Technical Committee and Subcommittee Reports

2012–2013 Report of the Technical Committee

Committee members: C. Powell, *chair*; M. Eurich, C. Benedict; S. Brendecke; L. Chadwick; J. Cornell; Guerdrum; R. Jennings; K. Lakenburges; A. Porter; A. MacLeod; C. Pachello, J. Palausky; D. Maradyn; and B. Foster (*senior advisor*)

The ASBC Technical Committee and Subcommittee chairs coordinated 18 subcommittees during 2012–2013. As a result, the following method is being recommended for inclusion in the ASBC *Methods of Analysis* (MOA):

- The Determination of Gluten using the R5 Competitive ELISA Method, chaired by Lindsay Guerdrum (New Belgium Brewing Co).

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:

- Isomerized Alpha Acids in Beer by Solid Phase Extraction and Subsequent Spectrophotometric Measurement, chaired by Tom Shellhammer and Phillip Wietstock (Oregon State University).

The following methods will continue for another year of collaborative study:

- Wort Amino Acids by HPLC, chaired by Chris Baugh (Sierra Nevada Brewing Co).
- Headspace Gas Chromatography/Electron Capture Detector Analysis of Total Vicinal Diketones in Beer, chaired by Grant Ruehle (New Belgium Brewing Co).

The review of one section of the ASBC Methods of Analysis will also be continued:

- Beer, chaired by Karl Lakenburges (Anheuser-Busch InBev) and Mark Eurich (MillerCoors)

In 2010, the ASBC Board of Directors initiated a grant program to be administered by the ASBC Technical Committee for the development of methods or value products for inclusion in Methods of Analysis. This was continued in 2012–2013 with Mark Zunkel (Weihenstephan, hop flavor wheel development), Philip Wietstock (Oregon State University, new method for the analysis of IBU in beer and wort), and Alex Mott (University of Nottingham, method video development) receiving grants.

The Coordination of New and Alternate Methods of Analysis Subcommittee submitted a survey to members in 2013. 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 2013 Annual Convention in Tucson, Arizona. Based on the polling results and feedback at this meeting, a number of methods have been recommended for collaborative study in 2013–2014:

- Review of ASBC Methods of Analysis section: Barley, chaired by Rebecca Jennings (Rahr Malting Co).
- Determination of Beta Glucan in Beer, chaired by Unju Kim (Novozymes).
- Rapid Immunoassay for Deoxynivalenol (DON) analysis in Barley, chaired by Theresa Chicos (Rahr Malting Co).
- Statistical analysis of samples, chaired by Aaron MacLeod (Canadian Grain Commission).
- Determination of Phenolic Characteristic in Yeast, chaired by Trevor Cowley (SABMiller Ltd).
- Microbiological Methods in Brewing, chaired by Caroline Pachello (MillerCoors)

As in previous years, the following eight 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 Luke Chadwick (Bell's Brewery)
- 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)
- Soluble Starch, chaired by Rebecca Jennings (Rahr Malting Co)
- Check Services, chaired by Rebecca Jennings (Rahr Malting Co) and Jodi Grider (ASBC)

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.

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.

We would also like to thank Boulevard Brewing Co, and in particular Joe Palausky, for hospitality and assistance in domestic arrangements during the ASBC Technical Committee meeting in Kansas City this fall.

Finally, I would like to recognize the dedication and hard work put forth by the Technical Committee over the previous year. The continual enthusiasm and commitment demonstrated by the team is sincerely appreciated and is key to ensuring future developments to ASBC Methods of Analysis, along with associated technical content.

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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
(*Luke Chadwick, lchadwick@bellsbeer.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
(*Chris Baugh, cbaugh@sierranevada.com*)

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 Caroline directly.

