Technical Committee and Subcommittee Reports

2013–2014 Report of the Technical Committee

Committee members: C. Powell, *chair*; S. Brendecke; M. Eurich; L. Guerdrum; R. Jennings; K. Lakenburges; A MacLeod; D. Maradyn; C. Pachello; J. Palausky; A. Porter; F. Strachan 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 2013–2014. As a result, the following methods are being recommended for inclusion in the ASBC *Methods of Analysis* (MOA):

- Rapid Immunoassay Method for Deoxynivalenol Analysis in Barley, chaired by Theresa Chicos (Rahr Malting).
- Headspace Gas Chromatography/Electron Capture Detector Analysis of Total Vicinal Diketones in Beer, chaired by Grant Ruehle (New Belgium Brewing).
- β-Glucan in Wort – Spectrophotometric Method, chaired by Unju Kim (Novozymes).

Two methods were evaluated but not recommended for inclusion in MOA at this time. Additional development and further evaluation is required prior to recommendation of the following methodology:

- Method for the Identification of Phenolic Character in Yeast, chaired by Caroline Pachello (MillerCoors).
- Wort Amino Acids by HPLC, chair TBD.

In 2013/14, the Technical Committee were involved in webinar/video development to provide additional content to MOA:

- The Sensory Science subcommittee (Lindsay Guerdrum, New Belgium Brewing Co) produced six webinars covering a range of sensory related topics.
- The Packaging subcommittee (Scott Brendecke, Ball Corporation) produced a webinar on aspects of packaging quality control that will be repeated in the coming year.

This series will continue in the coming year and the Craft Brew Subcommittee (Fred Strachan, Sierra Nevada) has produced a series of "Grow your Own Lab" webinars that will be launched in early 2015.

In order to gather information on the method requirements of the ASBC membership, the Coordination of New and Alternate Methods of Analysis Subcommittee submitted a survey to members in 2014. 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 2014 Brewing Summitt in Chicago, Illinois. Based on the polling results and feedback at this meeting, a number of methods have been recommended for collaborative study in 2014–2015:

- Rapid method for malt color, chaired by Betsy Roberts (Briess Malt).
- NIBEM for foam stability, chaired by Mark Eurich (MillerCoors).
- Hop analysis by GCMS, chaired by Kimberley Bacigalupo (Sierra Nevada).

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, chaired by Allen Eastlund (Rahr Malting).
- Beer filtration task force, chaired by Aaron MacLeod (Canadian Grain Commission) 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).
- International Methods, chaired by Chris Powell (University of Nottingham).
- Craft Brew, chaired by Fred Strachan (Sierra Nevada).
- Sensory Science, chaired by Lindsay Guerdrum (New Belgium Brewing).
- 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).
- Check Services, chaired by Rebecca Jennings (Rahr Malting) and Jodi Grider (ASBC SciSoc).

The ASBC Technical Committee also regularly reviews each section of MOA, including a partial review of MOA-Beer in 2013/14. In 2014/15, the review of this section will be completed and additional reviews initiated:

- Beer, chaired by Karl Lakenburges (Anheuser-Busch InBev) and Mark Eurich (MillerCoors).
- Barley, chaired by Rebecca Jennings (Rahr Malting).
- Statistical analysis of samples, chaired by Aaron MacLeod (Canadian Grain Commission).

Two student grant proposals have been submitted for consideration by ASBC BOD for 2014/15. Interested individuals should contact the Technical Committee Chair (Chris Powell, University of Nottingham) or the relevant Subcommittee Chairs below:

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

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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).

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. In particular, Jim Munroe (retired member, formerly of Anheuser-Busch) continues to provide statistical input and recommendations to the Check Services program and his input continues to be gratefully appreciated.

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 is key to ensuring that the ASBC Methods of Analysis remains contemporary, relevant, and of exceptional practical value to the brewing community.

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
(*Fred Strachan, fred@sierranevada.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 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.

Method 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 which 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
(*Mark Eurich, mark.eurich@millercoors.com*)

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

Hop Analysis by GCMS
(*Kimberly Bacigalupo, Kimberly@sierranevada.com*)

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
(*Allen Eastlund, aeastlund@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 aims to evaluate the techniques which are currently being utilized across laboratories and to develop a standard procedure.

MOA Review: Statistical Analysis of Samples
(*Aaron MacLeod, aaron.macleod@grainscanada.gc.ca*)

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, mark.eurich@millercoors.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.

MOA Review: Barley
(*Rebecca Jennings, rjennings@rahr.com*)

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

β-Glucan in Wort – Spectrophotometric Method

Subcommittee members: U. Kim, *chair*; C. Adam; D. Brown; T. Chicos; S. Harasymow; T. Henderson; S. Jensen; R. Lahlum; C. Martens; E. Roberts; M. Rodriquez; G. Spedding; A. Stern; and A. MacLeod (*ex-officio*).

