommittee have successfully completed their review of the Hops section of the *Methods of Analysis* and will pass their recommendations on to the Technical and Publications Committees. Sue has assembled her subcommittee, conducted monthly conference calls and a needs and opportunities analysis, and prioritized areas of focus going forward. Congratulations are in order to both Bob and Sue for successfully launching these new initiatives this past year and demonstrating significant progress. Bob and Sue will continue as chairs in 2007–2008.

As in previous years, the following four standing subcommittees continued activities in 2006–2007: Soluble Starch, chaired by Karen Churchill (Prairie Malting Ltd.); New and Alternate Methods of Analysis, chaired by Jeff Cornell (Coors Brewing Company); International Methods, chaired by David Maradyn (InBev nv/sa); and Craft Brewers, chaired by Gina Kelly (New Belgium Brewing Company). Karen and Jeff are both new to the role of chair for their respective subcommittees and will be continuing their leadership in 2007–2008. Gina has completed her second year as chair of the Craft Brewers subcommittee and will continue in that role for 2007–2008.

The Check Sample Service Program continued under the stewardship of Rob McCaig (Canadian Malting Barley Technical Centre) in 2006–2007. With the goal of maximizing value to current subscribers and attracting potential new subscribers, the focus this year has been on preparing for the transition from mailed to online data reporting and the launch of the Craft Brewers Beer Check Service. Scientific Societies has been working with AACC International for the past two years regarding the specifics for an online reporting tool, and we plan to adopt their system once implemented. The Craft Brewers Beer Check Service was officially launched in the fall of 2006. The number of subscribers to the service has been below expectations, but strategies to increase membership are being investigated in conjunction with the Craft Brewers Subcommittee. Rob stepped down as director of the Check Sample Service Program at the conclusion of the Victoria meeting, having served his 3-year term. We are currently looking for Rob's replacement. I, along with the Technical Committee, would like to thank Rob for his service to the Society as director of the Check Sample Service for the past three years.

Tim Moore (Scientific Societies), Stephen Kenny (Washington State University), and John Barr (North Dakota State University) 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!

Special recognition goes to the subcommittee chairs for their hard work and dedication in conducting their collaborative studies throughout the past year. I hope that this experience has been rewarding for them from both a personal and professional point of view. I would also like to recognize the many members who participated on the various subcommittees that were active during the past year. As the trends of consolidation, downsizing, and focusing on efficiencies continue in the brewing industry, many companies are working with technical staffs that are greatly reduced from even five years ago. Thus, many subcommittee members are doing collaborative work on their own time—after hours, in the morning, and on weekends. However, we are always able to find enough willing subcommittee members to run successful collaboratives each and every year. This really speaks to the character of our membership and the spirit of volunteerism that is alive and well in the ASBC. I congratulate and thank you all!

Last, I would like to personally thank the members of the Technical Committee for their time, dedication, enthusiasm, and sense of humor during the past year. It has certainly been a pleasure to work with such a talented group of brewing scientists! Thanks to Dana Sedin, Bob Foster, Gina Kelly, Sue Thompson, Cynthia Henson, Chris Powell, Karl Lakenburges, and Chaz Benedict. All will continue on the Technical Committee for the upcoming year. Of special note, Bob Foster agreed to assume the role of senior advisor to the Technical Committee in the spring of 2007. Bob brings a wealth of experience, wisdom, and love of brewing science to our committee that we simply don't want to lose.

Coordination of New and Alternate Methods of Analysis
(Jeff Cornell, Coors Brewing Company, *Chair*)

This is a standing subcommittee that collects suggestions for new methods, determining their potential for collaborative testing by identifying published methodology, and polling the membership for interest in suggested methods. Membership polling resulted in the formation of one new subcommittee for collaborative testing in 2006–2007: TBA Test as an Indicator for Flavor Stability, to be chaired by Katie McGivney (New Belgium Brewing Company).

doi:10.1094/ASBCJ-2007-1005-01

International Collaborative Methods
(David Maradyn, InBev nv/sa, *Chair*)

This subcommittee facilitates technical liaisons with our sister organizations: BCOJ, EBC, and IBD. The subcommittee has focused on ways to improve communication and coordination of international collaborative methods (ICM) and will continue to do so in the upcoming year. To that end, Technical Committee Chair David Maradyn attended the fall meeting of the EBC Analysis Committee in Pfaffikon, Switzerland, in November 2006. Likewise, two representatives of the BCOJ Analysis Committee, Chair Hiroto Kondo (Suntory Limited) and Vice Chair Shuso Sakuma (Kirin Brewery Company Ltd.), attended the ASBC Technical Committee meeting in Victoria, BC, held prior to the ASBC Annual Meeting.

Soluble Starch
(Karen Churchill, Prairie Malting Ltd., *Chair*)

This is a standing subcommittee that functions to procure and test lots of high-quality soluble starch for the Society. Collaborative testing is initiated by the chair when the supply falls below 100 kg. As of September 6, 2007, 20 lb (9.1 kg) remained for sale. A new lot of soluble starch will need to be evaluated in the fall of 2007. However, since our previous supplier of soluble starch no longer produces the product for sale, Karen must first find an alternate source before collaborative testing can be initiated.

Method for Reference Standard for Total Packaged Oxygen
(Mark Eurich, Coors Brewing Company, *Chair*)

This fifth-year collaborative study evaluated changes to the previous methodology to improve repeatability coefficients of variation, such as addition of a procedure to eliminate oxygen in the blank, standardization of the syringe type and needle size utilized, changes to the volume of the air spikes employed, inclusion of a series of video clips and digital photographs in the method description demonstrating the technique, and practice of the spiking procedure by collaborators prior to conducting the test. Unfortunately, repeatability coefficients of variation were judged unacceptable once again. It was recommended that the collaborative study continue for another year, incorporating additional changes to the methodology, including use of aluminum cans of beer from one source, large hose clamps to hold a septa against the can wall, and air injection utilizing an air-tight syringe with a hardened steel needle.

Determination of Alpha-Amylase by Automated Flow Analysis
(Jolanta Menert, Anheuser-Busch, Inc., *Chair*)

This was the second and final year of the subcommittee's existence, which was formed on the recommendation of the ASBC Technical Committee. The subcommittee is charged with evaluating automated flow analysis for the determination of alpha-amylase in malt. Guidelines for ASBC soluble starch specify that to be acceptable, the new starch lot must yield diastatic power and alpha-amylase results following the manual standard reference methods (Malt-6 and Malt-7) that are within 2 and 5% of the standard starch, respectively. During the evaluation of starch lot 29672A in 2003, it was recognized that only a small number of laboratories still routinely perform the manual methods. Thus, a need exists for the development or approval of a new protocol to determine the diastatic power and alpha-amylase values for soluble starch. A survey of subcommittee members prior to the first year of the collaborative study found that of those using automated flow instrumentation by Skalar, 80% were utilizing procedures based on iodine methodology. Therefore, this methodology was selected for collaborative study. In the first year, repeatability coefficients of variation were judged acceptable; however, reproducibility coefficients of variation were judged unacceptable. To improve the interlaboratory results, it was recommended that prior to the second year of the study all Skalar instruments should all be calibrated with the manual Malt-7A method. Analysis of the data from the second year of the study yielded acceptable repeatability and reproducibility coefficients of variation for the analysis of alpha-amylase in malt. No statistically significant differences were found between the Skalar iodine method and the manual method based on the paired *t* test for differences in means. The method has been recommended for inclusion in the *Methods of Analysis*.

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

This is a standing subcommittee charged with determining what packaging methodologies are currently in use and investigating new methodologies that could be useful to those in the brewing industry. Developing contacts and facilitating technical liaisons with beverage packaging associations is also an important mandate of this subcommittee. To this end, during the past year the EBC was contacted to obtain copies of their can methods for review; the Beverage Can Manufacturers of Europe were contacted to obtain an update on their activities and areas of interest; and the International Society of Beverage Technologists (ISBT) fall technical committee meeting in St. Paul, MN, was attended by the chair. Initial discussions were conducted on the drafting of a double-seam method, based on methodologies currently published. The subcommittee has also initiated the planning of a Packaging Community on the ASBC*net* website, including methodology, troubleshooting, and technical forum sections.

Method for Measurement of Resistance of Oxidation in Beer by Electron Paramagnetic Resonance
(David Barr, Bruker BioSpin, *Chair*)

This second-year subcommittee evaluated electron paramagnetic resonance (EPR) to determine the 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 beer shipping 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. The recommendation from the subcommittee was that the collaborative study be repeated for a third year, with a focus on changes to standard selection, sample selection, and sample shipping aimed at improving the unacceptable repeatability and reproducibility coefficients of variation.

