Technical Committee and Subcommittee Reports

2014–2015 Report of the Technical Committee

Committee Members: M. Eurich, *chair*; S. Brendecke; L. Guerdrum; R. Jennings; E. Jorgenson; K. Lakenburges; A MacLeod; D. Maradyn; C. Pachello; J. Palausky; A. Porter; C. Powell; N. Rettberg; E. Welten (EBC); and B. Foster (*senior advisor*).

The ASBC Technical Committee and Subcommittee chairs conducted a number of method evaluations through collaborative study and coordinated a range of additional activities during 2014–2015. For 2015 there are no new methods recommended for inclusion in the ASBC *Methods of Analysis* (MOA):

One method was evaluated but not recommended for inclusion in MOA at this time. Additional development and further evaluation is required for the following methodology:

- Rapid method for malt color, chaired by Betsy Roberts (Briess Malt).

In addition, the following methods will continue for another year of collaborative study:

- Wort amino acids by HPLC, chair TBD.
- Medium for the identification of phenolic character in yeast, chaired by Caroline Pachello (MillerCoors).

The ASBC Technical Committee regularly reviews each section of MOA. In 2015–2016 reviews of two sections of ASBC Methods of Analysis will be continued:

- Beer, chaired by Karl Lakenburges (Anheuser-Busch InBev) and Mark Eurich (New Belgium Brewing Co.)
- Statistical analysis of samples, chaired by Aaron MacLeod (Hartwick College).

In order to gather information on the requirements of the ASBC membership, the Coordination of New and Alternate Methods of Analysis Subcommittee submitted surveys to members in 2015. Joe Palausky (subcommittee chair) worked closely with the Technical Committee to design the questions, and a number of topics were polled for interest in future subcommittees. The results were presented at the 2015 ASBC Annual Conference in La Quinta, California. Based on the polling results and feedback at this meeting, a number of methods have been recommended for collaborative study in 2015–2016:

- NIBEM for foam stability, chaired by Aaron Golston (Lagunitas Brewing Co,).
- Hop analysis by GCMS, chair TBD.
- Tetrahydro-iso-alpha acids in hop products by spectrophotometer, chaired by Bob Smith (Hopsteiner).

In addition, the following topics will undergo preliminary analysis and ruggedness testing prior to collaborative study in 2015:

- Wort color turbidity correction, chaired by Theresa Chicos (Rahr Malting).
- Lipoxygenase activity in malt, chair TBD.
- Beer filtration task force, chaired by Aaron MacLeod (Hartwick College) and assisted by Unju Kim (Novozymes), Chris Swersey (Brewers Association), and Tom Neilson (Sierra Nevada).

As in previous years, the following standing subcommittees continue:

- Coordination of New and Alternate Methods of Analysis, chaired by Joe Palausky (Boulevard Brewing Co.).
- International Methods, chaired by Chris Powell (University of Nottingham).
- Craft Brew, chaired by Eric Jorgenson (Highland Brewing Co.).
- Sensory Science, chaired by Lindsay Guerdrum (New Belgium Brewing Co.).
- International Hop Standards Committee, chaired by Bob Foster (MillerCoors).
- Packaging Methods, chaired by Scott Brendecke (Ball Corporation).
- Microbiological Methods in Brewing, chaired by Caroline Pachello (MillerCoors).
- Soluble Starch, chaired by Rebecca Jennings (Rahr Malting Co).
- Check Services, chaired by Rebecca Jennings (Rahr Malting Co.) and Jodi Grider (ASBC SciSoc).

In 2014–2015 the Technical Committee was involved in webinar/video development to provide additional content to MOA:

- The Sensory Science subcommittee (Lindsay Guerdrum, New Belgium Brewing Co.) produced another webinar for sensory-related topics.
- The Packaging subcommittee (Scott Brendecke, Ball Corporation) produced a webinar on aspects of packaging quality control which will be repeated in the coming year.
- The Craft Brew Subcommittee (Eric Jorgenson, Highland Brewing Co.) has produced another in the webinar series "Grow Your Own Lab."

One student grant proposal was submitted for consideration by ASBC BOD. Interested individuals should contact the Technical Committee Chair (Mark Eurich, New Belgium Brewing Co.):

- Comparison of Package Analyzers for Total Package Oxygen, chair TBD.

