Technical Committee and Subcommittee Reports

2016–2017 Report of the Technical Committee

Committee Members: M. Eurich, *Chair*; S. Brendecke; L. Barr; R. Jennings; E. Jorgenson; K. Lakenburges; A MacLeod; C. Pachello; J. Palausky; A. Porter; 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 2016–2017. For 2017 one new method is recommended for inclusion in the ASBC *Methods of Analysis* (MOA):

One method was evaluated and is recommended for inclusion in the MOA for 2017.

- Lipoxygenase activity in malt, chaired by Bobby Monsour (Rahr Malting Co.)

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

- Hop aroma compound analysis by GCMS, international method, chaired by Nils Rettberg (VLB-Berlin).
- Beer Method 25B- Diacetyl, collaboration for an update to the method for alignment using calibration standards for current industry products, chaired by Robert Fulwiler (Fremont Brewing Co.).

The ASBC Technical Committee regularly reviews each section of MOA. In 2016/17 reviews of one section of the ASBC Methods of Analysis will be completed:

- Beer, chaired by Karl Lakenburges (Anheuser-Busch InBev) and Mark Eurich (New Belgium Brewing Co.)

In order to gather information on the requirements of the ASBC membership, the Innovative Methods, formerly titled Coordination of New and Alternate Methods of Analysis, organized roundtable discussions at the annual meeting in Sanibel, FL. Joe Palausky (subcommittee chair) worked closely with the Technical Committee chairs to collect feedback for these breakout sessions and input from these roundtable discussions. Please review the Innovative Methods report submission for additional details. Aaron Porter will chair the Innovative Methods subcommittee starting in 2018.

In addition, the following topic will undergo preliminary analysis and ruggedness testing prior with the possibility of collaborative study in 2018:

- FastOrange™ Brett and Yeast Agar Detection, chaired by Guy Stewart (New Belgium Brewing Co.).

This report is published as submitted. The pages were numbered at the ASBC headquarters office, but the report was not edited.

http://dx.doi.org/10.1094/ASBCJ-2017-4918-01

As in previous years, the following standing subcommittees continue:

- Innovative Methods, formerly titled, Coordination of New and Alternate Methods of Analysis, chaired by Joe Palausky (Boulevard Brewing Co.).
- International Methods, chaired by Mark Eurich (New Belgium Brewing Co.)
- Craft Brew, chaired by Eric Jorgenson (Victory Brewing Co.).
- Sensory Science, chaired by Lindsay Barr (New Belgium Brewing Co.).
- International Hop Standards Committee, chaired by Bob Foster (MillerCoors).
- Packaging Methods, chaired by Scott Brendecke (MicroStar Logistics).
- Microbiological Methods in Brewing, chaired by Caroline Pachello (MillerCoors).
- Soluble Starch, chaired by Rebecca Jennings (Rahr Malting Co).
- Check Services which is changing to Lab Proficiencies, chaired by Rebecca Jennings (Rahr Malting Co) and Carol Ericson (ASBC- Scientific Societies).

In 2016/17 the Technical Committee collaborated with the Brewers Association in video development to provide additional content to MOA. A cobranded webinar was also completed:

Videos

- Calibration and Use of a Hydrometer
- Calibration and Use of a Density Meter
- Wort and Beer Sample Filtration
- Setting up a Microscope
- Yeast Cell Counting

Webinar

- Malt Hot Steep Sensory Method

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

- Updated photos for Common Brewery-Related Microorganisms.

No student grant evaluations were conducted in 2016/17.

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 would like to formally acknowledge the many subcommittee members who have participated over the past year.

I would like to recognize the dedication and hard work put forth by all members of the Technical Committee over the previ-

ous year. The continual enthusiasm and commitment demonstrated by the team is sincerely appreciated and I firmly believe is key to ensuring that the ASBC Methods of Analysis remains contemporary, relevant, and of exceptional practical value to the brewing community.

Finally, I would like to thank Eric Welten for his time and efforts as the Chairman of the EBC Analysis Committee and his active participation on the ASBC Technical Committee. We welcome Lene Bech Lene, Carlsberg Breweries, as the newly elected Chairperson of the EBC Analysis Committee.

Innovative Methods, formerly 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.

Lab Proficiencies, formerly Check Services
(*Rebecca Jennings, rjennings@rahr.com* and *Carol Ericson, cericson@scisoc.org*)

This is a standing subcommittee to ensure value and relevancy of the ASBC Check Sample Service. In 2018 the ASBC will move to an update software platform and is being retitled Lab Proficiencies. 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@victorybeer.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 Barr, lBarr@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, in process evaluation, beer lexicon, 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 un-isomerized hop standards for the brewing, hops, and related industries.