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the determination of β-glucan content by the spectrophotometric method ranged from 2.5 to 8.2% and 13.5 to 18.8%, respectively, and were judged acceptable.
2. Based on a comparison of means using a paired *t*-test, the spectrophotometric method was significantly different from the fluorescence method (Wort 18-B) at the 95% confidence level.

RECOMMENDATIONS

1. The subcommittee recommends that the spectrophotometric method for determination of β-glucan, as given in the Appendix, be included in *Methods of Analysis*.
2. Discharge the subcommittee.

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This was the first year of the subcommittee's existence. The subcommittee was formed to evaluate a spectrophotometric method for β-glucan determination in wort using the GlucaTest reagent supplied by R-Biopharm. The currently accepted method for β-glucan determination in wort is by fluorescence detection using calcofluor (Wort-18). However, many laboratories do not have this capability. Therefore, the method was developed to offer a simple, rapid, and low-cost alternative to the fluorescence method. A collaborative test was required to determine repeatability and reproducibility coefficients of variation for the new method prior to inclusion in the *Methods of Analysis*.

PROCEDURE

Four sample pairs of commercial malt, labeled A/B, C/D, E/F, and G/H, were sent to each collaborator covering a range of β-glucan levels. Each collaborator also received one bottle of GlucaTest Reagent plus β-glucan standards for construction of a calibration curve. Collaborators were asked to prepare a Congress mash (Malt-4) for each malt sample and determine the β-glucan content using the GlucaTest method. Those collaborators who also routinely run the calcofluor method were asked to determine

TABLE I
β-Glucan (mg/L) in Wort by Spectrophotometric Method

	Sample pair		Sample pair		Sample pair		Sample pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	58	56	96	102	138	150	266	283
2	93	93	153	146	200	202	383	387
3	72	75	123	114	172	176	217	294
4	55	61	102	102	143	138	272	280
5	72	81	132	125	171	178	341	351
6	71	80	111	109	151	147	154[a]	313[a]
7	95	108	191	147	184	194	311	307
8	69	77	112	108	157	160	289	306
9	60	74	113	108	153	149	253	255
10	85	95	135	129	206	202	313	333
11	85	94	119	133	181	188	315	369
12[b]	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-
Mean[c]	74	81	120	118	169	171	296	317
Grand mean[c]	78		119		170		306	

[a] Outlier at $P \leq 0.01$ based on differences (1).
[b] Data excluded due to known deviations from the method protocol.
[c] Calculated excluding outliers.

TABLE II
β-Glucan (mg/L) in Wort by Fluorescence Method (Wort-18B)

	Sample pair		Sample pair		Sample pair		Sample pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	81	82	93	95	170	170	244	292
3	65	67	86	87	163	172	250	249
4	91	99	118	117	247	238	360	365
5	117	128	149	143	320	328	471	481
6	60	67	78	84	161	177	269	256
7	92	96	110	119	182	185	339	360
8	86	95	97	101	238	245	345	412
9	84	90	99	95	182	168	237	252
Mean	85	91	104	105	208	210	314	333
Grand mean	88		104		209		324	

TABLE III
Statistical Summary of Results[a]

Sample pair	No. of labs	Grand mean	Repeatability			Reproducibility		
			S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Spectrophotometric								
A/B	11	78	3.6	4.6	10.0	14.6	18.8	40.9
C/D	11	119	10.1	8.2	28.4	22.3	18.1	62.5
E/F	11	170	4.3	2.5	12.1	23.0	13.5	64.5
G/H	11	308	17.9	5.8	50.4	44.8	14.6	125.6
Fluorescence								
A/B	8	85	2.3	3.2	6.4	18.6	22.1	52.1
C/D	8	105	3.5	3.4	9.8	19.8	19.7	55.3
E/F	8	208	9.7	5.5	27.2	53.0	25.4	148.4
G/H	8	322	18.1	5.6	50.6	77.9	24.2	218.2

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

TABLE IV
Comparison of Methods for Determination of β-Glucan in Wort Using the Paired *t*-Test for Differences Between Means

	Spectrophotometric	Fluorescence
Mean	159.6	180.5
Variance	7173	12085
Observations	58	58
Pearson correlation	0.9807	
Hypothesized mean difference	0	
df	57	
t stat	–3.22[a]	
P (T ≤ *t*) two-tail	0.002	
t critical two-tail	2.00	

[a] *t* statistic is greater (sign ignored) than *t*-critical indicating that the methods are significantly different at the 95% confidence level.

β-glucan by that method and submit both results on the data sheet. Results were evaluated by Youden unit block design (1) and methods compared using a paired *t*-test (1).

RESULTS AND DISCUSSION

Results from 13 collaborators were received for the four sample pairs. Results from two collaborators were excluded prior to statistical analyses due to failure to follow the method protocol. Data for the β-glucan content in wort, determined using the GlucaTest method, are presented in Table I. Data for the β-glucan content in wort, determined by calcofluor method, are presented in Table II. Outliers were identified using Dixon's ratio test (1).