Isomerized Alpha Acids in Beer by Solid Phase Extraction, and Subsequent Spectrophotometric Measurement
(*Tom Shellhammer, tom.shellhammer@oregonstate.edu*)

This collaborative is based on a method developed at Oregon State University in Tom Shellhammer's lab. The method utilizes solid-phase extraction followed by spectrophotometric detection for rapid and accurate bitterness analysis. The method correlates well with the IAA method using HPLC, thus providing a more accurate assessment of bitterness compared with the standard spectrophotometric method. The method utilizes methanol and water as solvents and can be run by any laboratory with a spectrophotometer.

Headspace Gas Chromatography/Electron Capture Detector Analysis of Total Vicinal Diketones in Beer
(*Grant Ruehle, gruehle@newbelgium.com*)

This subcommittee was initiated on the recommendation of the subcommittee for Coordination of New and Alternate Methods and enters its first year. The subcommittee will evaluate the use of the headspace/gas chromatography/electron capture detection (GC/ECD) for measuring total vicinal diketones (VDKs) in beer.

Determination of Beta Glucan in Beer
(*Unju Kim, ujk@novozymes.com*)

This subcommittee was initiated based on the recommendation of the subcommittee for Coordination of New and Alternate Methods. It is anticipated that this subcommittee will evaluate the use of test kits for the determination of Beta Glucan in beer.

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, larry.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.

Rapid Immunoassay Method for Deoxynivalenol (DON) in Barley
(*Theresa Chicos, tchicos@rahr.com*)

This subcommittee was formed based on the recommendation of the *ASBC Methods of Analysis: Malt Review*. The subcommittee aims to evaluate rapid immunoassay-based methods which can be used as alternatives to the existing DON method currently recommended for use by ASBC Methods of Analysis.

Determination of Phenolic Characteristic 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.

The Determination of Gluten using the R5 Competitive ELISA Method

Subcommittee members: L. Guerdrum, *chair*; D. Sedin (*ex officio*), J. Ang, B. Bell, S. Bolin, J. Casey, X. Castane (EBC), C. Christis, J. Gelroth, C. Gößwein, T. Koerner, P. Köhler, C. Mena, N. Parker, D. Ryu, Y. Sjögren, D. Thompson, M. Lombardia Uria, R. Walden, and Y. Zhang

Keywords: Celiac, Enzyme-linked immunosorbent assay, R5 Mendez

CONCLUSIONS

1. Repeatability and reproducibility coefficients of variation for the determination of gluten by R5 Competitive ELISA ranged from 11.9% to 25.8% and 25.0% to 34.9%, respectively, and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends that the method for gluten determination by R5 Competitive ELISA be included in the Methods of Analysis.
2. Discharge the subcommittee.

This was the first year of the subcommittee's existence. This subcommittee was formed based on the Technical Committee's recommendation to evaluate the R5 competitive ELISA method of gluten determination. Review of the available methodology concluded that the most reliable procedure that ensures quantification of all the relevant proteinaceous material (including degradation products) is the R5 competitive ELISA method based on the monoclonal R5 antibody (8).

PROCEDURE

Five sample pairs of commercial beers were sent to each collaborator. Each pair was of the same brand but from different production dates. Beer samples were chosen to span the range of gluten content from approximately 0 mg/L to 100 mg/L. The samples were obtained from: Anheuser-Busch Inbev, Sierra Nevada, New Belgium Brewing Company, Guinness, and Bard's Breweries. Fish gelatin was obtained from Sigma-Aldrich in bulk, aliquot by the subcommittee chair, and sent to the individual laboratories. The fish gelatin was used to overcome the binding between protein and polyphenol, which results in an effective extraction matrix when paired with ethanol (5). The samples were analyzed using the R5 Competitive ELISA method from R-Biopharm (7). The R5 antibody utilized in the ELISA has the ability to detect the consensus sequence Q-X-P-W/F-P (6) corresponding to multiple immunoreactive regions in α/β-, γ-, and ω-gliadins; the R5 monoclonal antibody can be used to detect and quantify partially hydrolyzed and heat-treated food samples with great specificity.