Two student grant evaluations were conducted in 2014–2015. Results will be shared in 2016 once they are submitted and evaluated by the Technical Committee. The relevant evaluations and subcommittee chairs are as follows:

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- Can fill calculator, chaired by Scott Brendecke (Ball Corporation).
- NIR for hop analysis, chaired by Bob Foster (MillerCoors) and Aaron Porter (Sierra Nevada).

The Technical Committee also has a role in the collection of information regarding industry-related concerns pertinent to the brewing community or to the ASBC membership:

- Emerging Issues Subcommittee, chaired by Dave Maradyn (Novozymes).

I would like to thank Chris Powell and all the efforts he provided as the Technical Committee chair these past few years. We wish him the best of luck in his continuing ASBC journey.

The Technical Committee would like to thank the current subcommittee chairs for their hard work and dedication in conducting their respective collaborative studies during the past year. Furthermore, we formally acknowledge the many subcommittee members who have participated over the past year.

Finally, I would like to recognize the dedication and hard work put forth by all members of the Technical Committee over the previous year. The continual enthusiasm and commitment demonstrated by the team is sincerely appreciated, and I firmly believe it is key to ensuring that the ASBC Methods of Analysis remains contemporary, relevant, and of exceptional practical value to the brewing community.

I would also like to thank Dave Maradyn for his years of service as the MOA Editor-in-Chief. Those duties will now lie in the Technical Committee itself. As well, Dave has done an outstanding job as the Subcommittee Chair for Emerging Issues as he steps away from this role.

Coordination of New and Alternate Methods of Analysis
(*Joe Palausky, j.palausky@boulevard.com*)

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

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

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

Check Services
(*Rebecca Jennings, rjennings@rahr.com,* and *Jodi Grider, jgrider@scisoc.org*)

This is a standing subcommittee to ensure value and relevancy of the ASBC Check Sample Service. This service provides subscribing members an opportunity to evaluate method accuracy and precision and instrument performance on a scheduled, regular basis. By comparing internal laboratory data to results from other laboratories around the world, a critical assessment of the analytical data generated by subscriber labs can be made, and identification of areas for method improvement can be identified.

Craft Brew
(*Eric Jorgenson, ericj@highlandbrewing.com*)

The mandate of this subcommittee is to engage the craft brewing members of ASBC and explore opportunities to make the society more relevant to these individuals. Additionally, the subcommittee aims to explore opportunities and pursue strategies to bring craft brewers who are not members of the society into the ASBC.

Sensory Science
(*Lindsay Guerdrum, lguerdrum@newbelgium.com*)

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

International Hop Standards Committee
(*Bob Foster, robert.foster@millercoors.com*)

This subcommittee was formed in 1996 between the ASBC and EBC and is a standing committee whose goal is to produce, purify, and verify isomerized and unisomerized hop standards for the brewing, hops, and related industries.

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

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

International Methods
(*Chris Powell, chris.powell@nottingham.ac.uk*)

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

Wort Amino Acids by HPLC
(*TBD*)

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

Microbiological Methods in Brewing
(*Caroline Pachello, caroline.pachello@millercoors.com*)

This subcommittee aims to evaluate novel methods for analysis of microbiological samples in brewing, including yeast and bacteria related assays. During the coming year information on innovative methodology and techniques will be collected and assessed. Individuals interested in contributing and/or participating in collaborative work are encouraged to contact Chris Powell or Caroline Pachello directly.

Medium for the Identification of Phenolic Character in Yeast
(*Trevor Cowley, trevor.cowley@sabmiller.com*)

This subcommittee aims to evaluate simple techniques to determine the potential for yeast strains to produce phenolic compounds during fermentation. It is anticipated that this may assist in strain characterization, and for selection of novel strains that may be suitable for the production of beers where such compounds are desirable.

Rapid Method for Malt Color
(*Betsy Roberts, betsy.roberts@briess.com*)

This subcommittee aims to evaluate methods for the analysis of malt color. Full details of this subcommittee will be confirmed in due course.

NIBEM for Foam Stability
(*Aaron Golston, aaron.golston@lagunitas.com*)

This subcommittee will evaluate the Nibem T foam analyzer as a means of assessing foam in beer. This will be performed in conjunction with the EBC Analysis Committee to create an internationally recognized technique.