MOA Methods Review
(Bob Foster, Coors Brewing Company, *Chair*)

This is a new standing subcommittee formed on the recommendation of the ASBC Technical Committee to provide an ongoing review of the published analytical methods in the *Methods of Analysis*. Each method will be reviewed by a subcommittee of selected experts to identify obsolete equipment and hazardous chemicals and reagents and to evaluate usefulness (if the method is still used by anyone in the industry). Recommendations will be forwarded to the Technical Committee for review and the Publications Committee for implementation. One section of the MOA will be reviewed each year. The first section reviewed was Hops.

Sensory Science
(Sue Thompson, Miller Brewing Company, *Chair*)

This is a new standing subcommittee formed on the recommendation of the ASBC 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 method-

ology, and propose new methodology for collaborative testing. Activities this past year included recruiting members for the subcommittee, establishing monthly teleconference meetings, conducting a needs and opportunities assessment, and prioritizing its focus going forward. The subcommittee identified panelist performance and evaluation, up-to-date threshold levels, train the trainer, updating the flavor wheel (including oxidation flavor wheel), and the relationship of sensory attributes to chemical analysis as priorities. Immediate focus will be on panelist performance and evaluation. The EBC has revised the triangle test methodology, and this subcommittee is currently reviewing the proposed changes in order for this method to retain its ICM status.

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

This standing subcommittee has been in existence for two years. The mandate of this subcommittee is to connect with the craft brewing membership 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 this past year include the launch 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.

TBA Test as an Indicator for Flavor Stability
(Karl Lakenburges, Anheuser-Busch, Inc., *Chair*)

This first-year subcommittee was charged with evaluating the TBA test for the prediction of beer flavor stability. Collaborative testing was not initiated this year, but discussions with interested collaborators on appropriate methodology and preliminary ruggedness testing were conducted in the chair's laboratory. Going forward, Katie McGivney (New Belgium Brewing Company) will assume the role of chair of this subcommittee, and collaborative testing will commence.

Coordination of New and Alternate Methods of Analysis

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

Corresponding Members: A. Mundy, European Brewery Convention (EBC); J. Murray, Institute of Brewing and Distilling (IBD); and Y. Nara, Brewery Convention of Japan (BCOJ).

Keywords: Accelerated aging, Allergens, Arabinoxylans, Carbohydrate, Carbon dioxide, Decarbonation, Foam, Gluten, Malting quality, Original Extract, Proteins, Rho, Tetra, Thiobarbituric acid, Total polyphenol

RECOMMENDATIONS

1. Poll the membership in 2007/2008 for interest in accelerated aging of seed as a predictor for viability loss in barley.
2. Conduct polling in 2007/2008 of users of ATP luminescence methods to better understand what methods/devices are in use and for what applications.
3. If a reference method can be found, conduct polling of the membership in 2007/2008 for interest in participating in a collaborative study on molecular weight distribution of arabinoxylans in beer.
4. Form a subcommittee to evaluate thiobarbituric acid test as a predictor for flavor stability in beer.
5. Technical Committee review of the list of archived ASBC methods for UV spectrophotometer-colorimetric methods that may be suitable and useful for craft brewers.
6. Poll the membership in 2007/2008 on existing instrumentation and methodologies utilized for volatile sulfur analyses.

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 Subcommittee and plans to explore further work together at annual meetings.

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 (12). The third and final year of a long-term storage study is underway at North Dakota State University, and the methodology (based on that of the Canadian Grain Commission) appears promising. The subcommittee recommends polling the membership in 2008 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 Subcommittee and this subcommittee. The allergen receiving the most attention related to malt beverages is gluten, which is linked to celiac disease. The TTB published an interim rule, effective July 26, 2006, allowing the voluntary listing of major food allergens on the labels of wines, distilled spirits, and malt beverages. To date, there is no mandatory requirement.

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" could be used.
- Defines "prohibited grains" such that to use the term "gluten-free" the product ingredients must not contain any wheat, barley, or rye (and hybrids).
- States wherever 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 has been suggested in the past as a method for testing surface swabs or rinse waters for CIP validation. Although several breweries are actively using this technology, the methods and outputs vary depending on the instrument manufacturer. If a collaborative test 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 recommends polling users of ATP luminescence methods in 2007/2008 to better understand what methods and devices are in use and for what applications.

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

Four members and two vendors showed interest in a potential collaborative study as a result of polling at the La Quinta meeting in 2006. Both *Analytica–EBC* and the *IOB Methods of Analysis* contain methods for the determination of carbon dioxide in beer by an instrumental procedure using the Mettler Toledo 965D (Corning) carbon dioxide analyzer (5,9). This instrument is no longer manufactured and, therefore, is not being considered for ASBC collaborative testing.

A major soft drink company indicated that they are using the Zahm and other temperature/pressure-based devices. A second soft drink company is using CarboQC (Anton Paar), as are some breweries. The EBC is preparing to conduct a collaborative study to evaluate the CarboQC instrument in the near future, and there are four North American labs that have committed to participating. If the study is successful, the method would meet the criteria as an international collaborative method (ICM) that could be incorporated into the ASBC *Methods of Analysis* (MOA). This subcommittee recommends that the Technical Committee monitor the progress of the EBC study and evaluate the proposed method for inclusion in the MOA.

doi:10.1094/ASBCJ-2007-1005-02

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 ASBC MOA contains three methods for the decarbonation of beer: Beer-1A and Beer-1D, decarbonate by shaking in a flask or rotary shaker, respectively (1).

Degassing is a sample preparation technique—the typical application is to remove enough CO_2 from the sample to minimize measurement 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 CO_2 removal and ethanol retention (3, 15). An oral presentation was given on the subject at the 2006 La Quinta meeting by Karl Siebert. Given the current body of work and interest expressed, the subcommittee recommends the following and is seeking someone to lead the effort:

- Summarize the findings from the existing work.
- Based on the summary, work with the subcommittee to pull together a recommendation for a collaborative study 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 (2). A membership poll conducted in 2004 to determine which foam analysis methods were currently in use 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 (two labs). The EBC recently adopted a NIBEM-T method for foam stability testing into *Analytica-EBC*. This subcommittee will check the list of participating laboratories in that study to determine whether it qualifies as an ICM under the recently revised guidelines. Polling at the 2006 La Quinta meeting failed to generate enough potential participants for a collaborative study on the Lg-Automatic instrument. Polling questions were submitted to Scientific Societies for distribution to the membership in February 2007, to obtain an update on the number of members using the Lg-Automatic and their willingness to participate in a collaborative study. Also, a signup sheet gauging interest in a potential collaborative study was posted at the 2007 Victoria meeting. The membership did not show sufficient interest in conducting a collaborative study on the foam tester from Lg-Automatic at this time; therefore, this item 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 Savannah meeting used size exclusion, but it is not appropriate for a lab method. The subcommittee is also aware that VLB is working on a dye-based method. This subcommittee will continue to search the scientific literature for appropriate methodology in 2007/2008. If a method is found, then polling of the membership for interest in participation in a collaborative study will occur. If a method is not found, the idea will be archived.

Rapid Carbohydrate Determination in Beer

Interest in a new rapid method for the determination of total carbohydrate in beer was expressed by the membership at the 2003 Albuquerque meeting. Currently, Beer-6D, Calculated Carbohydrate (recommended for use in label statements) (1) is available in the MOA. Beer-41, Total Carbohydrates (phenol-sulfuric method) states that this method is not to be used for labeling purposes because 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 was limited use of HPLC for profiling. Polling questions were sent to Scientific Societies in February 2007 for distribution to membership to better understand carbohydrate analyses currently in use and the desire for a rapid method as well. If no alternate methods are discovered, or if the membership no longer desires such a method, this idea will be archived.

Spectrophotometric Method for the Determination of Rho and Tetra Hops

A spectrophotometric method for the quantification of isomerized alpha-acids in hop products published by Maye et al (11) was suggested for possible collaborative study at the 2005 Savannah meeting. Polling of the membership in 2006 indicated insufficient interest in this method for hop products; however, there was significant interest expressed for such a method in finished beer. Since the published paper does not include finished beer as a matrix for this analysis, the Technical Committee has decided to archive this idea until a refereed publication appears in the literature.