The statistical summary of the β-glucan content in wort data are shown in Table III. Repeatability and reproducibility coefficients of variation for the determination of β-glucan content by the spectrophotometric method ranged from 2.5 to 8.2% and 13.5 to 18.8%, respectively, and were judged acceptable. These are similar results to those achieved in a previous ring test conducted by the EBC Analysis Committee (2).

Eight collaborators also submitted results using the fluorescence method (Wort-18). Repeatability and reproducibility coefficients of variation for the determination of β-glucan content by the fluorescence method ranged from 3.2 to 5.6% and 19.7 to 25.4%, which were similar to those obtained in a previous ASBC study (3).

The comparison of means between the spectrophotometric method and the reference method data using the paired *t*-test is shown in Table IV. With the results of all four sample pairs included, the means were significantly different between the spectrophotometric method and the fluorescence method for the β-glucan content based on the *t*-test at the 95% confidence level; however, the difference between the methods is small in comparison to the difference among laboratories.

LITERATURE CITED

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3. American Society of Brewing Chemists. Report on Determination of β-glucan in Congress Wort by Segmented Flow Analysis . *J. Am. Soc. Brew. Chem.* 69:296-297, 2011.

Rapid Immunoassay Method for Deoxynivalenol Analysis in Barley

Subcommittee members: T. Chicos, *chair;* B. Francisco; S. Jensen; J. Johnson; R. Lahlum; A. MacLeod; N. McMaster; E. Roberts; M. Rodriguez; A. Stern; T. Wijetuna; and R. Jennings (*ex officio).*

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the determination of deoxynivalenol (DON) using the Diagnostix EZ-Tox homogeneous enzyme immunoassay (HEIA) kit ranged from 9.4 to 11.8% and 19.4 to 27.2%, respectively, and were judged acceptable.
2. Based on an insufficient number of participants, the Neogen enzyme linked immunosorbent assay (ELISA) kit could not be qualified.
3. Based on the unpaired *t*-test, Barley-11 and the Diagnostix EZ-Tox HEIA kit were not significantly different at the 95% confidence level.

RECOMMENDATIONS

1. The subcommittee recommends that the rapid immunoassay method for DON in barley be included in *Methods of Analysis*.
2. The subcommittee recommends no further study on the Neogen ELISA kit due to insufficient number of participants.
3. Discharge the subcommittee for rapid immunoassay for DON analysis in barley.

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This subcommittee is in its fourth year of existence, started on the recommendation of the subcommittee for *Methods of Analysis* Malt Review (4). In its first year, this subcommittee only evalu-

TABLE I
Diagnostix EZ-Tox Homogeneous Enzyme Immunoassay Method for Detecting Deoxynivalenol (mg/L) in Barley

	Sample pair		Sample pair		Sample pair		Sample pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	0.6	0.6	0.9	1.1	2.1	1.8	2.7	2.4
2	0.5	0.7	1.3	1.1	2.7	2.6	3.2	2.5
3	0.7	0.8	1.2	1.4	3.0[a]	2.1[a]	3.9	3.3
4	0.5	0.5	1.1	1.0	1.6	1.4	2.3	2.5
5	0.3	0.4	0.8	0.7	1.8	1.6	2.2	2.3
6	0.6	0.6	1.3	1.1	1.8	1.7	2.0	2.3
7	0.4	0.4	1.0	1.0	2.2	1.9	2.6	2.9
Mean	0.51	0.57	1.09	1.06	2.17	1.87	2.70	2.60
Grand mean	0.54		1.08		2.02		2.65	

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

TABLE II
Neogen ELISA Method for Detecting Deoxynivalenol (mg/L) in Barley

	Sample pair		Sample pair		Sample pair		Sample pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	0.6	0.7	1.3	1.1	2.3	1.7	2.6	2.3
2	0.8	0.8	1.3	1.2	2.9	2.0	2.8	2.7
3	0.8	0.8	1.4	1.2	2.7	2.1	3.6	3.9
4	0.4	0.6	1.1	1.0	3.2	3.3	3.6	2.8
Mean	0.65	0.73	1.28	1.13	2.78	2.28	3.15	2.93
Grand mean	0.69		1.21		2.53		3.04	

TABLE III
GC Assay Method (Malt-13) for Detecting Deoxynivalenol (mg/L) in Barley

	Sample pair		Sample pair		Sample pair		Sample pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
1	0.5	0.6	1.1	1.0	2.1	1.8	2.3	2.2
2	0.6	0.7	1.0	0.9	2.4	1.5	2.1	2.1
3	0.6	0.8	1.2	1.1	2.5	2.1	3.0	3.1
4	1.0	0.7	1.2	1.3	2.1	1.9	2.0	2.6
5	0.8	0.9	1.3	1.1	2.1	1.8	2.4	2.5
6	0.6	0.8	1.2	0.8	2.0	2.2	2.0	2.0
7	0.4	0.6	1.0	0.8	1.6	1.2	2.0	2.4
Mean	0.64	0.73	1.14	1.00	2.11	1.79	2.25	2.41
Grand mean	0.69		1.07		1.95		2.33	