The raw data from the individual laboratories was sent to the subcommittee chair and analyzed using the RIDASOFT Win program provided by R-Biopharm. This software uses the cubic spline calculation model and could not be used for results below 10 ppm gluten. Samples below the lowest level were calculated by use of a different model where absorbance was transformed and a linear fit was made vs. concentration. Results were evaluated using the Youden block design (1).

RESULTS AND DISCUSSION

Results from 15 collaborators were received for the 5 sample pairs. No outliers were removed prior to statistical analysis to display the variability that can be expected from this method. Potential sources of variation can come from pipetting technique, the amount of time allowed between ending the reaction and reading the results, homogenization and extraction of the sample, and laboratory dust contamination. Repeatability and reproducibility coefficients of variation for the determination of gluten by R5 Competitive ELISA ranged from 11.9 to 25.8% and 25.0 to 34.9%, respectively (Table II), and were judged acceptable.

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TABLE I
Gluten Concentration (ppm) in Beer by R5 Competitive ELISA

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5[a]	
Collaborator	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
1	31.1	32.1	39.2	73.3	91.1	78.1	30.1	30.6	22.4	26.8
2	41.7	45.2	66.3	66.8	85.2	83.4	28.0	31.3	BDL	BDL
3	40.7	40.2	47.0	116.1	85.1	84.6	49.1	49.1	BDL	BDL
4	50.2	56.0	81.0	82.0	102.3	112.9	100.3	93.9	BDL	BDL
5	51.2	62.2	96.3	83.5	105.8	91.7	63.5	66.3	BDL	BDL
6	22.4	25.0	46.5	54.8	64.8	62.9	39.9	42.6	BDL	BDL
7	39.5	45.7	76.0	73.8	87.5	95.6	73.3	73.5	BDL	BDL
8	67.1	62.8	83.7	80.5	102.1	233.6	87.0	96.4	11.9	12.0
9	36.7	59.4	77.1	85.1	101.2	120.4	54.1	90.8	BDL	BDL
10	29.3	33.4	55.6	57.2	60.8	71.3	47.0	53.0	15.2	16.8
11	40.1	49.6	75.4	67.2	85.1	113.9	85.7	80.3	BDL	BDL
12	29.0	26.0	53.3	80.6	103.9	126.4	56.5	53.5	BDL	BDL
13	35.3	34.7	55.5	39.7	71.9	90.1	52.1	60.4	BDL	BDL
14	26.2	36.7	50.2	70.2	70.2	75.6	45.1	53.9	BDL	BDL
15	29.9	38.7	58.5	58.3	72.8	91.3	58.2	58.7	BDL	BDL
Mean	38.02	43.17	64.10	72.61	85.98	102.12	57.99	62.28	BDL	BDL
Grand mean	40.60		68.35		94.05		60.13		4.83	

[a] Below detection limit.

TABLE II
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}
1	15	40.60	4.84	11.9	13.54	12.07	29.7	33.79
2	15	68.35	15.39	22.5	43.10	17.07	25.0	47.80
3	15	94.05	24.24	25.8	67.86	30.76	32.7	86.14
4	15	60.13	7.18	11.9	20.11	20.97	34.9	58.72
5[b]	15	4.8[b]	1.7[b]	35.0[b]	4.7[b]	8.5[b]	175.7[b]	23.7[b]

[a] All calculations were made based on Table I.
[b] Below detection limit, based on results from Table I.

Results below the 10 ppm quantification limit (1) are not reported, but can be calculated using a reciprocal transform model (RIDASOFT Win software cannot be used for calculating gluten results below the detection limit). For samples with low mean values, the variance makes up a greater portion of the mean. Therefore, these results were not included in the reported conclusions listing the repeatability and reproducibility coefficients of variation.