Packaging Methods
(*Scott Brendecke, sbrendecke@microstarkegs.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
(*Mark Eurich, meurich@newbelgium.com*)

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.

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 Caroline Pachello directly.

FastOrange™ Brett and Yeast Agar Detection Brewing
(*Guy Stewart, gstewart@newbelgium.com)*

This subcommittee aims to analyze the effectiveness of PIKA FastOrange Brett and Yeast Agar media types. FastOrange Brett Agar is designed to selectively culture and detect Brettanomyces/Dekkera yeast, while suppressing the growth of brewer's yeast and bacteria. FastOrange Yeast Agar is designed to detect contamination of brewery products by wild yeast or mold.

Hop Aroma Analysis by GCMS
(Nils Rettberg, nrettberg@vlb-berlin.org)

This subcommittee aims to develop methods for the analysis of hop aroma compounds using GCMS. Full details of this subcommittee will be confirmed in due course as well as international collaboration with the European Brewing Convention Analytical Committee.

Beer Method 25B- Diacetyl Update
(Robert Fulwiler, robert@fremontbrewing.com)

This subcommittee will complete a full collaboration for an update to the method for alignment using calibration standards for current industry products. Please contact Robert Fulwiler for information or if you wish to participate.

Lipoxygenase Activity in Malt
(Bobby Monsour, rmonsour@rahr.com)

LOX is a family of enzymes that catalyze the oxygenation of poly-unsaturated 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 produced a method for inclusion into the MOA.

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 multi-laboratory 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 non-expert 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.

AMERICAN SOCIETY OF BREWING CHEMISTS, INC.
Report of SubCommittee

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INNOVATIVE METHODS

Subcommittee Members: J. Palausky, *Chair*; L. Barr; S. Brendecke; R. Foster; R. Jennings; E. Jorgenson; K. Lakenburges; A. Macleod; C. Pachello; A. Porter; N. Rettberg; and M. Eurich (*ex officio*).

Associate Members: J. Masschelin (TTB)

Corresponding Members: E. Welten (EBC); and Masahito Muro (BCOJ).

RECOMMENDATIONS

1. Obtain input on new methods from the ASBC membership
2. Evaluate feasibility and potential interest for new methodologies.
3. Evaluate potential changes to existing methodologies.

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

STATUS OF SUBCOMMITTEE

Membership and Meetings

Given the very close tie this subcommittee has with the Technical Committee, the Innovative Methods subcommittee is an integral part of the Technical Committee's activities. Additional subject matter experts will be added to this subcommittee, or consulted with on an as needed basis.

The Innovative Methods subcommittee held an open meeting at the 2017 ASBC meeting in Fort Meyers, FL. The status of current subcommittee tasks were presented and discussed with the attendees. Following the discussion of status, the attendees were encouraged to join one of seven subgroup

http://dx.doi.org/10.1094/ASBCJ-2017-4924-01

meetings to continue discussions on more specific topics. The subgroup discussions are summarized below.

Advanced Instrumentation - 15 participants
There is currently a draft methodology for analyzing aroma compounds using dynamic headspace-GC/MS and there is also a draft methodology for analyzing hop compounds using solid phase micro extraction or SPME-GC/MS. An analytical method that would address both of these techniques would be valuable. The discussion at this meeting was primarily centered on the dynamic headspace-GC/MS. The main discussion in this meeting was on the draft methodology and the experiences of the attendees in using the technique. Many ideas were discussed to clarify the draft methodology. There was also considerable discussion on the target compound list that should be included in the methodology as many laboratories focus on different compounds.

Beer and Analytical Methods – 12 participants
The main focus of the discussion was the analysis of Viscinal Diketones or VDKs by distillation and how the current method (Beer 25B) should be revised. The meeting was beneficial in that it brought together a good number of laboratories that perform the method and identified potential participants for collaborative analysis. Through the discussions and clarifications brought forth at this meeting, a new collaborative will be scheduled for 2017/2018.

Microbiology Methods -13 participants
The items discussed at this session were:

- General open discussion on beer microbiology and methodologies.
- Discussion of issue of dextrin-fermenting wild yeasts.
- Discussion of potential collaborations with BCOJ.
- Request documentation and images for potential update to microbiology catalog.
- A new collaborative will be conducted in 2017/2018 using Fast Orange ™ media for rapid detection of brettanomyces.

Raw Materials – 10 participants
The items discussed at this session were:

- General open discussion on raw materials and malt testing.
- A new collaborative will be conducted in 2017/2018. DON in barley by Lateral Flow Assay.
- Discussion of potential interest for determining the gelatinization temperature of cereal grains by differential scanning calorimetry.