Hop Analysis by GCMS
(*TBD*)

This subcommittee aims to develop methods for the analysis of hop compounds using GCMS. Full details of this subcommittee will be confirmed in due course.

Lipoxygenase Activity in Malt
(*TBD*)

Lipoxygenase (LOX) is a family of enzymes that catalyze the oxygenation of polyunsaturated acids. In combination with other degrading enzymes, they produce flavor-active compounds and lead to a decrease in the shelf life and stability of beer. This subcommittee aims to evaluate the techniques that are currently being utilized across laboratories and to develop a standard procedure.

MOA Review: Statistical Analysis of Samples
(*Aaron MacLeod, macleoda@hartwick.edu*)

This subcommittee has been initiated to provide guidelines for the statistical analysis of data related to brewery samples. The subcommittee will focus on comparison and validation of analytical methods through single and multilaboratory studies. It will address topics such as identifying the appropriate statistical test to apply, dealing with outliers, and interpreting results. The primary goal is to prepare a set of methods and guidelines to assist the nonexpert in correctly analyzing data.

MOA Review: Beer
(*Karl Lakenburges, Karl.Lakenburges@anheuser-busch.com*, and *Mark Eurich, meurich@newbelgium.com*)

This subcommittee is charged with reviewing the Beer section of ASBC Methods of Analysis to ensure that all methods are relevant and are consistent with modern techniques.

Malt Color—Rapid Microwave Method

Subcommittee members: E. Roberts, *chair*; D. Griggs; R. Jennings; Y. Li; C. Martens; M. Miller; P. Schwarz; S. Theriot; R. Thiel; and A. MacLeod (*ex officio*).

CONCLUSIONS

1. Repeatability coefficients of variation for the determination of malt color by the rapid microwave method ranged from 1.9 to 7.1% and were judged unacceptable.
2. Reproducibility coefficients of variation for the determination of malt color by the rapid microwave method ranged from and 4.5 to 9.0% and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends that the collaborative study be repeated with more collaborators and including a comparison with the standard reference method (Wort-9).

This was the first year of the subcommittee's existence. The subcommittee was formed to evaluate a rapid method for malt color analysis. On the basis of polling by the subcommittee for Coordination of New and Alternate Methods, it was determined that there was interest in a method for determination of malt color that did not require the use of a traditional mashing bath (3). Such a method could be used by a broader range of laboratories as an alternative to the standard method, Wort-9. A rapid method for malt color using a microwave oven extraction and subsequent spectrophotometric measurement of color has been proposed by Li et al. (2) A collaborative test was required to determine repeatability and reproducibility coefficients of variation for the rapid method prior to inclusion in the ASBC *Methods of Analysis*.

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PROCEDURE

Five sample pairs of commercial base and specialty malt, labeled A/B, C/D, E/F, and G/H, covering a range of color levels, were sent to each collaborator. Collaborators were asked to determine the color for each malt sample as follows. Malt (25 g) was combined with 400 g of water in a 250 mL Pyrex bottle, shaken to mix contents thoroughly, and microwaved for 2 min on 50% power. After shaking the bottle to mix, the bottle was returned to the microwave for an additional 2 min at 50% power. The extract was then filtered through fluted filter paper, clarified with a 0.45 µm nylon filter, and the absorbance recorded at 430 nm. Color was reported as absorbance multiplied by 25.4. Results were evaluated using the Youden unit block design (3).

RESULTS AND DISCUSSION

Results from nine collaborators were received for the five sample pairs. Data for the malt color determination using the rapid microwave method are presented in Table I. Outliers were identified using Dixon's ratio test (1). Laboratories 8 and 9 reported using higher microwave power settings to achieve the required