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 for finished beer only was recommended for publication in the MOA. It was recommended that a subcommittee be formed to investigate the applicability of this method for hopped wort. Polling of the membership for participation in collaborative testing was conducted in 2005/2006 and 2006/2007; however, insufficient interest was expressed, and the study was not initiated. Additional polling was conducted at the 2007 Victoria meeting, and again insufficient interest was expressed by the membership in initiating a collaborative study. This method will be archived.

Thiobarbituric Acid Test

Thiobarbituric acid (TBA) test may be a predictor for oxidation and flavor stability (7,14,16). Discussions over the past several years have indicated that there is limited interest in this methodology. Polling of the membership in 2004 and 2005 yielded insufficient potential collaborators. However, polling at the 2006 La Quinta meeting yielded 10 interested participants. During 2006/2007 the ex officio conducted ruggedness testing on a potential methodology. This subcommittee recommends the formation of a subcommittee to evaluate the TBA test in 2007/2008.

Total Polyphenol Method

Interest was expressed at the 2005 Savannah meeting in understanding interferences with the total polyphenol spectrophotometric analysis (Beer 35). No interest was expressed in the topic at the 2006 La Quinta meeting. It is possible that this topic could have come from someone getting higher than expected results from highly hopped beers. Discussions with several of the method users, the Technical Committee, and this subcommittee 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 Savannah meeting for some simple colorimetric methods for craft brewers. This was reiterated at the 2006 La Quinta meeting. It was not clear which methods were of interest, but some of the old archived ASBC methods may be worth bringing back and possibly updating. This subcommittee recommends that the Technical Committee review the list of archived methods in 2007/2008 and recommend which would be appropriate for craft brewers.

Volatile Sulfur Compounds in Beer

Although volatile sulfur compounds contribute in a positive manner to the character of many beers, because of their low sensory thresholds and powerful, often unpleasant characteristics they are frequently the cause of off-odors and -flavors. Therefore, to have an analytical method available to the brewer that can quantitate these compounds to 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]) (8,10,13). Sulfur-selective detectors are mandatory for this type of analysis due to the low levels of these compounds within the beer matrix. Various sampling techniques have been employed for volatile sulfur analyses, ranging from simple headspace to purge and trap to solid-phase microextraction. This subcommittee will poll the membership in 2007/2008 for what sampling techniques, sulfur-selective detectors, and methodologies they use for volatile sulfur analysis of beer, as the first step in setting up a collaborative study on an appropriate methodology.

Yeast Vitality

A poster was presented at the 2005 Savannah meeting by Boris Stambuk on a new method to evaluate yeast vitality. However, to date the full paper has not been published in a refereed scientific journal. He was contacted in July 2007 and replied that a manuscript was in preparation and would be submitted for publication shortly. Once the paper is published, the Technical Committee will review the methodology and make a recommendation on whether it should be submitted for polling to the membership.

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*, 9th ed. Malt–4 Extract; Wort-9 Color; Beer–1A Preparation of sample for chemical and physical analysis, -1D Decarbonation by rotary shaker, -6D Calculated carbohydrate, -35 Total polyphenol, -41 Total carbohydrate. The Society, St. Paul, MN, 2004.
2. Anger, H., Glaro, L., and Scholz, M. A new process for foam stability measurement. *Brauwelt Int.* 3:146, 2002.
3. Constant, M., and Collier, J. Alternative techniques for beer decarbonation. *J. Am. Soc. Brew. Chem.* 51:29-35, 1993.
4. de la Vega, P. Spectrophotometric analysis of protein in wort and beer. *Proc. Conv. Inst. Brew. Aust. N.Z. Sect.* 21:100-103, 1990.
5. European Brewing Convention. *Analytica-EBC*, 5th ed. Section 9 Beer Method 9.28.2 Carbon dioxide in beer: Instrumental Method. Verlag Hans Carl Getranke Fachverlag, Nürnberg, Germany, 1998.
6. Food and Drug Administration. 21 CFR Part 101. Food labeling; Gluten-free labeling of foods. *Fed. Reg.* 72(14):2795-2817.
7. Grigsby, J., and Palamand, S. Studies on the staling of beer: The use of 2-thiobarbituric acid in the measurement of beer oxidation. *J. Am. Soc. Brew. Chem*. 34:49-55, 1976.
8. Hill, P. G., and Smith, R. M. Determination of sulphur compounds in beer using headspace solid phase microextraction and gas chromatographic analysis with pulsed flame photometric detection. *J. Chromatogr. A* 872:203-213, 2000.
9. Institute of Brewing, Analysis Committee of the IoB. *Recommended Methods of Analysis.* Beer 9.15 Carbon dioxide in beer by thermal conductivity (EM). The Institute, London, 1997.
10. Kancler, J. Low-level analysis of sulfur compounds in beer by purge and trap. Tekmar Application Note, 2003.
11. Maye, J. P., Mulqueen, S., Xu, J., and Weis, S. A spectrophotometric analysis of isomerized α-acids . *J. Am. Soc. Brew. Chem*. 60:98-100, 2002.
12. Modarresi, R., Rucker, M., and Tekrony, D. M. Accelerating ageing test for comparing wheat seed vigor. *Seed Sci. Technol*. 30:683-687, 2002.
13. OI. Low-level sulfur compounds in beer by purge and trap with a pulsed flame photometric detector (PFPD). OI Analytical Application Note 16181100, 2005.
14. Parsons, R., and Cop, R. The assessment and prediction of beer flavour stability. *Proc. Congr. Eur. Brew. Conv.* 19:279-286, 1983.
15. Smith, P. A., and Marinelli, L. J. Evaluation of established methods of decarbonating beer. *J. Am. Soc. Brew. Chem.* 50:102-105, 1992.
16. Thalacker, R., and Birkenstock, B. Eine neue Kennzahl in der brauttechnischen Analyze—Die Thiobarbitursäurezahl (TBZ). *Brauwissenschaft* 6:133-137, 1982.

Soluble Starch

Subcommittee Members: K. Churchill, *Chair*; A. Budde; S. Chan; D. Christopher; C. Eckermann; P. Gualdoni; R. Hills; B. Johannes; M. Joyce; D. Langrell; E. Kouhi-Lavender; E. Martens; J. Menert; J. Nawrocki; G. Smith; M. Walters; and C. Henson (*ex officio*).

Keywords: Soluble starch

RECOMMENDATIONS

1. Source a new supplier of soluble starch for the Society.
2. Initiate a collaborative study in 2007/2008 to evaluate a new lot 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. Starch lot 29672A was collaboratively tested and approved for sale by the Society during the spring of 2004 (1).

The subcommittee recommends evaluation of a new lot of starch when the current supply falls below 100 kg (220 lb). As of September 6, 2007, only 9.1 kg (20 lb) of starch lot 29672A was available for sale. A new lot of soluble starch will need to be evaluated as soon as possible. However, since our former supplier of soluble starch no longer produces the product, we must source a new supplier first. Once we have found a new supplier, we will commence the study immediately.

LITERATURE CITED

1. American Society of Brewing Chemists. Report of Subcommittee on Soluble Starch. *J. Am. Soc. Brew. Chem.* 62:173-174, 2004.

doi:10.1094/ASBCJ-2007-1006-01

Determination of Alpha-Amylase by Automated Flow Analysis

Subcommittee Members: J. Menert, *Chair*; B. Amundsen; A. Baroch; A. Brown; A. Caruso; D. Christopher; K. Churchill; P. Gualdoni; K. Isomaki; R. Jennings; B. Johannes; R. Joy; D. Kremer; D. Langrell; M. Omillian; P. Rich; O. Salonen; H. Sweins; and D. Sedin (*ex officio*).

Keywords: Enzymes, Iodine, Skalar

CONCLUSIONS

1. Repeatability coefficients of variation for the determination of alpha-amylase by the automated Skalar iodine method ranged from 1.7 to 4.9% and were judged acceptable.
2. Reproducibility coefficients of variation for the determination of alpha-amylase by the automated Skalar iodine method ranged from 11.7 to 18.1% and were judged acceptable.
3. Based on the paired *t* test for differences in means, no statistically significant differences were found between the automated Skalar iodine method and the manual method in the determination of alpha-amylase in malt.

RECOMMENDATIONS

1. The method Determination of Alpha-Amylase in Malt by Automated Flow Analysis be adopted for inclusion in the *Methods of Analysis*.
2. The subcommittee be discharged.
3. The Subcommittee for Coordination of New and Alternate Methods of Analysis poll the membership to determine if additional automated methods for the determination of alpha-amylase in malt need to be tested.