Packaging Methods - 8 participants
The items discussed at this session were:

- CO2 purity – Taste/Sampling/Testing. ISBT Standards-2017 Guideline is currently being written. Can ASBC link with them? Frank Voerklen and Ken Jennings are resources and Frank sits on ISBT Technical Committee.
- TPO Studies – What are next steps? How can we use the TPO Verifier?
- Standardized sampling methodology for microbiology for all package types (kegs, bottles, cans, growlers, etc.)
- Nitrogen measurement for packages (e.g., Ball widget cans). Do we have or can we get a direct measurement method?

- Testing of glue strength/overall package testing/color standards. Can guidance documents be written?

Craft Brewing – 19 participants
The items solicited from the group are:

- Yeast rehydration guide
- Yeast selection guide
- Elaborating on media selection/micro regimen by brewery size
- KOH test as a replacement for Gram staining. Create MOA and incorporate into GYOL.
- Guide to allergens, glass & chemical HAACP
- Guide to market withdraw/recall
- Hands-on demonstration or video of plating
- Guide to how scale up staff/hiring
- Short videos/potential BA collaboration ideas include; titratable acidity, yeast propagation, database management, Excel templates, dissolved oxygen

Sensory Analysis – 18 participants
The Sensory Analysis subcommittee modified/produced two methods this year. Discussion was related to the activities of the Sensory subcommittee. The items discussed in the group included:

- New malt sensory method. Sensory Analysis 14 – Hot Steep Malt Sensory Evaluation Method. The method presents a standardized preparation for evaluating raw malt.
- New hop sensory method. Sensory Analysis 15 – Hop Tea Sensory Method. The method presents a standardized preparation for evaluating raw hop cones and pellets.
- Lexicon for malt and hop descriptors.
- Sensory webinars have been very successful. The next scheduled webinar will be November, 2017.

AMERICAN SOCIETY OF BREWING CHEMISTS, INC.
Report of SubCommittee

DETECTION OF LIPOXYGENASE IN MALTED BARLEY BY SPECTROPHOTOMETER (IM)

Subcommittee Members: B. Monsour, *Chair;* I. Anaya; J. Ashlock; H. Babkhan; B.Bazaluk; C. Carvalho; D. Griggs (EBC); S. Harasymow (EBC); T. Henderson; M. Izydorczyk; T. Kishimoto (BCOJ); R. Lahlum; JD. Lowe; L. McIntyre; T. McMillan; S. Millard (*EBC*); G. Powell (*EBC*); M. Rodriguez; M. Schmitt (*EBC*); S. Schwebel; J. Testi; T.Ueda (BCOJ); M. Walters; and R. Jennings (*ex-officio*).

Keywords: Flavor, LOX, Stability, Staling, Lipoxygenase

CONCLUSIONS

1. Repeatability coefficients of variation for the determination of lipoxygenase activities in malted barley by spectrophotometry ranged from 7.09 to 13.5% and were judged acceptable.
2. Reproducibility coefficients of variation for the determination of lipoxygenase activities in malted barley by spectrophotometry ranged from 20.30 to 67.60% and were judged acceptable.
3. Using the CV is not the best determination of statistical analysis for diminutive value data sets.

RECOMMENDATIONS

1. The subcommittee recommends that the method for Detection of Lipoxygenase in Malted Barley by Spectrophotometer be included in *Methods of Analysis.*

This report is published as submitted. The pages were numbered at the ASBC headquarters office, but the report was not edited.

http://dx.doi.org/10.1094/ASBCJ-2017-4932-01

2. Discharge the subcommittee.

This is the third year of the subcommittee's existence. The subcommittee was started on the recommendation from industry professionals that there was a need for collaboration on a unified procedure for lipoxygenase (LOX) in malted barley to be used across multiple laboratories.

In the first year, a questionnaire was distributed to all subcommittee members to determine methodologies currently in the malting industry. Based on the response to the questionnaire, a method of analysis for the detection of LOX in malted barley was constructed.

In the second year, the method was distributed and evaluated by the subcommittee members through collaborative testing. A total of eight samples representing four sample pairs (similar but distinct) plus two check samples were sent to each collaborator with a range of LOX activity values. It was noted that one of the buffer protocols was incorrect, this was corrected and the method was redistributed. Based on recommendations in the second year, a second control was added to the set of samples to increase the reliability of testing. The collaborators were asked to follow the method included with samples and to note any deviations from the prescribed protocol.