The results demonstrate that no false negatives were measured in this sample set (i.e., no collaborator reported results below 20 ppm gluten for samples 1–4). Only one collaborator reported a false positive result for sample 5 where the reported results were above 20 ppm gluten for a gluten-free beer.

The statistical results from this study are in line with a study conducted by AACC International using the R5 ELISA antibody test for gluten analysis in food matrices (4). From the FDA proposed rule (72 FR 2795) for ELISA methods based on the R5 antibody: "the Agency has tentatively determined that enzyme-linked immunosorbent assay (ELISA)-based methods can be used reliably and consistently to detect gluten at the level of 20 ppm in a variety of food matrices" (3). Additionally, this method has received a *Certificate of Performance Tested Status* from the AOAC Research Institute (Certificate No. 12061) (2).

ACKNOWLEDGMENTS

We would like thank R-Biopharm for generously supplying the kits to the collaborators and Paul Wehling for consulting with us on this project.

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Isomerized Alpha Acids in Beer by Solid Phase Extraction and Subsequent Spectrophotometric Measurement

Subcommittee members: T. Shellhammer, *chair*; P. Wietstock, *chair*; P. Aron, L. Barber, M. Biendl (EBC), M. De Rechter, J. Demaniuk, M. Guilford, C. Guy, K. Hollar, B. Jaskula-Goiris, J. Kelter, R. Martin, I. McLaughlin, S. Mulqueen, C. O'Neill, J.Palausky, M. Raver, G. Ruehle, J. Schier, R. Schmidt (EBC), G. Spedding, S. Theriot, and A. Porter (*ex officio*)

Keywords: Bitterness, Hops, HPLC, IAA, Iso-octane, SPE, UV

CONCLUSIONS

1. Repeatability coefficients of variation for the determination of IAA in beer ranged from 9.9 to 15.2% and were judged unacceptable.
2. Reproducibility coefficients of variation for the determination of IAA in beer ranged from 30.5 to 69.4% and were judged unacceptable.

RECOMMENDATIONS

1. The repeatability and reproducibility were judged unacceptable. The subcommittee therefore recommends improving the method in 2013.
2. The subcommittee recommends testing the method's ruggedness according to *Methods of Analysis*, Statistical Analysis – 3 (1) in 2014.
3. The subcommittee furthermore recommends repeating the study in 2015.

This was the second year of the subcommittee's evaluation of measuring iso alpha acids (IAA) in beer by solid phase extraction (SPE) followed by ultraviolet (UV) absorbance. The committee was started with the goal of approving a new and alternate method for determining IAA in beer using the SPE technique from Beer-23C. It eliminates the usage of iso-octane and can be used to more accurately assess beer bitterness in dry-hopped beers relative to results obtained via the traditional iso-octane BU method, Beer-23A. SPE followed by washing removes polyphenolic material, which can be particularly high in dry-hopped beers, and which can also absorb at 275 nm and thereby interfere with the measurement of IAA in the sample. Furthermore, multiple wavelength measurements can result in estimations of the alpha acid concentration in the sample, which can also absorb at 275 nm and therefore interfere with the BU reading. These features along with the elimination of iso-octane make this new method attractive to many labs.

In the first year of the study, repeatability coefficients of variation for the determination of IAA in beer ranged from 2.7 to 6.0% and were judged acceptable. Reproducibility coefficients of variation for the determination of IAA in beer ranged from 9.4 to 18.2% and were judged unacceptable. Before starting the second year of the ring study, the method's procedure was simplified by skipping washing step A. Furthermore, a video with a detailed procedure of the method was produced, which was an improved and more informative version than the first year's video. Collaborators were instructed to view the video prior to commencing the study.

This year, the subcommittee recommends improving the method. The method's sensitive features such as flowrate, the effect of the washing steps, and the filtration step prior to measuring the sample should be elaborated and identified. Furthermore, a non-beer standard for validating the method shall be provided. In 2014, the method's ruggedness shall be tested according to *Methods of Analysis*, Statistical Analysis – 3 (1) in the co-chair's laboratories. Once a refined method has been established, the subcommittee will repeat the collaborative study in 2015.