This is the second year of the subcommittee's existence. Based on a recommendation by the Technical Committee, this subcommittee was formed to evaluate the determination of alpha-amylase in malt by automated flow analysis. Guidelines for ASBC soluble starch specify that to be acceptable the new starch lot must yield diastatic power and alpha-amylase results using the manual standard reference methods Malt-6 and Malt-7 (1) that are within 2 and 5% of those from the standard starch, respectively (3). The majority of laboratories perform the determination of diastatic power and alpha-amylase in malt by automated flow analyses.

In the first year, a number of methods were tested, but statistical analysis was only carried out on the automated Skalar iodine (ASI) method (2). The ASI method produced acceptable repeatability coefficients of variation and unacceptable reproducibility coefficients of variation. The subcommittee recommended that the collaborative testing be repeated with a focus on manual methods Malt-7A and -7B and the ASI method.

PROCEDURE

Collaborators received eight malt samples representing four sample pairs, A/B, C/D, E/F, and G/H, that represented low-alpha malt (<25 DU), typical two-row malt (55–70 DU), typical six-row malt (40–60 DU), and distillers' malt (>70 DU), respectively. The instruments were calibrated based on a curve provided by the chair and by following the published Skalar method (catalog no. 164-001, issue 062303/EK/99225404). Alpha-amylase (Type VIII, catalog no. A2771, Sigma Chemical Co., St. Louis) was supplied by the chair. Beta-limit dextrin (P-BLDX, lot 00901) and beta-amylase (E-BABL, lot 50301) were generously supplied by Megazyme International Ltd. (Bray, Ireland). Samples were analyzed via ASBC manual methods (Malt-7A and -7B) and the ASI method. Results were evaluated using the Youden unit block design (1).

RESULTS AND DISCUSSION

Seventeen collaborators participated in the study. Alpha-amylase data from the manual and ASI methods are presented in Tables I and II, respectively. Outliers were identified using Dixon's ratio test (1).

The statistical summary and *t*-test comparison of the manual and ASI methods are presented in Tables III and IV, respectively. Repeatability coefficients of variation for alpha-amylase determined by the ASI and manual methods ranged from 1.7 to 18.0% and 1.3 to 29.6%, respectively, and were judged unacceptable. Reproducibility coefficients of variation for alpha-amylase determined by ASI and manual methods ranged from 11.7 to 30.1% and 4.5 to 47.9%, respectively, and were judged unacceptable. The poor repeatability and reproducibility were concluded to be due to the nature of the samples, rather than the analytical methodology. Alpha-amylase was measured in samples A and B at low levels, but the results varied considerably between collaborators. The low level of alpha-amylase in both samples was not representative of commercial malt; therefore, the provided calibration curve was not valid for this sample pair. By removing the data associated with samples A and B, repeatability coefficients of variation for samples C through H for the ASI and manual methods ranged from 1.7 to 4.9% and 1.3 to 5.7%, respectively, and were judged acceptable. Reproducibility coefficients of variation for the ASI and manual methods ranged from 11.7 to 18.1% and 4.5 to 16.4%, respectively, and were judged acceptable. The higher reproducibility coefficients of variation for the ASI method versus the manual methods were most likely due to differences in standardization of instrument operation. Differences between collaborators in tubing size, how the system is primed, and sampling time are the most obvious sources of error. Although the calibration data were provided to the collaborators, standardization details were not specified. The comparison of means of the ASI and manual methods using the paired *t* test showed no significant differences between the two utilized methods when individual sample pairs are compared at a 95% confidence level. The mean of the difference (D) and standard error of the difference (S_D) between the two methods were quite low except for the G/H sample pair (distillers' malt).

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*, 8th ed. Malt-6A Diastatic power, -6C Diastatic power, automated flow analysis, -7A Alpha-amylase by fixed color and variable time method, -7B Alpha-amylase by fixed time and variable color method; Statistical Analysis-4 Youden unit block collaborative testing procedure, -5 Comparison of test methods; Appendix-IA Methods for calibration of spectrophotometer. The Society, St. Paul, MN, 1992.
2. American Society of Brewing Chemists. Report of the Subcommittee on Determination of Alpha-Amylase by Automated Flow Analysis. *J. Am. Soc. Brew. Chem.* 64:248-249, 2006.
3. American Society of Brewing Chemists. Report of the Subcommittee on Soluble Starch. *J. Am. Soc. Brew. Chem.* 62:173-174, 2004.

doi:10.1094/ASBCJ-2007-1007-01

TABLE I
Determination of Alpha-Amylase (DU) in Malt by Manual Methods

	Sample Pair[a]		Sample Pair		Sample Pair		Sample Pair	
Collaborator/Method	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
3/7A	4.6	20.1	51.7	51.7	63.4	65.1	74.8	91.1
4/7B	21.9	21.6	45.9	48.4	59.0	60.3	64.1	75.7
6/7B	18.0	20.3	46.8	47.6	59.4	59.9	65.3	65.4
7/7A	4.3	19.0	46.8	47.6	61.9	60.1	72.6	73.2
8/7B	17.5	18.7	44.4	49.7	59.6	59.1	62.4	68.5
9/7B	0	12.1	38.4	47.3	67.0	67.5	43.3	52.5
12/7B	23.1	25.4	50.2	49.4	60.4	61.6	73.6	79.6
13/7B	1.2	10.3	73.7[b]	74.0[b]	92.5[b]	87.9[b]	92.3	90.7
15/7A	4.4	18.5	45.8	49.1	58.0	59.7	68.0	72.1
17/7A	4.3	15.9	40.9	42.0	50.1[b]	50.8[b]	65.5	63.8
18/7A	10.7	19.1	49.4	48.3	61.5	62.4	73.5	81.4
Mean	9.98	18.27	46.01[c]	48.11[c]	60.22[c]	60.77[c]	68.67	74.01
Grand mean	14.13		47.06[c]		60.49[c]		71.34	

[a] Not included in the results.
[b] Outlier at $P \leq 0.01$ based on totals and/or differences (1).
[c] Calculated excluding outliers.

TABLE II
Determination of Alpha-Amylase (DU) in Malt by Automated Skalar Iodine Method

	Sample Pair[a]		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	13.9	20.6	48.1	50.5	61.5	62.2	69.3	81.2
2	12.3	17.3	43.1	45.5	56.2	56.8	64.8	72.7
3	6.5	20.0	49.3	50.8	60.8	64.7	73.9	82.1
5	12.5	20.7	42.5	39.6	46.9	48.0	52.6	59.6
6	4.6	15.2	45.6	46.5	61.3	60.5	72.5	73.7
8	11.7	21.5	47.7	52.7	63.2	64.0	69.2	81.4
10	15.0	19.1	54.7	55.7	68.0	70.0	73.4	78.4
13	–[b]	–[b]	59.8	57.1	111.8[c]	82.2[c]	106.2	116.8
14	15.9	29.4	60.5	57.4	66.7	68.0	69.5	71.1
15	10.1	24.4	55.1	62.5	63.6	66.7	79.9	81.3
16	0.6	15.2	50.0	55.6	72.7	76.5	85.5	94.2
Mean	10.29[d]	20.32[d]	50.57	52.17	62.09[d]	63.74[d]	74.24	81.14
Grand mean	15.31[d]		51.37		62.91[d]		77.69	

[a] Not included in the results.
[b] Negative numbers removed at the discretion of the chair.
[c] Outlier at $P \leq 0.01$ based on totals and/or differences (1).
[d] Calculated excluding outliers.

TABLE III
Statistical Summary of Results[a]

			Repeatability			Reproducibility		
Sample Pair	**No. of Labs**	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Manual method								
A/B[b]	11	14.13	4.18	29.6	11.70	6.77	47.9	18.94
C/D	10	47.06	2.17	4.6	6.08	3.37	7.2	9.45
E/F	9	61.42	0.81	1.6	2.28	2.79	4.5	7.81
G/H	11	71.34	4.08	5.7	11.41	11.67	16.4	32.67
Automated Skalar iodine method								
A/B[b]	10	15.31	2.75	18.0	7.70	4.60	30.1	12.89
C/D	11	51.37	2.50	4.9	6.99	6.34	12.4	17.75
E/F	10	62.91	1.09	1.7	3.04	7.37	11.7	20.64
G/H	11	77.69	2.89	3.7	8.10	14.07	18.1	39.40

[a] All calculations were made based on ASBC methods (1).
[b] Not included in the results.