TABLE I
Malt Color (°ASBC) by Rapid Microwave Method

	Sample pair		Sample pair		Sample pair		Sample pair		Sample pair	
Collaborator	**A**	**B**	**C**	**D**	**A**	**F**	**G**	**H**	**I**	**J**
1	2.54	2.39	3.81	3.63	9.65	9.47	18.72	22.15	61.7	57.4
2	2.64	2.39	3.68	3.61	9.25	9.73	17.42	20.09	65.0	56.1
3	2.46	2.34	3.56	3.28	9.73	9.75	17.27	21.06	62.5	51.5
4	2.49	2.36	3.48	3.45	9.35	9.55	17.50	21.20	57.1	54.2
5	2.54	2.28	3.61	2.89	9.55	9.03	17.52	21.41	66.7	59.2
6	2.62	2.53	3.73	4.24	10.95	11.56	17.63	21.21	65.4	58.9
7	2.74	2.64	3.86	3.56	9.75	9.86	15.95	21.54	63.5	55.5
8[a]	3.05[b]	4.39[b]	3.86	3.38	11.86	11.28	18.90	23.37	72.6	64.4
9[a]	4.20[b]	3.90[b]	5.20[b]	4.70[b]	11.10	10.90	19.40	22.60	66.3	59.2
Mean	2.81	2.80	3.87	3.64	10.13	10.13	17.81	21.63	64.5	57.4
Grand mean	2.81		3.76		10.13		19.72		60.9	

[a] Results from these laboratories were excluded due to deviations from the method.
[b] Identified as an outlier using Dixon's test.

TABLE II
Statistical Summary of Results

			Repeatability			Reproducibility		
Sample pair	**Number of labs**	**Grand mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Malt color								
A/B	9	2.81	0.05	1.9	0.14	0.11	4.5	0.31
C/D	9	3.76	0.26	7.1	0.72	0.29	8.0	0.81
E/F	9	10.1	0.29	2.9	0.81	0.91	9.0	2.54
G/H	9	19.7	0.59	3.0	1.64	1.00	5.1	2.80
I/J	9	60.9	1.68	2.8	4.72	3.97	6.5	11.1

temperatures, which appears to have resulted in higher color values, especially at the lower color levels. The results from these laboratories were excluded from the statistical analysis.

The statistical summary of the malt color data is shown in Table II. Repeatability and reproducibility coefficients of variation for the determination of malt color by the rapid method ranged from 1.9 to 7.1% and 4.5 to 9.0%, respectively. The ASBC official method for malt color does not contain any precision data for comparison purposes. A collaborative study conducted by the EBC Analysis Committee reported repeatability and reproducibility coefficients of variation for the spectrophotometric determination of malt color on congress mash to be 1.9–3.8% and 4.1–17.8%, respectively (4).

LITERATURE CITED

1. American Society of Brewing Chemists, *Methods of Analysis*. Statistical Analysis-4 Youden unit block collaborative testing procedure. ASBC, St. Paul, MN, 2014.
2. Li, Y., and Maurice, M. Development of a fast and reliable microwave-based assay for measurement of malt color. *J. Am. Soc. Brew. Chem.* 71:144-148, 2013.
3. Powell, C., Brendecke, S., Eurich, M., Guerdrum, L., Jennings, R., Lakenburges, K., MacLeod, A., Maradyn, D., Pachello, C., Palausky, J., Porter, A., Strachan, F., and Foster, B. Report of the Technical Committee. *J. Am. Soc. Brew. Chem.* 72:296-298, 2014.
4. White, F. H. Spectrophotometric determination of malt colour. *J. Inst. Brew.* 101:431-433, 2013.

Coordination of New and Alternate Methods of Analysis

Subcommittee members: J. Palausky, *chair*; S. Brendecke; M. Eurich; R. Foster; L. Geurdrum; R. Jennings; A. Macleod; C. Pachello; A. Porter; C. Powell; and K. Lakenburges (*ex officio*).
Associate members: J. Masschelin (TTB)
Corresponding members: E. Welten (EBC); and Y. Hida (BCOJ).

RECOMMENDATIONS

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

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

STATUS OF SUBCOMMITTEE

Membership and Meetings

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

The subcommittee held a meeting at the 2015 ASBC meeting in La Quinta, California. Topics of interest and discussion included the following:

- There is a need/interest in more rapid methods and instrumental methods for microbiology. Polymerase chain reaction (PCR) was briefly discussed. Information that is needed includes the number of laboratories performing PCR analysis and the instruments that are in use currently.
- "Fast Orange" for *Brettanomyces* detection was discussed. Collaborative analysis will be performed this year.
- Is there interest to pursue creation of an ASBC method for the analysis of bisphenol A (BPA) in canned beer?
- Is there interest in establishing a method for the determination of the energy values of beer (i.e., calories, carbohydrates, etc.)?
- Is there a need for developing a method for congress mash in high-gravity brewing?
- Is there a need to develop a method for the determination of organic acids in beer?