PROCEDURE

Three sample pairs, similar but distinct, were sent to 31 collaborating labs and were selected to cover a range of IAA concentrations and levels of dry hopping. The samples consisted of non-dry hopped pale lager beers A/A_1, mid-range hopped beers that had not been dry-hopped B/B_1, and two highly dry-hopped beers C/C_1. A fourth beer, which was a medium hopped, amber lager, was included as a reference sample for which the analytical result was provided. This allowed participants to practice the method with a beer of a known SPE-IAA value and helped reveal potential difficulties encountered by individual labs. Participants were asked to follow the method as closely as possible. If any adjustments or modifications were made in the method, they were to document this with the subcommittee chairmen prior to submitting their results. Results were evaluated using the Youden unit block design (1).

TABLE I
Summary of IAA Method Results from the Collaborative Study for IAA (units)

	Sample pair		Sample pair		Sample pair	
Collaborator	**A**	**A_1**	**B**	**B_1**	**C**	**C_1**
1	2.4	–4.6	10.6	12.2	25.8	23.1
2	10.5	6.4	38.3	30.4	59.8	51.7
3	11.5	10.5	29.6	32.9	59.8	59.7
4	11.4	10.1	11.1	24.0	14.8	56.9
5	12.5	13.3	34.7	37.2	59.8	56.7
6	10.3	9.2	28.2	29.6	49.5	48.6
7	13.9	3.4	28.5	30.4	33.2	45.3
8	9.8	6.4	29.0	28.3	51.5	52.2
9	16.1	14.7	41.8	40.4	62.8	66.6
10	14.4	11.9	35.0	32.6	57.0	60.0
11	17.6	19.9	47.7	40.3	63.5	79.0
12	10.9	10.5	26.7	26.2	46.6	43.2
13	10.6	9.0	31.4	28.5	47.6	45.9
14	10.7	9.8	37.0	37.8	61.6	55.4
15	7.6	5.7	26.5	27.8	41.5	37.6
16	46.4	44.0	70.0	61.2	104.1	96.4
17	11.1	11.4	31.0	33.6	52.7	53.3
18	16.2	14.5	36.7	36.4	56.3	53.1
19	21.8	20.4	44.2	46.2	66.6	64.7
20	12.6	12.6	31.1	31.3	51.0	51.0
Mean[a]	13.91	11.96	33.45	33.37	53.27	55.02
Grand mean[a]	12.94		33.41		54.14	

[a] Calculated excluding outliers.

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TABLE II
Statistical Summary of IAA Method Results[a]

			Repeatability			Reproducibility		
Sample pair	No. of labs	Grand mean	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
A/A_1	20	12.94	1.96	15.18	5.50	8.98	69.41	25.14
B/B_1	20	33.41	3.31	9.90	9.26	11.21	33.56	31.39
C/C_1	20	54.14	7.86	14.52	22.01	16.50	30.47	46.20

[a] Calculations were made based on Table I.

TABLE III
Summary of Unfiltered and Filtered Samples for IAA (units)