TABLE IV
Comparison of Automated Skalar and Manual Methods for Determination of Alpha-Amylase (DU) in Malt Using the Paired *t* Test for Differences in Means[a]

Statistical Parameter	Sample A[b]	Sample B[b]	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H
Number of paired results (N)	10	10	11	10	9	9	11	11
Mean of differences (D)	–0.57	1.26	2.05	3.57	0.81	1.69	5.58	7.13
Standard error of difference (S_D)	3.35	1.98	2.68	2.53	2.53	2.55	3.85	4.50
Calculated t	–0.169[c]	0.634[c]	0.799[c]	1.410[c]	0.303[c]	0.630[c]	1.521[c]	1.661[c]
$t_{0.05}$[d]	2.262	2.262	2.228	2.262	2.306	2.306	2.228	2.228

[a] All calculations were based on ASBC methods (1), and outliers were excluded.
[b] Not included in the results.
[c] Not significant at 95% confidence level.
[d] Two-tail test.

Method for Reference Standard for Total Package Oxygen

Subcommittee Members: M. Eurich, *Chair*; S. Carswell; X. Castañé (EBC); B. Couillard; M. Hamilton; D. Hutchinson; A. Kasprzyk; G. Kelly; T. Lawrence (EBC); C. Mendryshca; J. Parrell; A. Porter; K. Pryor; 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 4.5 to 65.6% and were judged unacceptable.
2. Repeatability coefficients of variation for the determination of the 200-µL oxygen spike recovery ranged from 3.4 to 32.0% and were judged unacceptable.

RECOMMENDATIONS

1. Repeat the collaborative study, incorporating a revised aluminum can procedure.

This is the fifth year of the subcommittee's evaluation of a method to produce a reference standard for total package oxygen analysis. In its first year, ruggedness testing of the proposed methodology was conducted (3). In its second year, different laboratories measured canned water and obtained statistically similar results (1). In its third year, the method produced unacceptable repeatability and reproducibility coefficients of variation (2). Based on feedback from members of the subcommittee, a number of modifications to the protocol were identified to correct the deficiencies, as well as improve communication with collaborators. The recommended changes to the 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. Further, to improve communication of how the preparation of the spiked samples should be carried out, the committee recommended that a series of photos demonstrating the technique be included with the method. Finally, the committee recommended that collaborators be advised to practice the spiking procedure 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, and a report was not submitted.

PROCEDURE

Each collaborator collected one case of bottled beer, either pasteurized or unpasteurized. Unpasteurized bottles were required to be a minimum of 1 month old prior to use to ensure there was no free oxygen in the bottle. Collaborators were instructed to practice the technique for injecting the samples with air, as outlined in the procedure. Following the methodology outlined, samples were prepared at the 0, 100-µL spike, and 200-µL spike levels. Samples were then measured for total package oxygen following the manufacturer's instructions for calibration and operation of each specific collaborator's instrument. Results were evaluated using SPSS Inc. software and mixed-effects model ANOVA test criteria.

RESULTS AND DISCUSSION

Results from 13 collaborators were received, with some collaborators submitting more than one set of data. In total, 17 sets of data were obtained for statistical analysis. These included the 0, 100-µL spike, and 200-µL spike levels. Results were measured on each collaborator's individual instrument and sampling device. Data for the mass of oxygen measured and percent recovery at the 0, 100-µL spike, and 200-µL spike levels 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 4.5 to 65.6% and were judged unacceptable. Repeatability coefficients of variation for the percent recovery of oxygen addition at the 200-µL injection level ranged from 3.4 to 32.0% and were judged unacceptable.

The ANOVA statistical summary of the 100- and 200-µL spike level data are shown in Table IV. The within-lab coefficient of variability (repeatability) was 23.2%. The between-lab coefficient of variability (reproducibility) was 18.9%. Combined, the total variance for the study was 29.9%.

To provide representative and meaningful variability estimates, the data were examined to determine the influence of suspect data points using two methods. The first was based on knowledge of the measurement system and physical properties and what type of values could be physically expected. The second method eliminated specific values that would have an undue influence on the final statistics (i.e., outliers). Because subsequent statistical techniques to find the estimated variances for reproducibility and repeatability use averages in the calculation, only one discrepant point, or outlier, would provide a false impression of the true variability. The screening method for detecting outliers was to compare the average with the median. The median would not be influenced by one discrepant point and would agree closely with an average with no influential outliers. Closeness of agreement of the estimated mean within a lab and within a spike level to the respective medians, integrated with expert knowledge, revealed seven total outliers that were removed from the dataset.

Estimated marginal means of recovery are illustrated in Figure 1. Estimated marginal means refer to the average of multiple readings (recovery rates) 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 the difference between collaborators and levels. The greater the dispersion observed in the graph between collaborators, the greater the collaborator variability. Additionally, looking at the graph one can see that collaborator 12 consistently read between the 100- and 200-µL level, whereas collaborator 11 had disparate readings by level. The value of the graph is that it shows precisely which collaborators were farthest from the others (perhaps having the most trouble) and which ones could determine recovery rates accurately at either the 100- or 200-µL levels. The graph provides a level of immediate and clear detail to supplement what caused the magnitude of between and within variability numbers.