Topics for Polling

Polling questions were developed for online polling to gather information on potential new methods for collaborative study. These questions were formatted into two web-based surveys with assistance and administration by Scientific Societies staff. The topics in the online poll along with background information are described here and in the Appendix.

Input on new and alternative methods. This subcommittee and the Technical Committee receive input on potential new and alternative methods throughout the year. Much of the input comes through the ASBC Annual Meeting, but the poll is another valuable tool to gather additional information. This year's poll included questions concerning use of modifications of existing methods in the *Methods of Analysis* (*MOA*).

Spectrophotometric analysis. Discussions during the New and Alternative Methods session at the 2014 annual meeting indicated that new spectrophotometric methods were desired. Without extensive input during the session, it was decided to use polling to gather information on the current state of instrument capability and member needs to identify potentially new methods that could be examined.

Microbiology. As with the spectrophotometric analysis, discussions during the New and Alternative Methods session at the 2014 annual meeting indicated that new and faster microbiological methods were desired. Without extensive input during the session, it was decided to use polling to gather information on the current state of instrument capability, strict anaerobe testing, rapid method use, and yeast strain purity testing to identify potentially new methods that could be examined (Caroline Pachello).

Topics to archive. None.

APPENDIX
SUMMARIZED RESULTS FROM 2015 ONLINE POLLING

Top Line Results

- The new poll design of making shorter polls targeting specific subject matter resulted in a higher percentage completion. The completion rate, measured as the percentage of polls completed to polls started, on the 2014 poll was 49%.
- Over 750 individuals received invitations to complete the two 2015 polls.
- 108 individuals started the 2015 microbiology poll. The completion rate of the poll was 91%.
- 98 individuals started the 2015 analytical poll. The completion rate of the poll was 74%.
- 7 respondents submitted information on new and/or alternative methods.
- 4 respondents submitted information on modifications of existing methods.

Summary of Microbiology Poll

- **Microbiology instrument capabilities.** A significant percentage of laboratories have fluorescent microscope and thermocycler/electrophoresis/PCR capability. Based on this information, focus should be directed toward developing methods relating to these instruments.
- **Strict anaerobe testing.** A significant percentage of laboratories testing for strict anaerobes are not using the current ASBC methods available. This is an indication that we need to provide updated methods for strict anaerobe detection.
- **Rapid methods.** Respondents indicated they were using PCR and other methods. Identification seemed to be a common interest. This issue will need additional exploration.
- **Yeast strain purity.** Most people are not using the current *MOA* or any methodology for yeast strain purity.

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Summary of Analytical Poll

The following is a summary of comments submitted regarding new and/or alternative methods that are not currently in the *MOA*.

- **Replacement for malt DP analysis.** Limit dextrinase, alpha-amylase, and beta-amylase Megazyme methods undertaken together may be used to better predict potential malt fermentability. Evans, A more cost- and labor-efficient assay for the combined measurement of the diastatic power enzymes beta-amylase, alpha-amylase, and limit dextrinase, *J. Am. Soc. Brew. Chem.* 66:215-222, 2008. Evans et al., Refining the prediction of potential malt fermentability by including an assessment of limit dextrinase thermostability and additional measures of malt modification, using two different methods for multivariate model development, *J. Inst. Brew.* 116:86-97, 2010.
- **Total β-glucan.** AACC International Method 32-22.01.
- **Alpha- and beta-acids.** The general technique has the potential for resolving the alpha- and beta-acid contents without necessarily measuring spectral absorbance at wavelengths where the absorbance is rapidly changing with wavelength. The specific technique used measurements taken from strip-chart recordings and were not well adaptable to a universal method. However, with modern spectrometer capabilities, it might be worth taking another look at this technique. Gutierrez, Derivative spectroscopy applied to the determination of alpha- and beta-acids in hops, *J. Inst. Brew.* 98:277-281, 1992.
- **Isomerized and reduced alpha acids.** Spectral method for determination of reduced iso-alpha acids. It would be useful because there is no official spectral method for the determination of tetrahydro-iso-alpha acids (Tetra), and a number of breweries purchase Tetra products by spectral analysis. Maye et al., Spectrophotometric analysis of isomerized alpha-acids, *J. Am. Soc. Brew. Chem.* 60:98-100, 2002.
- **Isomerized and reduced alpha acids.** This method would measure the concentrations of isomerized and reduced alpha-acids in alkaline methanol. Isomerized and reduced alpha-acids absorb ultraviolet and visible light, making quantitative analysis by spectrophotometric analysis a simple and accurate tool. J. P. Maye, S. Mulqueen, J. Xu, and S. Weis, Spectrophotometric analysis of isomerized alpha-acids, Haas Hop Products, Washington, DC 20016.
- **Yeast vitality.** Gabriel et al., Optimised acidification power test of yeast vitality and its use in brewing practice, *J. Inst. Brew.* 114:270-276, 2008.