	Sample pair				Sample pair				Sample pair			
	A		A_1		B		B_1		C		C_1	
Collaborator	Unfiltered	Filtered	Unfiltered	Filtered	Unfiltered	Filtered	Unfiltered	Filtered	Unfiltered	Filtered	Unfiltered	Filtered
1	10.8	12.5	10.9	13.3	31.4	34.7	33.1	37.2	56.3	59.8	53.0	56.7
2	9.6	10.3	8.7	9.2	27.2	28.2	28.9	29.6	48.2	49.5	47.3	48.6
3	14.4	12.2	9.0	11.9	32.1	35.0	32.9	32.6	57.1	57.0	60.0	60.0
4	7.3	7.6	6.2	5.7	25.7	26.5	27.1	27.8	41.1	41.5	36.5	37.6
5	11.7	16.2	11.7	14.5	32.6	36.7	32.5	36.4	51.7	56.3	46.1	53.1
6	16.3	21.8	16.9	20.4	40.0	46.2	33.8	36.7	63.5	66.6	59.6	64.7
7	9.5	12.6	9.0	12.6	46.6	51.0	46.9	51.0	28.4	31.3	28.5	31.2
8	10.8	10.5	6.9	6.4	33.3	38.3	34.9	30.4	66.6	59.8	54.8	51.7
9	11.3	16.1	10.6	14.7	37.3	41.8	36.0	40.4	58.9	62.8	60.4	66.6
Mean	11.3	13.3	10.0	12.1	34.0	37.6	34.0	35.8	52.4	53.8	49.6	52.2

RESULTS AND DISCUSSION

Results from 20 collaborators were received for the sample pairs A/A_1, B/B_1, and C/C_1.

The results for the IAA method are presented in Table I. Outliers were identified using Dixon's ratio test (1).

The statistical summary of the IAA data is presented in Table II. Repeatability and reproducibility coefficients of variation for the IAA method range from 9.9 to 15.2% and 30.5 to 69.4% respectively and were both judged unacceptable.

Despite best efforts to simplify the method, a number of labs continued to have difficulty. In discussing the method with one collaborator, it was discovered that filtering the sample after eluting it in the last step can yield higher absorbencies in some cases. The collaborators were therefore asked to submit data for both unfiltered and filtered samples. The summary of the unfiltered and filtered data is presented in Table III. Data for unfiltered and filtered samples were received from nine collaborators. For this set of data, the filtration step increased the IAA values by 6.6%. For single sample pairs, the filtration step yielded an increase of the IAA values by 16.2% for A/A_1, 7.3% for B/B_1, and 3.9% for C/C_1, respectively. It is suspected that a wetting agent in the filtration media may be the source of the higher spectrophotometric readings. Discussing this effect with a manufacturer for syringe filters revealed that the first 2–3 mL during filtration should be discarded prior to measuring the samples.

Further problems with the method were mostly related to some collaborators having never performed SPE before or having problems maintaining the proper flow rate. Observations from trials in the chairmen's laboratories suggests that results are potentially dependent on flowrate. High flowrates yield lower values. The manufacturer recommendation for SPE cartridge flow rate should be no more than 2 mL/min.

TABLE IV
Summary of HPLC Results and IBU

	Sample pair		Sample pair		Sample pair	
	A	A_1	B	B_1	C	C_1
IBU mean values[a]	9.4	8.2	33.7	34.9	54.9	54.2
HPLC mean values[a]	10.3	8.8	24.8	26.2	41.1	44.2
IAA mean values	13.9	12.0	33.5	33.4	53.3	55.0

[a] Mean was calculated from a duplicate measurement.

It appears that the measures for simplifying and improving the method by skipping washing step A from the previous (2012) method's year procedure yielded poorer results than in the first study. The role of the first washing step will be recommended starting a third trial. Furthermore, a stable, non-beer standard will be provided for validating the method in the respective labs.

For a comparison and judgment of the results as obtained from the SPE-based IAA analysis, the isohumulone concentration of the samples was determined by HPLC in the chair's laboratories. Furthermore, IBU analysis (1) was performed. The average HPLC and IBU results are presented in Table IV.

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

Subcommittee members: G. Ruehle, *chair*; F. X. Castañé (EBC); M. Qian; K. Troxell; K. Viljanen; 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 GC-ECD ranged from 7 to 32% and 24 to 38%, respectively, and were judged unacceptable.
2. Repeatability coefficients of variation for the determination of 2,3-pentanedione by headspace GC-ECD ranged from 3 to 8% and were judged acceptable, but reproducibility coefficients of variation ranged from 59 to 65%, and were judged unacceptable.