doi:10.1094/ASBCJ-2007-1006-02

TABLE I
Mass of Oxygen Measured and Percent Recovery

Collaborator	Blank (mg)	100 µL (mg)	Percent Recovery, 100 µL	200 µL (mg)	Percent Recovery, 200 µL	Collaborator	Blank (mg)	100 µL (mg)	Percent Recovery, 100 µL	200 µL (mg)	Percent Recovery, 200 µL
1	0.015	0.048	121.4	0.052	67.9	9	0.002	0.026	100.7	0.062	128.6
1	0.016	0.030[a]	57.1[a]	0.059	80.4	9	0.002	0.048[a]	196.4[a]	0.028[a]	54.7[a]
1	0.013	0.036	78.6	0.056	75.0	9	0.005	0.005[a]	9.4[a]	0.051	104.7
1	0.014	0.033	67.9	0.058	78.6	10	0.012	0.036	102.9	0.050	83.7
1	0.011	0.034	71.4	0.055	73.2	10	0.011	0.036	102.9	0.061	108.1
1	0.015	0.127[a]	403.6[a]	0.264[a]	446.4[a]	10	0.013	0.030	76.8	0.057	99.3
2	0.004[a]	0.005[a]	5.60[a]	0.045[a]	75.5[a]	10	0.012	0.045	142.0	0.059	103.7
2	0.005[a]	0.013[a]	35.2[a]	0.005[a]	2.7[a]	10	0.013	0.038	111.6	0.055	94.8
2	0.006[a]	0.006[a]	9.3[a]	0.006[a]	4.5[a]	10	0.013	0.037	107.2	0.059	103.7
2	0.003[a]	0.007[a]	13.0[a]	0.014[a]	19.1[a]	11	0.017	0.032	90.2	0.052	88.5
2	0.001[a]	0.003[a]	–1.9[a]	0.006[a]	4.5[a]	11	0.011	0.037	112.9	0.051	86.3
2	0.002[a]	0.009[a]	20.4[a]	0.011[a]	13.6[a]	11	0.016	0.036	108.3	0.051	86.3
3	0.029	0.040	41.4	0.076	85.8	11	0.009	0.035	103.8	0.070	128.5
3	0.025	0.035	22.8	0.061	58.5	11	0.012	0.048	162.9	0.065	117.4
3	0.026	0.317[a]	1,067.3[a]	0.263[a]	425.8[a]	11	0.008	0.036	108.3	0.060	106.3
3	0.025	0.045	59.9	0.062	60.3	12	0.013	0.036	92.3	0.064	96.1
3	0.039	0.040	41.4	0.165[a]	247.6[a]	12	0.006	0.034	85.1	0.058	85.4
3	0.029	0.079[a]	185.8[a]	0.063	62.1	12	0.006	0.036	92.3	0.085[a]	133.6[a]
4	0.003[a]	0.039[a]	135.8[a]	0.010[a]	13.9[a]	12	0.006	0.034	85.1	0.063	94.3
4	0.004[a]	0.005[a]	9.9[a]	0.005[a]	4.8[a]	12	0.019	0.032	78.0	0.070	106.8
4	0.002[a]	0.006[a]	13.6[a]	0.004[a]	3.0[a]	12	0.011	0.046	128.0	0.067	101.5
4	0.002[a]	0.003[a]	2.5[a]	0.011[a]	15.8[a]	13	0.003[a]	0.003[a]	0.0[a]	0.009[a]	9.1[a]
4	0.001[a]	0.008[a]	21.0[a]	0.007[a]	8.5[a]	13	0.009[a]	0.009[a]	18.5[a]	0.009[a]	9.1[a]
4	0.002[a]	0.008[a]	21.0[a]	0.009[a]	12.1[a]	13	0.003[a]	0.012[a]	29.6[a]	0.006[a]	3.6[a]
5	0.078[a]	0.077[a]	29.6[a]	0.091[a]	40.0[a]	13	0.003[a]	0.018[a]	51.9[a]	0.009[a]	9.1[a]
5	0.030[a]	0.070[a]	3.7[a]	0.190[a]	220.0[a]	13	0.003[a]	0.009[a]	18.5[a]	0.012[a]	14.5[a]
5	0.043[a]	0.055[a]	–51.9[a]	0.110[a]	74.5[a]	13	0.003[a]	0.006[a]	7.4[a]	0.012[a]	14.5[a]
5	0.074[a]	0.063[a]	–22.2[a]	0.280[a]	383.6[a]	14	0.041[a]	0.065[a]	82.1[a]	0.049[a]	12.5[a]
5	0.059[a]	0.095[a]	96.3[a]	0.073[a]	7.3[a]	14	0.053[a]	0.054[a]	42.9[a]	0.054[a]	21.4[a]
5	0.130[a]	0.073[a]	14.8[a]	0.067[a]	–3.6[a]	14	0.054[a]	0.039[a]	–10.7[a]	0.051[a]	16.1[a]
6	1.744[a]	0.065[a]	72.5[a]	0.107[a]	73.1[a]	14	0.034[a]	0.039[a]	–10.7[a]	0.058[a]	28.6[a]
6	2.412[a]	0.063[a]	69.0[a]	0.097[a]	64.3[a]	14	0.030[a]	0.047[a]	17.9[a]	0.045[a]	5.4[a]
6	0.023[a]	0.055[a]	55.0[a]	0.106[a]	72.2[a]	14	0.040[a]	0.057[a]	53.6[a]	0.053[a]	19.6[a]
6	0.160[a]	0.063[a]	69.0[a]	0.117[a]	81.9[a]	15	0.004	0.023	87.1	0.036	71.5
6	0.015[a]	0.061[a]	65.5[a]	0.096[a]	63.5[a]	15	0.005	0.028	109.8	0.049	100.4
6	0.014[a]	0.057[a]	58.5[a]	0.092[a]	60.5[a]	15	0.003	0.027	105.3	0.047	95.9
7	0.042	0.065	72.5	0.107	73.1	15	0.004	0.029	114.4	0.034[a]	67.0[a]
7	0.022	0.063	69.0	0.097	64.3	15	0.004	0.024	91.7	0.040	80.4
7	0.028	0.055	55.0	0.106	72.2	15	0.003	0.027	105.3	0.031[a]	60.4[a]
7	0.015	0.063	69.0	0.117	81.9	16	0.006	0.032	114.4	0.047	89.3
7	0.016	0.061	65.5	0.096	63.5	16	0.007	0.028	96.2	0.053	102.6
7	0.019	0.057	58.5	0.092	60.5	16	0.004	0.028	96.2	0.050	95.9
8	0.002[a]	0.087[a]	306.0[a]	0.147[a]	260.1[a]	16	0.008	0.031	109.8	0.058	113.7
8	0.002[a]	0.069[a]	241.7[a]	0.141[a]	249.4[a]	16	0.007	0.032	114.4	0.056	109.3
8	0.002[a]	0.083[a]	291.7[a]	0.188[a]	333.3[a]	16	0.009	–[b]	–[b]	0.058	113.7
8	0.002[a]	0.080[a]	281.0[a]	0.164[a]	290.5[a]	17	0.002	0.028	94.4	0.058	102.8
8	0.002[a]	0.075[a]	263.1[a]	0.151[a]	267.3[a]	17	0.003	0.030	101.9	0.054	95.4
8	0.002[a]	0.085[a]	298.8[a]	0.173[a]	306.5[a]	17	0.003	0.029	98.1	0.056	99.1
9	0.003	0.027	105.1	0.076[a]	159.1[a]	17	0.002	0.028	94.4	0.058	102.8
9	0.002	0.015[a]	52.9[a]	0.047	96.0	17	0.002	0.031	105.6	0.054	95.4
9	0.003	0.028	109.4	0.052	106.9	17	0.003	0.030	101.9	0.055	97.2

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

The following sources are most likely the cause of the inability of the method to pass the statistical criteria: instrument-to-instrument variability, including calibration and/or maintenance requirements; injection and fobbing technique of the bottled product as described in the method; zero or minimal oxygen levels in the bottled product for the blanks; and a single source of bottled product not being used. The revised procedure that will be implemented going forward will use aluminum cans of beer from one source, large hose clamps to hold a septa against the can wall, and air injection utilizing an air-tight syringe with a hardened steel needle.

LITERATURE CITED

1. American Society of Brewing Chemists. Report of the Subcommittee on Method for Reference Standard for Total Package Oxygen. *J. Am. Soc. Brew. Chem.* 62:202-203, 2004.
2. American Society of Brewing Chemists. Report of the Subcommittee on Method for Reference Standard for Total Package Oxygen. *J. Am. Soc. Brew. Chem.* 63:216-217, 2005.
3. American Society of Brewing Chemists. Report of the Subcommittee on Oxygen and Carbon Dioxide Measurement in Packaged Beer. *J. Am. Soc. Brew. Chem.* 61:241-242, 2003.

TABLE II
Statistical Summary of 100-µL Injection Results

Collaborator	Valid *N*	Mean	Median	Min	Max	*S*	*cv*
1	4	0.69	0.70	0.57	0.79	0.09	13.0
3	4	0.41	0.41	0.23	0.60	0.15	36.6
7	6	0.65	0.67	0.55	0.73	0.07	10.5
9	6	0.96	1.03	0.09	1.96	0.63	65.6
10	6	1.14	1.08	0.90	1.63	0.25	21.9
11	6	1.07	1.05	0.77	1.42	0.21	19.5
12	6	0.93	0.89	0.78	1.28	0.18	19.0
15	6	1.02	1.05	0.87	1.14	0.11	10.4
16	5	1.06	1.10	0.96	1.14	0.09	8.8
17	6	0.99	1.00	0.94	1.06	0.04	4.5

TABLE III
Statistical Summary of 200-µL Injection Results

Collaborator	Valid *N*	Mean	Median	Min	Max	*S*	*cv*
1	5	0.75	0.75	0.68	0.08	0.05	6.5
3	4	0.67	0.61	0.58	0.86	0.13	19.2
7	6	0.69	0.68	0.60	0.82	0.08	19.2
9	6	1.08	1.06	0.55	1.59	0.35	32.0
10	6	1.02	0.97	0.86	1.29	0.18	17.7
11	6	0.99	1.01	0.84	1.08	0.09	8.8
12	6	1.03	0.99	0.85	1.34	0.17	16.2
15	6	0.79	0.76	0.60	1.00	0.16	20.3
16	6	1.04	1.06	0.89	1.14	0.10	9.6
17	6	0.99	0.98	0.95	1.03	0.03	3.4

TABLE IV
Precision Within and Between Collaborators

Term	Variance S^2	cv_r (%)
Within-lab repeatability variance (S_r^2)	0.045	23.2
Between-lab variance (S_b^2)	0.030	18.9
Reproducibility variance ($S_R^2 = (S_r^2 + S_b^2)$)	0.075	29.9
Grand mean = 0.916		
n = 112		

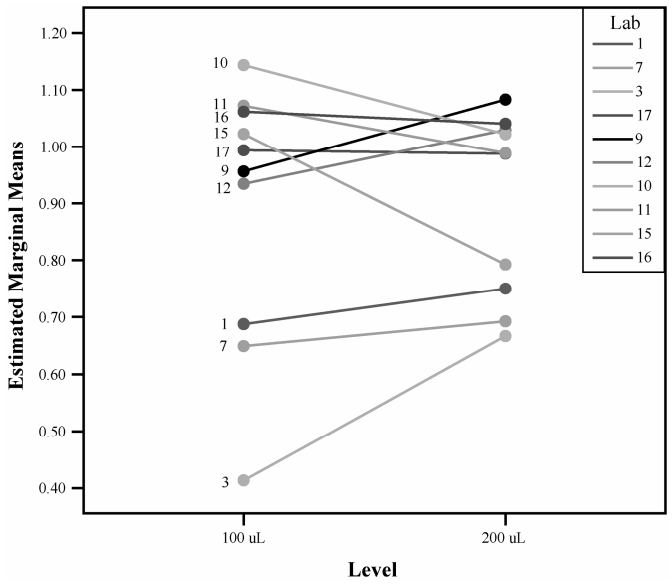

Fig. 1. Average collaborator recovery rates and ability to reproduce the method at both the 100- and 200-µL injection levels.