Packaging Subcommittee Annual Meeting Report

Subcommittee members: S. Brendecke, *chair*; A. Porter; C. Benedict (*ex officio*).

RECOMMENDATIONS

The subcommittee was formed to update methodology that related to packaging. Existing ASBC methods for packaging described parts of cans, bottles, and ends. There are additional existing methods also describing some of the tools used to measure packaging materials and measurement of operations performed on packaging materials. The current focus of the subcommittee is the technical aspects of the packaging filling operations; the first presentation was given for receiving palletized cans at the brewery, to the monthly craft brewers subcommittee in late 2013. This presentation can be given again if it decided that a permanent record of it is needed.

Assessment is needed at this point to determine the direction of the subcommittee. There has been discussion in the past about microbiological measurement of packaging lines. The new and alternate polls did show an interest by members to pursue this. At this point it is unclear if the information produced should be a provisional method, guideline documentation, video, or appendix to other media being produced by ASBC.

There has also been interest in the calculations of fill volumes, correcting for the dissolved CO_2. ASBC has a method, Fills-2, for making these calculations. The method uses partial molar volume (PMV) as a variable for the calculations. PMV is described in *J. Am. Soc. Brew. Chem.* 49:23, 1991, and the article provides a method for determining PMV.

A publication in *J. Inst. Brew.* 112:4, 2006, gave a PMV of 0.73 for standard European beers. The article also provides a calculation for determining PMV using only % ethanol and real extract.

PRELIMINARY CONSIDERATION OF METHODOLOGY

The microbiological information that is to be put forward to interested parties should describe aerobic and anaerobic testing methods used on packaging lines. The information should include recommended sampling rates on the filling lines and areas associated with the packaging lines. The information should also include known key locations on the filling line for sampling. Differences between monitoring glass filling, keg filling, and can filling lines also will need to be addressed.

For determination of fill volumes, more background research needs to be done to ensure more recent work has not been done for calculating PMV. Assuming that there has not been any, then there may be an opportunity for research to be conducted on additional beer types (IPAs, stouts, barley wines, etc.) to determine their PMVs. The work could also determine if the EBC calculation for PMV using % ethanol and real extract works for these different types of beers.

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Phenolic Yeast Detection

Subcommittee members: Trevor Crowley, *chair*; Caroline Pachello (*ex officio*).

This was the second year of the subcommittee's existence. The purpose of the subcommittee is to evaluate the use of yeast medium supplemented with ferulic acid to detect phenolic yeast strains able to decarboxylate ferulic acid, resulting in the formation of this 4-vinyl guaiacol (4-VG or 2-methoxy-4-vinylphenol) providing an aromatic smoky or clove-like compound.

During its first year, the subcommittee performed testing to detect phenolic yeast using ferulic acid yeast medium. The test results were not acceptable owing to some of the collaborators identifying nonphenolic yeast strains as phenolic. Additionally, some collaborators were not able to correctly distinguish phenolic producing yeast. Recommendations resulting from the first year were to include prescreening of collaborators to ensure that they can correctly detect 4-VG spiked into the media matrix compared with nonmedia matrix. Collaborators who cannot correctly identify the spiked sample will not be included in the next round of testing. It was also recommended that adding additional incubation time be investigated to aid in detecting the 4VG aroma.