TABLE IV
Statistical Summary of Results[a]

Sample pair	No of labs	Grand mean	Repeatability			Reproducibility		
			S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
EZ Tox								
A/B	7	0.54	0.06	10.4	0.16	0.15	27.2	0.15
C/D	7	1.08	0.13	11.8	0.35	0.21	19.4	0.58
E/F	7	2.02	0.19	9.4	0.53	0.46	23.1	1.30
G/H	7	2.65	0.29	10.8	0.80	0.54	20.3	1.51
GC								
A/B	7	0.69	0.12	17.9	0.34	0.16	23.8	0.45
C/D	7	1.07	0.11	10.0	0.30	0.16	14.4	0.43
E/F	7	1.95	0.25	16.5	0.69	0.32	16.5	0.90
G/H	7	2.33	0.20	8.4	0.55	0.37	16.1	1.05

[a] All calculations were made based on Tables I and III.

TABLE V
Comparison of Diagnostix EZ-Tox Homogenous Enzyme Immunoassay and Barley-11 for the Determination of Deoxynivalenol (mg/L) in Barley 2013 Using the Unpaired *t*-Test[a]

Statistical parameter	
Number of sample pairs, N	56
Mean of differences, D	0.059
Standard error of differences, S_D	0.053
Calculated t	–1.11[b]
$t_{0.05}$	2.00

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

ated malted barley (3). It was recommended by the subcommittee to include barley for the following year's study.

In the second year of study, the method produced unacceptable repeatability and reproducibility coefficients of variation for both the Diagnostix EZ-Tox HEIA kit, and the Neogen ELISA kit for malted barley. At the time of study, both kits produced acceptable repeatability and reproducibility coefficients of variation for barley. The subcommittee recommended that the malted barley portion of the study be repeated with more homogenous malt samples that possess DON levels in the desirable range. No further testing was recommended for the barley portion of the study (2).

After its third year, it was determined that the method in general needed to be further evaluated with the manufacturers and would not be recommended for either barley or malted barley (2). With recommendations from the malting industry, the rapid methods for barley were submitted for testing again. Malted barley is still undergoing research to determine some underlying concerns with the rapid methods.

PROCEDURE

Collaborators were provided a total of eight samples of barley for testing, representing sample pairs A/B through G/H. Sample pairs were chosen to represent barley with varying levels of DON, ideally ranging from 0.5 to 3.0 ppm. Barley samples were prepared in accordance to Barley-4. Ground samples were weighed and vacuum sealed to prevent moisture gain and sent to each collaborator. Barley sample pair A/B was obtained from 6-row Lacey barley. Sample pair C/D was obtained from 2-row Pinnacle barley. Sample pair E/F was obtained from 6-row Tradition barley, and sample pair G/H was obtained from a separate lot of 6-row Tradition barley. Results were evaluated using the Youden unit block design (1) and the unpaired *t*-test at the 95% confidence level.

RESULTS AND DISCUSSION

Results for the Diagnostix EZ-Tox HEIA kit were received from seven collaborators for sample pairs A/B to G/H; four collaborators reported results for Neogen ELISA kit and seven collaborators reported results for Barley-11. Collaborators used varying lots for the test kits. The results for the EZ-Tox, Neogen, and Barley-11 methods are summarized in Tables I, II, and III, respectively. Outliers were identified using Dixon's ratio test (1); however, no outliers were removed from statistical analysis. Outliers were not excluded because of the low values that were observed and no known deviations from protocols were noted.

The statistical summary of the data for the Diagnostix HEIA kit and Barley-11 methods are represented in Table III. There were not enough participants for the Neogen ELISA method to perform statistical analysis. The results of the unpaired *t*-test are represented in Table IV. Based on the unpaired *t*-test, Barley-11 and the Diagnostix EZ-Tox HEIA kit were not significantly different at the 95% confidence level.

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Phenolic Yeast Detection

Subcommittee members: T. Cowley, *chair*; E. Belden; K. Boneburg; W. Box; M. Orive Camprubi; G. Castelijn; R. Eideman; K. Tafoya Gibbs; A. Emeline Gless; D. Götsch (EBC); D. Harms, L. Marques; S. Nicholls; K. Pawlowsky; E. Schuermeyer; K. Shepard; P. Zeegers; and C. Pachello (*ex-officio*).

Keywords: 2-Methoxy-4-vinylphenol, 4-Vinyl guaiacol, 4-VG, Contamination, Microbiology, POF, Wild yeast

CONCLUSIONS

1. The sensitivity for correct identification for the unknown phenolic yeast strain B was 53.8% at 24 hr and 69.2% at 48 hr, which is deemed unacceptable.
2. The specificity for non-phenolic unknown yeast strain A was 100% at 24 hr and 100% at 48 hr, which is deemed acceptable.
3. The specificity for non-phenolic unknown yeast strain C was 86.7% at 24 hr, which is deemed unacceptable. The specificity was 80.0% at 48 hr, which was deemed unacceptable.