RECOMMENDATION

Due to the unacceptable statistical results, the subcommittee recommends that the collaborative study be repeated in 2013/2014 after performing a ruggedness analysis for critical factors.

The subcommittee was formed on the recommendation of the subcommittee for Coordination of New and Alternate Methods. The subcommittee was formed to develop an updated method for the measurement of vicinal diketones in beer using modern supplies, instrumentation, and techniques.

PROCEDURE

Three sample pairs were sent to collaborators. Sample pair 1-2 was a commercially available lager, sample pair 3-4 was a commercially available ale, and sample pair 5-6 was an in-process fermenter sample that was collected, filtered, and frozen. These pairs covered the concentration range of the method. All samples were sent frozen for next day air. Results were evaluated using the Youden unit block design (1).

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RESULTS AND DISCUSSION

While samples were dispatched to 16 collaborators, results were received from only six collaborators. The data for 2,3-butanedione and 2,3-pentanedione are presented in Tables I and II, respectively. Statistical analysis is based on data from all collaborators.

The statistical summary of the data is shown in Table III. The repeatability coefficient of variation for 2,3-butanedione by GC-ECD ranged from 7 to 32% and were judged unacceptable. The repeatability coefficient of variation for 2,3-pentanedione by GC-ECD ranged from 3 to 8% and were judged acceptable.

Reproducibility coefficients of variation for 2,3-butanedione by GC-ECD ranged from 24 to 38% and were judged unacceptable. Reproducibility coefficients of variation for 2,3-pentanedione ranged from 59 to 65% and were judged unacceptable.

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

	Sample pair		Sample pair		Sample pair	
Collaborator	**1**	**2**	**3**	**4**	**5**	**6**
1	24	25	65	180	67	130
2	46	39	102	279	158	226
3	33	31	81	185	173	213
4	36	38	88	251	149	177
5	48	52	112	251	163	215
6	47	45	77	72	195	229
Mean	39.0	38.1	87.3	202.8	150.8	198.4
Grand mean	38.6		145.1		174.6	

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

	Sample pair		Sample pair		Sample pair	
Collaborator	**1**	**2**	**3**	**4**	**5**	**6**
1	28	28	33	12	20	38
2	52	53	31	40	78	102
3	24	36	19	10	45	47
4	17	18	13	15	31	35
5	77	83	41	49	119	139
6	25	26	15	14	42	41
Mean	37.0	40.8	25.2	23.2	55.8	66.8
Grand Mean	38.9		24.2		61.3	

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									
	1/2	6	38.6	2.6	6	7.1	9.6	25	26.9
	3/4	6	145.1	46.1	32	129.0	54.6	38	152.8
	5/6	6	174.6	11.5	6	32.1	41.3	24	115.7
2,3-Pentanedione									
	1/2	6	38.9	8.1	3	8.9	23.3	60	65.3
	3/4	6	24.2	33.6	8	22.8	14.3	59	39.9
	5/6	6	61.3	12.3	7	21.1	39.9	65	111.8

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

There are a number of challenges inherent in measuring compounds at low (<20 µg/L) levels that could contribute to such high levels of variation. In particular, finding commercially available, stable beer with these compounds over the range that the method is developed to measure poses a challenge. The method is intended to measure the low levels expected in finished beer as well as high levels usually only found in beer that is in process. Additionally, in-process beer contains both compounds and their immediate precursors that must be converted and measured. For future collaboration, it will be necessary to re-evaluate the samples that are distributed to collaborators to determine the best way to cover the necessary concentration range and evaluate the ability of the method to convert precursors with a stable sample that can be shipped internationally.

The recommendation of the subcommittee is to evaluate the method of sample stabilization and shipping by ruggedness testing. This is necessary to better evaluate the method across laboratories.