There was no activity with the subcommittee this year to allow time to coordinate participant screening for phenolic recognition and to redesign test regime to reflect a more typical sensorial test design. The subcommittee will be reassembled in fall of 2015. The new testing will involve a modified two-alternative forced choice test for prescreening to determine which laboratories pass and can participate. Two prescreened participants will be required per laboratory. Blind test yeast strain samples will be evaluated using paired testing with nonphenolic and phenolic producing yeast strains Collaborators will be asked to select which sample within a set is a phenolic-producing yeast.

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Report of 2014-BCOJ Collaborative Work

Determination of Wheat Protein in Beer by FASPEK Wheat/Gluten (Gliadin) ELISA Kit II

Subcommittee Members: T. Watanabe (Sapporo Breweries Ltd.), *chair*; T. Akiba (Wakodo Co., Ltd.); R. Arai (Kirin Company, Ltd.); Y. Ishizuka (Suntory Beer, Ltd.); M. Kanauchi (Miyagi University); M. Kato (Kirin Company, Ltd.); K. Kusaka (National Research Institute of Brewing); K. Kuwahara (Morinaga Institute of Biological Science, Inc.); T. Miyagi (Orion Breweries, Ltd.); S. Mori (Pokka Sapporo Food & Beverage Ltd.); A. Ohuchi (Asahi Breweries, Ltd.); S. Tatsu (Suntory Global Innovation Center, Ltd.)

Keywords: ELISA, Wheat protein

CONCLUSIONS

1. Relative repeatability standard deviation (RSD_r) and relative reproducibility standard deviation (RSD_R) for determination of wheat protein content using FASPEK Wheat/Gluten(Gliadin) ELISA Kit II ranged from 2.0 to 4.7% and from 7.5 to 20.1%, respectively, and were judged acceptable.
2. Recovery of wheat protein was 83.8%, and was judged acceptable.

RECOMMENDATIONS

1. It was concluded that the ELISA method using FASPEK Wheat/Gluten(Gliadin) ELISA Kit II is capable of determining wheat protein content in beer containing 20 µg/mL or less.
2. The subcommittee recommends that the ELISA method using FASPEK Wheat/Gluten(Gliadin) ELISA Kit II be adopted for inclusion in *the Methods of Analysis of BCOJ*.
3. Discharge the subcommittee.

Barley malt is the main ingredient in most Japanese beer but wheat is also used as an ingredient in some types of beer. It is desired to establish the quantitative method for allergic wheat protein. The method would enable the detection of wheat protein contamination into wheat-free beer through a production line. It will be useful to assess the allergic influence on consumers.

The Consumer Affairs Agency in Japan indicates that the ELISA method is applicable to quantitate allergic wheat protein, and foods with wheat protein contents exceeding 10 µg/g are evaluated positive. The ELISA method has not been conducted with beer in Japan until now.

The BCOJ subcommittee was charged with evaluating the ELISA method. We evaluated wheat protein contents in beer with FASPEK Wheat/Gluten(Gliadin) ELISA Kit II, which met the guidelines determined by the Consumer Affairs Agency (1).

The collaborative work was performed by 12 collaborators. The statistical summary of results were shown as follows: RSD_r ranged from 2.0 to 4.7%; RSD_R ranged from 7.5 to 20.1%. Recovery of wheat protein was 83.8%. We judged these results were acceptable. The subcommittee recommends that the ELISA method using FASPEK Wheat/Gluten(Gliadin) ELISA Kit II be adopted for inclusion in *the Methods of Analysis of BCOJ*.

PROCEDURE

The collaborative work was performed by 12 collaborators. Six sample pairs (A/B, C/D, E/F, G/H, I/J, and K/L) were provided for study. Each analysis was carried out in duplicate.

On the website (4), it is shown that barley malt also gives slightly positive results with FASPEK Wheat/Gluten(Gliadin) ELISA Kit II. Therefore, we chose one pair (A/B) as beer not containing wheat to evaluate the value of false positives. We also added one pair (K/L) which was spiked with standard wheat protein at about 10 µg/mL to sample pair A/B to evaluate recovery. The measured value of the spiked wheat proteins was 11.43 µg/mL. Recovery was calculated by the following equation: Recovery (%) = [(wheat protein of the sample pair K/L – wheat protein of the sample pair A/B)/11.43] × 100.