Method for Measurement of Resistance of Oxidation in Beer by Electron Paramagnetic Resonance

Subcommittee Members: D. Barr, *Chair*; S. Bobsen; T. Bradshaw; C. Giarratano; H. Hight; K. Holsopple; D. Loyet; C. Rhodes; K. Romanov; and R. Foster (*ex officio*).

Keywords: EPR, ESR, Lag time, PBN, T130, T150, Tempol

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the determination of lag time in beer ranged from 8.7 to 34.6% and 19.5 to 37.2%, respectively, and were judged unacceptable.
2. Repeatability and reproducibility coefficients of variation for the determination of T130 in beer ranged from 13.0 to 18.1% and 30.2 to 62.9%, respectively, and were judged unacceptable.

RECOMMENDATIONS

The subcommittee recommends repeating the collaborative study in 2007/2008, incorporating changes to sample shipping, standard selection, and analytical methodology aimed at improving the unacceptable repeatability and reproducibility coefficients of variation realized for the determination of lag time and T130 in beer.

This is the second year for this subcommittee's evaluation of a method for the measurement of resistance of free-radical oxidation in beer by electron paramagnetic resonance (EPR). The term EPR is frequently used interchangeably with electron spin resonance (ESR). In the first year, the method produced unacceptable repeatability coefficients of variation and unacceptable reproducibility coefficients of variation (4). The subcommittee recommended that the collaborative study be repeated, incorporating changes such as freezing samples before shipping, sending the *N-tert*-butyl-α-phenylnitrone (PBN) reagent as a solid with instructions on making up the standard (rather than sending a premade standard solution, which for many collaborators leaked during shipping), and sending samples with predetermined lag times.

PROCEDURE

Five sample pairs of beer (A/B, C/D, E/F, G/H, and I/J) were sent to each collaborator. Sample pair A/B was 100% light beer, sample pair C/D was a mixture of 75% light beer and 25% dark beer, sample pair E/F was a mixture of 50% light beer and 50% dark beer, sample pair G/H was a mixture of 25% light beer and 75% dark beer, and sample pair I/J was 100% dark beer. Beer samples were shipped frozen and packed on ice to each collaborator along with the Tempol standard PBN spin trap reagent. Collaborators were asked to freeze the samples upon receipt and run the analyses within 6 weeks. EPR oxidation values were determined as either lag times or T130. Results were evaluated using the Youden unit block design (1).

RESULTS AND DISCUSSION

Results from 10 collaborators were received for the five sample pairs. Data from two collaborators were excluded prior to statistical analyses because of known deviations from the prescribed experimental protocol. Data for lag time and T130 are presented in Tables I and II, respectively. Data were not screened for outliers.

The statistical summaries of the lag time and T130 data are shown in Table III. Repeatability and reproducibility coefficients of variation for the determination of lag time in beer ranged from 8.7 to 34.6% and 19.5 to 37.2%, respectively, and were judged unacceptable. Repeatability and reproducibility coefficients of variation for the determination of T130 in beer ranged from 13.0 to 18.1% and 30.2 to 62.9%, respectively, and were judged unacceptable. Although unacceptable, the repeatability and reproducibility coefficients of variation for the determination of lag time and T130 in beer improved in 2006/2007 compared with the first year of the collaborative study (2). The variance observed in lag time values could be due to differences in the time at which analyses were performed after receipt of the samples. Also, significant variability in the EPR intensity plots of the Tempol reference standards was observed, possibly due to different instruments and tubing sizes. This could have negatively impacted the reproducibility of the data. Another contributing factor to the unacceptable reproducibility coefficients of variation for the T130 data could be the E-Scan instrument, which only ran for 140 min instead of 180 min. Because of the difference in scan times, the EPR intensity values for the sample pairs normalized to the Tempol reference standard at T130 minutes showed more variability. Historically, the T150 minute limit is more stable than the T130. Yet another possible factor might have been the stability of the Tempol standard itself. Although considered relatively stable, better results may be obtained going forward with an inorganic reference standard, such as manganese (II). During the 2006/2007 study, shipping sample pairs frozen helped to minimize the sample storage variability that was a major issue in the first year that the collaborative study was performed. However, most participants still reported that they received samples thawed, but cold. Going forward, samples will be sent frozen and packed in dry ice to ensure that they arrive frozen and no oxidation occurs in transit.

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*. Statistical Analysis-2 Limits of detection and determination, -4 Youden unit block collaborative testing procedure. The Society, St. Paul, MN, 2006.
2. American Society of Brewing Chemists. Report of the Subcommittee on Method for Measurement of Resistance of Oxidation in Beer by Electron Paramagnetic Resonance. *J. Am. Soc. Brew. Chem.* 64:250-251, 2006.
3. Torline, P., and Grimmer, H. Is long shelf life possible? In: *Proc. 7th Conv. Inst. Brew. Afr. Sect*. Institute of Brewing, London, pp. 14-21, 2001.
4. Uchida, M., and Ono, M. Improvement for oxidative flavor stability of beer-rapid prediction method for beer flavor stability by electron spin resonance spectroscopy. *J. Am. Soc. Brew. Chem.* 54:198-204, 1996.

doi:10.1094/ASBCJ-2007-1007-02

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

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
1[a]	68.6	79.2	41.9	57.4	39.8	58.9	22.0	31.0	21.3	36.0
2[a]	108.5	109.3	44.2	46.5	20.5	59.6	24.2	52.1	26.6	41.3
3[a]	58.8	56.7	43.2	58.1	25.7	42.4	24.4	32.4	26.7	36.2
4[a]	97.6	99.3	38.9	81.3	28.9	63.1	21.8	44.4	23.8	23.6
5[b]	108.6	91.6	32.9	85.3	24.1	68.0	25.3	70.0	26.1	51.2
6[b]	95.0	87.1	32.9	90.8	25.8	77.8	17.3	58.7	17.2	51.2
7[b]	89.5	94.8	29.6	78.7	30.0	22.5	17.5	33.2	20.5	34.0
8[b]	112.2	90.3	60.8	101.7	41.0	99.3	21.1	76.2	19.2	65.4
Mean	92.35	88.54	40.55	74.98	29.48	61.45	21.70	49.75	22.68	42.36
Grand mean	90.45		57.78		45.44		35.69		32.52	

[a] EMX.
[b] E-Scan.

TABLE II
T130 (Normalized) in Beer Measured by Electron Paramagnetic Resonance

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
1[a]	47.2	44.1	62.6	48.8	73.0	54.5	82.7	61.1	88.2	61.2
2[a]	28.0	35.2	63.8	41.9	72.8	52.9	91.9	61.1	94.4	58.4
3[a]	52.2	39.0	56.0	48.9	59.7	48.2	64.8	48.5	64.4	51.1
4[a]	6.4	7.5	28.0	10.7	39.8	14.6	44.7	19.1	45.6	20.7
5[b]	15.7	19.2	49.9	21.2	68.4	26.0	82.1	30.1	89.0	32.8
6[b]	13.5	15.2	37.1	18.5	50.2	20.7	59.3	22.2	60.7	21.8
7[b]	15.4	19.4	45.0	24.5	53.9	30.8	68.2	37.0	60.1	36.0
8[b]	13.5	15.5	43.7	16.2	57.3	20.1	63.3	22.0	69.9	25.4
Mean	23.98	24.37	48.25	28.84	59.38	33.49	69.62	37.64	71.52	38.43
Grand mean	24.18		38.55		46.44		53.63		54.98	

[a] EMX.
[b] E-Scan.