RECOMMENDATIONS

1. The subcommittee recommends repeating the collaborative with more detailed selection of unknown yeast POF-producing yeast strains. Both high and low phenolic yeast strains will be included as unknowns.
2. Preliminary testing shall be performed to determine the value of adding extra incubation time to aid in detecting phenolic aromas by allowing more time for yeast decarboxylation of ferulic acid in the given media matrix.
3. Collaborators shall be pretested to ensure that they can correctly detect 4-vinyl guaiacol spiked into the media matrix compared to a non-spiked media matrix.

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This was the first year for the subcommittee to evaluate the use of yeast medium supplemented with ferulic acid to detect the

TABLE I
Phenolic Yeast Evaluation at 24 Hours[a]

Collaborator	Strain A Lager NP Unknown	Strain B Phen Unknown	Strain C Ale NP Unknown	Strain D Phen Control	Strain E NP Control
1	NP	no evaluation	NP	Phen	NP
2	NP	Phen	NP	Phen	NP
3	NP	NP	NP	Phen	NP
4	NP	Phen	NP	Phen	NP
5	NP	NP	NP	Phen	NP
6	NP	NP	Phen	Phen	no evaluation
7	NP	Phen	NP	Phen	NP
8	NP	Phen	NP	Phen	NP
9	NP	NP	NP	Phen	NP
10	NP	Phen	NP	Phen	NP
11	NP	NP	NP	Phen	NP
12	NP	NP	NP	Phen	NP
13	NP	Phen	Phen	Phen	NP
14	NP	NP	NP	Phen	NP
15	NP	Phen	NP	Phen	NP

[a] NP = non-phenolic, Phen = phenolic.

TABLE II
Phenolic Yeast Evaluation at 48 Hours[a]

Collaborator	Strain A Lager NP Unknown	Strain B Phen Unknown	Strain C Ale NP Unknown	Strain D Phen Control	Strain E NP Control
1	NP	no evaluation	NP	Phen	NP
2	NP	Phen	NP	Phen	NP
3	NP	NP	NP	Phen	NP
4	NP	Phen	Phen	Phen	NP
5	NP	NP	NP	Phen	NP
6	NP	NP	Phen	Phen	no evaluation
7	NP	Phen	NP	Phen	NP
8	NP	Phen	NP	Phen	NP
9	NP	Phen	NP	Phen	NP
10	NP	Phen	NP	Phen	NP
11	NP	Phen	NP	Phen	NP
12	NP	NP	NP	Phen	NP
13	NP	NP	NP	Phen	NP
14	NP	Phen	NP	Phen	Phen
15	NP	Phen	Phen	Phen	NP

[a] NP = non-phenolic, Phen = phenolic.

TABLE III
Specificity for Non-Phenolic Yeast Strains at 24 and 48 Hours

Strain	# of Correct Dispositions 24 Hr	Total # of Classifications 24 Hr	Specificity for Non-Phenolic 24 Hr	# of Correct Dispositions 48 Hr	Total # of Classifications 48 Hr	Specificity for Non-Phenolic 48 Hr
A	15	15	100%	15	15	100%
C	13	15	86.7%	12	15	80.0%

TABLE IV
Sensitivity for Phenolic Yeast Strains at 24 and 48 Hours

Strain	# of Correct Dispositions 24 Hr	Total # of Classifications 24 Hr	Specificity for Non-Phenolic 24 Hr	# of Correct Dispositions 48 Hr	Total # of Classifications 48 Hr	Specificity for Non-Phenolic 48 Hr
B	7	14	53.8%	9	1	69.2%

presence of 4-vinyl guaiacol (2-methoxy-4-vinylphenol) from yeast strains able to decarboxylate ferulic acid resulting in the formation of this clove-like compound (1,2). 4-vinyl guaiacol is considered to be an off-flavor and off-aroma note in beer if not intended for that particular style. This test method is subjective since it relies on the analyst's ability to detect the aroma. Additionally, the aroma notes may vary in intensity depending on the yeast strain. This method has some limitations but will provide an easy tool for laboratories to detect yeast production of phenolic compounds where appropriate instruments are not available.

PROCEDURE

Collaborators were sent five yeast strains on YM (yeast and mold) agar slants. Samples included three unknown yeast strains: strain A (lager non-phenolic), strain B (phenolic), and strain C (ale non-phenolic). Two known strains were included for reference to the collaborators as a yeast positive phenolic control (NCYC361) and a yeast negative non-phenolic control (University of Nottingham strain). Prior to performing testing, yeast slants were streaked for isolation onto YM agar and incubated for 3–7 days to enable starting with healthy cultures. Each yeast culture was inoculated in duplicate into test tubes containing 10 mL of yeast and mold broth supplemented with 0.1 mL of a 1% w/v ferulic acid solution. After 24 and 48 hr of incubation at 27–28°C, the cultures were subjectively analyzed for clove aroma resulting from 4-vinyl guaiacol production.