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TABLE I
Wheat Protein Content (µg/mL) Determined Using FASPEK Wheat/Gluten(Gliadin) ELISA Kit II[a]

	Sample pair		Sample pair		Sample pair		Sample pair		Sample pair		Sample pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	1.84	1.82	8.33	8.68	7.85	7.29	16.71	17.10	14.86	14.60	11.45	11.87
2	1.34[b]	1.08[b]	5.53	5.48	5.62	5.31	14.97	15.09	14.07	13.82	10.72	11.01
3	1.18	1.17	5.05	5.27	5.22	4.86	11.15	11.10	10.33	10.31	9.07	9.21
4	1.64	1.65	7.22	7.27	6.96	7.28	17.47	17.97	14.31	15.09	11.83	12.00
5	1.71	1.61	7.97	8.53	7.15	6.87	16.39	16.01	16.72	16.97	10.70	11.09
6	1.27	1.28	6.45	6.71	6.43	5.90	14.40	14.43	10.76	11.32	11.79	11.35
7	1.62	1.52	6.23	6.16	5.37	5.51	16.08	16.62	12.14	12.39	10.61	10.79
8	1.48	1.68	6.66	6.19	6.17	6.13	15.64	14.70	10.55	11.27	10.66	10.37
9	1.11	1.06	4.78	5.25	5.64	5.32	12.92	12.74	10.58	10.64	10.56	11.01
10	1.02	1.09	5.41	5.57	5.09	5.11	14.16	13.96	10.24	10.93	10.18	10.38
11	1.27	1.13	7.71	7.59	6.25	6.62	15.92	16.47	14.66	14.12	11.29	11.63
12	1.87[b]	2.29[b]	7.24[b]	7.63[b]	8.24	8.64	15.54	15.68	13.56	13.53	12.20	11.82
Mean	1.41	1.40	6.49	6.61	6.33	6.24	15.11	15.16	12.73	12.92	10.92	11.04
Grand mean	1.41		6.55		6.28		15.13		12.82		10.98	

[a] Data as false positive because sample A/B do not contain wheat.
[b] Outliers identified based on the Consumer Affairs Agency Notice and excluded from the statistical analysis.

TABLE II
Statistical Summary of Results

	Sample pair					
	A/B	**C/D**	**E/F**	**G/H**	**I/J**	**K/L**
Number of laboratories	10	11	12	12	12	12
Grand mean (m)	1.41	6.55	6.28	15.13	12.82	10.98
Repeatability standard deviation (S_r)	0.07	0.22	0.24	0.30	0.32	0.23
Relative repeatability standard deviation (RSD_r, %)	4.7	3.3	3.9	2.0	2.5	2.1
Reproducibility standard deviation (S_R)	0.28	1.24	1.08	1.84	2.16	0.83
Relative reproducibility standard deviation (RSD_R, %)	20.1	18.9	17.2	12.2	16.9	7.5
Recovery (%)	—	—	—	—	—	83.8

Four other pairs containing wheat proteins at ~5 to 20 µg/mL were chosen based on the results of the pretest.

The samples were degassed at 20 to 30°C, and analysis was performed using FASPEK Wheat/Gluten(Gliadin) ELISA Kit II. All procedures were performed based on the manufacturer's manual (5).

A microplate reader with filters of 450 nm and 600 to 650 nm was used for measuring absorbance.

The results were processed according to JIS Z 8401:1999 guidelines (2) and statistical analysis for the processed data was performed according to JIS Z 8402-2:1999 guidelines (3) and the Consumer Affairs Agency Notice.

RESULTS AND DISCUSSION

The results for wheat protein content are shown in Table I. The data of which coefficient of variance value is over 20% were excluded from the statistical analysis based on the Consumer Affairs Agency Notice. The other data were checked for outliers using Mandel's *h* and *k* statistics, and Cochran and Grubbs outlier test, and outliers were not detected. The statistical summary of results is shown in Table II.

Each of the calculated analytical values ranged as follows: RSD_r ranged from 2.0 to 4.7%, RSD_R ranged from 7.5 to 20.1%, and recovery was 83.8%. These values were judged acceptable.

It was concluded that the method is capable of determining wheat protein content in beer containing 20 µg/mL or less. The subcommittee recommends that the method should be adopted for inclusion in *the Methods of Analysis of BCOJ*.

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