TABLE III
Statistical Summary of Results[a]

		Repeatability			Reproducibility		
Sample Pair	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Lag time (min)							
A/B	90.45	7.85	8.7	21.99	17.63	19.5	49.37
C/D	57.78	14.55	25.2	40.74	15.19	26.3	42.52
E/F	45.44	15.25	33.6	42.70	16.91	37.2	47.35
G/H	35.69	12.36	34.6	34.62	12.59	35.3	35.24
I/J	32.52	10.44	32.1	29.22	9.57	29.4	26.79
T130 (normalized)							
A/B	24.18	4.37	18.1	12.24	15.20	62.9	42.57
C/D	38.55	4.99	13.0	14.00	13.95	36.2	39.05
E/F	46.44	7.18	15.5	20.11	14.00	30.2	39.21
G/H	53.63	8.05	15.0	22.55	16.31	30.4	45.67
I/J	54.97	9.55	17.4	26.74	16.82	30.6	47.08

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

Report of 2006 BCOJ Collaborative Work

Comparison of the Anton Paar Alcolyzer Method and the Official GC-FID Method of the National Tax Administration Agency Japan for the Evaluation of Alcohol Content in Beer, *Happo-Shu*, and Nonalcoholic Beer

Subcommittee Members: T. Kaneko (Sapporo Breweries Ltd.), *Chair (April 2006 – October 2006)*; S. Furusho (Sapporo Breweries Ltd.), *Chair (November 2006 – March 2007)*; R. Ganaha (Orion Brewery Co. Ltd.); T. Inui (Suntory Ltd.); A. Matsuyama (Kirin Brewery Co. Ltd.); A. Mizuno (National Research Institute of Brewing); M. Morimoto (Asahi Breweries, Ltd.); K. Takemoto (Kirin Brewery Co. Ltd.).

Keywords: Alcohol, Alcolyzer, GC-FID

CONCLUSIONS

1. The results were received from collaborators who analyzed a total of four sample pairs. The data were obtained using the Anton Paar Alcolyzer method and the GC-FID method, which is based on the official method of the National Tax Administration Agency Japan. No outliers were identified using Dixon's ratio test, and all results were used.
2. Based on the paired *t* test for the differences in the means of the alcohol content, no statistically significant difference was found between the Anton Paar Alcolyzer and GC-FID methods.
3. Reproducibility errors of the two methods were also measured using an *F* test, and there was a statistical difference between the two methods. The reproducibility error of the Anton Paar Alcolyzer method (pooled variance 0.00061) was smaller than that of the GC-FID method (pooled variance 0.01882).

RECOMMENDATIONS

1. No statistically significant difference was found between the Anton Paar Alcolyzer and GC-FID methods for the determination of alcohol content in beer, *happo-shu*, and nonalcoholic beer.
2. The Anton Paar Alcolyzer method excelled in the precision of its determination of alcohol content in beer, *happo-shu*, and nonalcoholic beer compared with the GC-FID method.
3. Discharge the subcommittee.

The method for the determination of alcohol content in beer, *happo-shu*, and nonalcoholic beer by the Anton Paar Alcolyzer was included in the *Methods of Analysis of BCOJ* in 2005 (2). The subcommittee also reported that no statistically significant difference was found between the Anton Paar Alcolyzer and SCABA (Foss Tecator AB) methods for the determination of the alcohol content in beer, *happo-shu*, and nonalcoholic beer based on a paired *t* test for the differences in the means. To verify the practical use of the Anton Paar Alcolyzer, the collaborative work was carried out by seven collaborators to compare the method with the GC-FID method authorized by the National Tax Administration Agency Japan (3).

doi:10.1094/ASBCJ-2007-1004-01

PROCEDURE

Four sample pairs (A/B, C/D, E/F, G/H) of packaged beer, *happo-shu*, and nonalcoholic beer were sent to each collaborator. The alcohol content in the selected samples ranged from 0.5 to 8.0%. They were selected as follows: nonalcoholic beer (A/B), *happo-shu* (C/D), pilsner beer (E/F), and dark beer (G/H). The Anton Paar Alcolyzer was calibrated according to the manufacturer's instruction manual. The samples were degassed at 20–22°C, filtrated using filter paper, and analyzed using the Anton Paar Alcolyzer and GC-FID methods. The results of the Anton Paar Alcolyzer and GC-FID methods were evaluated using the ASBC comparison of test methods (1).

Note

The Japanese liquor tax law prescribes the analysis of alcohol content in vol/vol% at 15°C. Therefore, the data for alcohol content (vol/vol%) using the Anton Paar Alcolyzer method, which were originally analyzed at 20°C, were converted into vol/vol% at 15°C as follows. Standard solutions of ethanol, which were prepared for the GC-FID method at 15°C, were also analyzed using the Anton Paar Alcolyzer method, and a calibration curve was drawn to convert the alcohol content from vol/vol% at 20°C to vol/vol% at 15°C.

RESULTS AND DISCUSSION

Results from seven collaborators who performed both methods were received for the four sample pairs. The alcohol content results for the Anton Paar Alcolyzer and GC-FID methods are shown in Tables I and II, respectively. No outlier was identified using Dixon's ratio test, and all results were used for the following statistical analysis.

The results of the paired *t* test are shown in Table III. No statistically significant difference was found between the Anton Paar Alcolyzer and GC-FID methods at the 95% confidence level. The pooled variances for each method are shown in Table IV. Based on the *F* statistic comparing the Anton Paar Alcolyzer and GC-FID methods, there was a statistical difference between the two methods. Further, the reproducibility error of the Anton Paar Alcolyzer method (pooled variance 0.00061) was smaller than that of the GC-FID method (pooled variance 0.01882).

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*, 9th ed. Statistical Analysis-4 Youden unit block collaborative testing procedure, -5 Comparison of test methods. The Society, St. Paul, MN, 2004.
2. Brewery Convention of Japan. Report of 2005 BCOJ collaborative work. *J. Am. Soc. Brew. Chem.* 64:252-254, 2006.
3. National Tax Administration Agency Japan. *Official Methods of National Tax Administration Agency Japan*, 4th ed. 3-4. Alcohol content. Brewing Society of Japan, Tokyo, 1993.

TABLE I
Alcohol Content (vol/vol%) Determined by the Anton Paar Alcolyzer Method

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	0.44	0.43	3.27	3.26	5.43	5.41	8.06	8.14
2	0.44	0.43	3.28	3.27	5.41	5.40	8.04	8.12
3	0.44	0.43	3.29	3.28	5.44	5.42	8.08	8.17
4	0.44	0.41	3.26	3.27	5.42	5.38	8.07	8.15
5	0.44	0.42	3.25	3.25	5.39	5.37	8.00	8.09
6	0.44	0.42	3.25	3.24	5.42	5.34	7.99	8.05
7	0.45	0.44	3.25	3.23	5.39	5.37	8.00	8.10
Mean	0.44	0.43	3.26	3.26	5.41	5.38	8.03	8.12
Grand mean	0.43		3.26		5.40		8.08	

TABLE II
Alcohol Content (vol/vol%) Determined by the GC-FID Method

	Sample Pair		Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	0.45	0.43	3.24	3.21	5.49	5.52	8.15	8.30
2	0.49	0.46	3.26	3.22	5.40	5.43	8.05	8.12
3	0.47	0.47	3.32	3.28	5.60	5.54	8.26	8.29
4	0.45	0.44	3.19	3.19	5.37	5.38	8.11	8.21
5	0.47	0.45	3.28	3.25	5.50	5.46	8.04	8.13
6	0.42	0.40	3.11	3.09	5.19	5.16	7.80	7.72
7	0.44	0.42	3.09	3.08	5.22	5.14	7.76	7.80
Mean	0.46	0.44	3.21	3.19	5.40	5.38	8.02	8.08
Grand mean	0.45		3.20		5.39		8.05	

TABLE III
Comparison of Means for the Anton Paar Alcolyzer and GC-FID Methods for Determination of Alcohol Using a Paired *t* Test[a]

Statistical Parameter	Alcohol (vol/vol%)
Number of paired results (*N*)	56
Mean of differences (*D*)	0.021
Standard error of differences (*SD*)	0.115
Calculated *t*	1.351[b]
$t_{0.05}$	2.004

[a] All calculations were made based on ASBC methods (1).
[b] Not significant at the 95% confidence level.

TABLE IV
Comparison of Method Precision[a]

Method	Sample Pair	Reproducibility Error	Pooled Variance	Degree of Freedom
Anton Paar Alcolyzer	A/B	0.007		
	C/D	0.017		
	E/F	0.024		
	G/H	0.039	0.00061	24
GC-FID	A/B	0.024		
	C/D	0.082		
	E/F	0.157		
	G/H	0.208	0.01882	24

[a] All calculations were made based on ASBC methods (1). Seven collaborators provided results. The calculated *F* statistic was 30.91 and was significant at the 95% confidence level ($F_{0.025;24,24} = 2.27$).