Testing for the presence and amount of LOX in malted barley is significant as it has shown to directly correlate to the stale tastes in beer and with decreased foam retention (Nieuwoudt 2014). The long-term effects that LOX has on wort and beer is a considerably shorter shelf-life and a "cardboard" stale flavor, which could lead negatively to the maltster's and brewer's brand image. Primarily, there are two isoenzymes that have been found to lead to these negative effects – LOX-1 and LOX-2 – with the more concerning of the two being LOX-1 as it has been found to catalyze the formation of trans-2-nonenal (flavor and smell) and trihydroxyoctadecenoic acids (foam stability). These Lipoxygenase enzymes have been found in the barley embryo and during the germination of the seed, but are also derived during the mashing of the malt. (Nieuwoudt 2014)

PROCEDURE

Four sample pairs plus two check samples were pre-ground using a Buhler DFLU mill set to fine grind (**Malt-4**), vacuum sealed and sent to each collaborator. Each sample pair was of the same barley variety, malted under the same conditions, but were from different production lots. The sample pairs were chosen to include a range of LOX enzymatic activity. Each malt sample was extracted with an acetate buffer, pH 5.0, for 20 minutes. The extract was reacted with a 2.5% linoleic acid solution in a phosphate buffer matrix, pH 6.8, for four minutes. The enzymatic activity was monitored by the change in absorbance at 234 nm. Absorbance readings were recorded at one and four minutes. Results were evaluated using the Youden unit block design (ASBC MoA Youden Unit Block Collaborative).

RESULTS AND DISCUSSION

Results were collected from 11 collaborators for four sample pairs and two check samples. Results from two collaborators were excluded prior to statistical analysis because of known deviations from the prescribed experimental protocol. Results from two collaborators for one sample pair were excluded because the incorrect sample set was provided. Data for the LOX activity is presented in Table I.

The statistical summary of the LOX activity data is presented in Table II. Repeatability and reproducibility coefficients of variation for the determination of LOX activity in malted barley by spectrophotometry ranged from 7.09 to 13.52% and 20.30 to 67.60%, respectively, and were judged acceptable. The last sample pair was of very low value which results in a very high cv. This sample set consisted of data ranging from 0-1.7 U/g. Specific to this assay, values <2 U/g are considered a “LOXless or low-LOX” varieties. Values this low do not have a linear correlation as far as time vs. concentration. Therefore, the coefficient of variation may not be the best guideline to use when analyzing a sample set that results in a very low value. As well as factoring in low values, the coefficient of variation takes into account both the standard deviation and the mean (Higgins 2015). The sample pairs made up in this collaboration used two different samples with similar, but different concentrations of LOX and will have

varying standard deviations and means. Therefore, it is suggested that the r-values, or the coefficient correlations, are a better means to determining if the collaboration was statistically robust.

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5. Hongxia Ye, Sequence variation and haplotypes of lipoxygenase gene LOX-1 in the Australian barley variety. BioMed Central, Ltd., 2014.
6. Higgins, Jim. The Radical Statistician, 5th Edition. Chapter 2, The Correlation Coefficient. Copyright 2015.

Table I

Lipoxygenase activity (U/g) in Malted Barley by Spectrophotmetry

	Sample Pair		Sample Pair		Sample Pair		Sample Pair		Check Samples	
Collaborator	**A**	**F**	**B**	**H**	**D**	**I**	**G**	**J**	**C***	**E***
1	7.5	8.8	12.6	7.9	15.4	15.4	1.6	1.6	21.1	1.7
2	5.4	5.3	7.2	6.7	9.9	9.4	0.5	0.6	15.3	0.6
3	…	…	…	…	…	…	…	…	…	…
4	11.8	11.7	14.6	14.5	16.7	17.8	1.6	1.6	23.7	2.0
5	9.0	8.3	11.3	9.7	12.3	12.1	1.6	1.7	19.8	2.1
6	7.7	9.1	10.2	11.9	13.72[a]	8.7[a]	0.1	0.4	25.1	0.3
7	7.8	8.5	10.3	11.0	13.13[a]	7.54[a]	0.1	0.2	22.6	3.8
8	…	…	…	…	…	…	…	…	…	…
9	9.3	11.2	12.9	15.0	18.1	15.2	0.9	0.5	22.8	1.2
10	7.3	8.4	9.8	9.1	16.3	13.8	1.4	1.3	20.4	1.5
11	9.9	11.4	12.9	14.3	17.3	17.6	0.5	0.5	22.3	0.9
Mean[b]	8.39	9.16	11.31	11.11	15.13	14.47	0.92	1.10	21.45	1.55
Grand Mean[b]	8.78		11.21		14.80		0.92			

[a] Different data set provided. Excluded from calculations.

[b] Calculated excluding outliers.

Table 2

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}
A/F	9	8.71	0.63	7.15	1.76	1.92	21.90	5.38
B/H	9	11.21	1.48	13.21	4.15	2.65	23.61	7.41
D/I	7	14.80	1.05	7.09	2.94	3.01	20.30	8.41
G/J	9	0.92	0.12	13.52	0.35	0.62	67.60	1.74

[a]All calculations were made based on Table I.

APPENDIX [Method Removed – will appear in *Methods of Analysis*]