RESULTS AND DISCUSSION

A total of 15 collaborators returned test results. Collaborators 1 and 6 did not fully analyze all yeast cultures; therefore, only partial data could be used from these collaborators (Tables I and II).

Results were analyzed and calculated using sensitivity and specificity. Sensitivity is the probability that the test will correctly identify a positive phenolic strain. Sensitivity = # of true positives / (# of true positives + # of false negatives) × 100. Specificity is the probability that the test will correctly identify a negative phenolic strain. Specificity = # of true negatives / (# of true negatives + # of false positives) × 100.

Collaborators were introduced to three unknown yeast strains for evaluation. Strain A was lager yeast, non-phenolic producer. The specificity of detecting non-phenolic for this strain was 100% at 24 and 48 hr (Table III). Strain C was ale yeast, non-phenolic producer. The specificity for detecting non-phenolic for this yeast strain was 86.7% at 24 hr and 80.0% at 48 hr, which is deemed unacceptable (Table III). One of the collaborators detected phenolic for this strain at 24 hr but not at 48 hr (Tables I and II). At 48 hr, two of the collaborators detected a phenolic aroma (Table II). The order of testing should possibly be considered. Sniffing the negative control between samples may have helped center people around non-phenolic. Strain B was the phenolic test yeast. This strain was a lower phenolic producer compared to the positive control when evaluated on GC/MS. The clove-like aroma appeared to be below threshold for several of the collaborators given the current test design. The sensitivity for correct identification for the given unknown phenolic yeast strain was 53.8% at 24 hr and 69.2% at 48 hr, which is deemed unacceptable (Tables I and II).

Future testing will include standardization of incubation vials in addition to including a wider range of unknown strains to provide a variety of phenolic intensities for the unknown samples. Increasing incubation time changes will also be considered. Collaborators will be screened blindly with non-spiked and 4-vinyl guaiacol spiked yeast media in test tubes to determine if they are sensitive and can correctly identify the spiked and non-spiked samples.

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Headspace Gas Chromatography/Electron Capture Detector Analysis of Total Vicinal Diketones in Beer

Subcommittee members: G. Ruehle *chair*; T. Bland; F. X. Castañé (EBC); K. Hofecker; B. Jordan; C. Stordeur; K. Troxell; B. Vordahl; A. Weygandt; and J. Palausky (*ex officio*).

Keywords: 2,3-Butanedione, 2,3-Pentanedione, Diacetyl, ECD, GC, VDK

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the determination of 2,3-butanedione by headspace gas chromatography/electron capture detection (GC-ECD) ranged from 2.9 to 8.5% and 6.8 to 30%, respectively, and were judged acceptable.
2. Repeatability and reproducibility coefficients of variation for the determination of 2,3-pentanedione by headspace GC-ECD ranged from 2.6 to 6.4% and 6.9 to 40%, respectively, and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends that the method for headspace GC-ECD analysis of total vicinal diketones in beer be included in the *Methods of Analysis*.
2. Discharge the subcommittee.

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This was the second year of this subcommittee's existence. The subcommittee was formed to develop an updated method for the measurement of vicinal diketones in beer using modern supplies, instrumentation, and techniques. In the first year, collaborative analysis showed unacceptable statistical data for both compounds tested (2). Prior to dispatching the samples, the sample preparation method was tested for stability over time and in shipping.

PROCEDURE

Three sample pairs were sent to collaborators. Each sample set consisted of a commercially available lager beer. The mid and high level samples were spiked with equal amounts of 2,3-butanedione and 2,3-pentanedione by opening the bottles, adding a concentrated solution, and re-crowning the bottles. Results were evaluated using the Youden unit block design (1).

RESULTS AND DISCUSSION

Results from 10 collaborators were received for the three sample pairs. Results from two collaborators were excluded prior to statistical analyses because of known deviations from the prescribed experimental protocol. Results from two additional collaborators at either low or high end of calibration were also excluded because the reported result was not bracketed by calibration standards. Data for 2,3-butanedione and 2,3-pentanedione are presented in Tables I and II, respectively. Outliers were initially identified using Dixon's ratio test (1).

TABLE I
2,3-Butanedione (µg/L) in Beer by Headspace Gas Chromatography-Electron Capture Detector

	Sample pair		Sample pair		Sample pair	
Collaborator	**C**	**F**	**A**	**D**	**B**	**E**
1	15	14	165	165	262	258
2	23	25	126	144	228	239
3	32	31	157	168	250	262
4	…[a]	…[a]	200	192	284	283
5	20	24	137	154	248	236
6	29	29	147	150	249	235
7	27	34	131	160	250	254
8	14.1	15.7	…[a]	…[a]	…[a]	…[a]
Mean	22.9	24.6	151.8	161.9	252.9	252.4
Grand mean	23.8		156.8		252.6	

[a] Data excluded from calculations due to known deviation from published method.

TABLE II
2,3-Pentanedione (µg/L) in Beer by Gas Chromatography-Electron Capture Detector

	Sample pair		Sample pair		Sample pair	
Collaborator	**C**	**F**	**A**	**D**	**B**	**E**
1	9	1	162	156	256	243
2	17	12	123	125	223	217
3	17	11	123	126	212	202
4	24	16	145	137	236	227
5	29	19	137	154	262	231
6	15	8	137	134	242	224
7	19	12	126	126	225	215
Mean	18.5	11.2	136.2	136.8	236.7	222.8
Grand mean	14.9		136.5		229.7	

TABLE III
Statistical Summary of Results[a]

				Repeatability			Reproducibility		
Compound	**Sample pair**	**# of labs**	**Grand mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
2,3-Butanedione	C/F	7	23.8	2.0	8.5	5.7	7.2	30.4	20.3
	A/D	7	156.8	8.8	5.6	24.7	21.1	13.4	59.0
	B/E	7	252.6	7.4	2.9	20.6	17.2	6.8	48.1
2,3-Pentanedione	C/F	7	14.9	1.0	6.4	2.7	6.0	40.4	16.8
	A/D	7	136.5	5.9	4.3	16.5	13.7	10.0	38.3
	B/E	7	229.7	5.9	2.6	16.6	15.8	6.9	44.2

[a] All calculations were made based on Tables I and II.

The statistical summary of the 2,3-butanedione and 2,3-pentanedione data are shown in Table III. The repeatability for 2,3-butanedione ranged from 2.9% at high concentrations to 8.5% at low concentrations and the reproducibility ranged from 6.8% at high concentrations to 30% at low concentrations. The repeatability for 2,3-pentanedione ranged from 2.6% at high concentrations to 6.4% at low concentrations and the reproducibility ranged from 6.9% at high concentrations to 40% at low concentrations. It is noted that the reproducibility of both compounds is elevated at low concentrations, but this is considered acceptable and consistent with external proficiency testing protocols (3).

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Report of 2013-BCOJ Collaborative Work

Determination of Ethanol in Low Alcohol Beer by Headspace GC-FID

Subcommittee members: A. Ohuchi (Asahi Breweries, Ltd.), *chair*; M. Asakawa (Shimadzu Co.); M. Kanauchi (Miyagi University); K. Kusaka (National Research Institute of Brewing); H. Takakuwa (Agilent Technologies Japan, Ltd.); S. Tatsu (Suntory Liquors, Ltd.); F. Tsuchiya (Thermo Fisher Scientific K.K.); Y. Tsukada (Kirin Company, Ltd.); and T. Watanabe (Sapporo Breweries Ltd.).

Keywords: Ethanol, Headspace GC-FID

CONCLUSIONS

1. Relative repeatability standard deviation (RSD_r) and repeatability limit (r_{95}) for determination of ethanol content using the headspace GC-FID method ranged from 0.6 to 2.3% and from 0.0003 to 0.0209 v/v%, respectively, and were judged acceptable.
2. Relative reproducibility standard deviation (RSD_R) and reproducibility limit (R_{95}) for determination of ethanol content using the headspace GC-FID method ranged from 1.5 to 4.3% and from 0.0006 to 0.0809 v/v%, respectively, and were judged acceptable.

RECOMMENDATIONS

1. It was concluded that the headspace GC-FID method is capable of determining ethanol content in low alcohol beer containing 0.005–1.0 v/v% ethanol.
2. The subcommittee recommends that the headspace GC-FID method be adopted for inclusion in the *Methods of Analysis of BCOJ*.
3. Discharge the subcommittee.

Recently, there has been increased consumer interest in the low alcohol beer category in Japan. Japanese major brewing companies have launched some low alcohol beer brands labeled "alcohol 0.00 v/v%." "Alcohol 0.00%" means that the concentration of alcohol is less than 0.005%. Therefore, it is necessary to develop an analytical method for accurate determination of 0.005% alcohol.

This subcommittee was charged with evaluating the headspace GC-FID method for analysis of ethanol in low alcohol beer.

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PROCEDURE

The collaborative work was performed by nine collaborators. Five sample pairs (A/B, C/D, E/F, G/H, and I/J) consisting of low alcohol beer (~0.005–0.8 v/v%) were provided for study.

Each sample or calibration standard solution of 10 mL (final conc. 0.005, 0.01, 0.025, 0.05, and 0.1 v/v% of ethanol) was placed in auto sampler vial. If the ethanol concentration in the sample excess 0.1 v/v%, the sample should be diluted to 10 times with distilled water. Internal standard solution of 0.1 mL (0.1 v/v% of 2-propanol) was added and capped the vial immediately. Then the samples were injected to headspace GC-FID systems. Each analysis was carried out in duplicate.

The GC method was modified from the analysis methods by the National Tax Agency Japan (4). GC analysis was performed based on the instrument manufacturer's manual.

Headspace Sampler Condition

Oven temp.: 60°C
Sample loop temp.: 110°C
Loop-fill time: 0.03 min
Loop-equilibrium time: 0.20 min
Transfer line temp.: 120°C
GC cycle time: 25 min
Sample vial equilibrium time: 20 min
Injection time: 0.50 min

GC-Condition

Column: Agilent DB-1, 30 m × 0.53 mm ID, 3 μm FT or equivalent
Carrier gas: He, 6 mL/min (constant flow)
Injection temp.: 120°C

TABLE I
Ethanol Content (v/v%) Determined Using the Headspace GC-FID Method

	Sample pair		Sample pair		Sample pair		Sample pair		Sample pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
1	0.0050	0.0050	0.0203	0.0203	0.4349	0.4230	0.3513	0.3672	0.7137	0.7211
2	0.0049	0.0047	0.0200	0.0201	0.4320	0.4297	0.3569	0.3561	0.7363	0.7311
3	0.0045	0.0047	0.0198	0.0201	0.4531	0.4368	0.3747	0.3669	0.7562	0.7488
4	0.0048	0.0047	0.0204	0.0203	0.4364	0.4463	0.3662	0.3763	0.7749	0.7819
5	0.0054	0.0051	0.0205	0.0207	0.4374	0.4382	0.3635	0.3671	0.7570	0.7578
6	0.0050	0.0050	0.0203	0.0204	0.4268	0.4272	0.3558	0.3706	0.7421	0.7250
7	0.0049	0.0049	0.0202	0.0203	0.4323	0.4347	0.3645	0.3621	0.7196	0.7258
8	0.0051	0.0051	0.0207	0.0207	0.4337[a]	0.4633[a]	0.3732	0.3674	0.7720[a]	0.7124[a]
9	0.0047	0.0049	0.0196	0.0199	0.4540	0.4488	0.3745	0.3770	0.8091	0.7897
Mean	0.0049	0.0049	0.0202	0.0203	0.4384	0.4356	0.3645	0.3679	0.7511	0.7477
Grand mean	0.0049		0.0203		0.4370		0.3662		0.7494	

[a] Outliers identified by outlier tests and excluded from the statistical analysis.

TABLE II
Statistical Summary of Results of the Headspace GC-FID Method

	Sample pair A/B	Sample pair C/D	Sample pair E/F	Sample pair G/H	Sample pair I/J
Number of laboratories	9	9	8	9	8
Grand mean (m)	0.0049	0.0203	0.4370	0.3662	0.7494
Repeatability standard deviation (S_r)	0.0001	0.0001	0.0058	0.0062	0.0075
Relative repeatability standard deviation (RSD_r, %)	2.3	0.6	1.3	1.7	1.0
Repeatability limit (r_{95})	0.0003	0.0003	0.0163	0.0174	0.0209
Predicted relative repeatability standard deviation ($PRSD_r$, %)	6.2	5.0	3.1	3.2	2.9
$HorRat_r$ ($RSD_r/PRSD_r$)[a]	0.3[c]	0.1[c]	0.4[c]	0.5[c]	0.3[c]
Reproducibility standard deviation (S_R)	0.0002	0.0003	0.0094	0.0077	0.0289
Relative reproducibility standard deviation (RSD_R, %)	4.3	1.5	2.2	2.1	3.9
Reproducibility limit (R_{95})	0.0006	0.0009	0.0264	0.0215	0.0809
Predicted relative reproducibility standard deviation ($PRSD_R$, %)	9.2	7.5	4.7	4.8	4.3
$HorRat_R$ ($RSD_R/PRSD_R$)[a]	0.5[c]	0.2[c]	0.5[c]	0.4[c]	0.9[b]

[a] HorRat values were calculated from w/w% of each ethanol concentration. w/w% data not shown.
[b] According to AOAC International Guidelines, HorRat values should be more than 0.5 and less than or equal to 2.0 (1).
[c] Accurate results although the HorRat values were under 0.5.

Split ratio: 1:30
Colum oven temp.: 50°C (isothermal)

FID-Condition

Detection zone temp.: 250°C
Hydrogen gas flow: 30 mL/min
Air flow: 400 mL/min
Mode: constant column + make up (30 mL/min)
Make up gas: He or N_2

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 AOAC International Guidelines (1).

RESULTS AND DISCUSSION

The results for ethanol content are shown in Table I. All of the samples were checked for outliers using Mandel's *h* and *k* statistics, and Cochran and Grubbs outlier test, and outliers were excluded from the statistical analysis (1,3). The statistical summary of results is shown in Table II.

Each of the calculated analytical values ranged as follows: RSD_r ranged from 0.6 to 2.3%; r_{95} ranged from 0.0003 to 0.0209 v/v%, respectively; RSD_R ranged from 1.5 to 4.3%; and R_{95} ranged from 0.0006 to 0.0809 v/v%, respectively, and were judged acceptable.

It was concluded that the method is capable of determining ethanol in low alcohol beer containing 0.005–1.0 v/v% of ethanol. The subcommittee recommends that the method should be adopted for inclusion in the *Methods of Analysis of BCOJ*.

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