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Report of 2012-BCOJ Collaborative Work

Quantitative Analysis of Total Purine Content Using the HPLC-UV Method in Beer, Low-Malt Beer, and Third-Category Beer

Subcommittee members: T. Hashimoto (Suntory Liquors, Ltd.), *chair*; T. Handa (Asahi Breweries, Ltd.); Y. Kakudo (Suntory Liquors, Ltd.); M. Kanai (National Research Institute of Brewing); K. Kaneko (Faculty of Pharma-Sciences, Teikyo University); C. Kenjo (Shimadzu Co.); M. Nakahara (Kirin Group Office Co. Ltd.); W. Nakamura (Orion Breweries, Ltd.); A. Ohuchi (Asahi Breweries, Ltd.); T. Watanabe (Sapporo Breweries, Ltd.)

Keywords: Total purine content, HPLC

CONCLUSIONS

1. Relative repeatability standard deviation (RSD_r) and repeatability limit (r_{95}) for determination of total purine content using the HPLC-UV method ranged from 0.8 to 4.6% and 1.7 to 7.6 mg/L, respectively, and were judged acceptable.
2. Relative reproducibility standard deviation (RSD_R) and reproducibility limit (R_{95}) for determination of total purine content using the HPLC-UV method ranged from 11.6 to 16.8% and 8.0 to 49.5 mg/L, respectively, and were judged acceptable.

RECOMMENDATIONS

1. It was concluded that the HPLC-UV method is capable of determining total purine content in beer, low-malt beer, and third-category beer containing a total purine content of approximately 20–140 mg/L.
2. The subcommittee recommends that the HPLC-UV method be adopted for inclusion in the Method of Analysis of BCOJ.
3. Discharge the subcommittee.

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This was the second year of this subcommittee's evaluation of the use of the HPLC-UV method for determining total purine content in beer, low-malt beer, and third-category beer. The method is a modification of the Kaneko's method (5).

In its first year, because some collaborators could not separate guanine peak completely, the method produced unacceptable relative reproducibility standard deviation (2). In this year, the subcommittee adopted several improvements in the HPLC conditions, such as using an absolute calibration method and selecting of an appropriate pH for the mobile phase.

PROCEDURE

The collaborative work was performed by 10 collaborators. Eight sample pairs consisting of low purine content beer (~20–30 mg/L; A/B, C/D, and E/F), moderate purine content beer (~40–90 mg/L; G/H, I/J, and K/L), and high purine content beer (~100–140 mg/L; M/N and O/P) were provided for study.

Each sample (4.5 mL) was degassed prior to analysis, placed in a test tube on ice, and then 70% perchloric acid (0.5 mL) was added; this was followed by heating above 95°C for 60 min in boiling water with stirring. The hydrolyzed sample solution was neutralized with 8.0 mol/L KOH, followed by centrifugation (3,000 rpm, 10 min). The supernatant of the solution was filtered through 0.45 μm hydrophilic filters and then injected into the HPLC system for analysis of adenine, guanine, hypoxanthine, and xanthine. Total purine content was calculated using an absolute calibration method.

The standard curves were prepared from adenine, guanine, hypoxanthine, and xanthine reagents (>99% purity). The concentrations of these standard mixtures were 1.0, 2.5, 5.0, 10.0, 50.0, and 100.0 mg/L (adenine and guanine) and 0.5, 1.25, 2.5, 5.0, 25.0, and 50.0 mg/L (hypoxanthine and xanthine).

HPLC analysis was performed under the following conditions: instrument, HPLC-UV system without regard to manufacturer; column, Shodex Asahi Pak GF-310 HQ (7.5 mm i.d. and 300 mm length) or GS-320 HQ (7.5 mm i.d. and 300 mm length); mobile phase, 150 m*M* sodium phosphate buffer (titrating a 150 m*M*

TABLE I
Total Purine Content (mg/L) Determined Using the HPLC-UV Method

	Sample pair		Sample pair		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**	**M**	**N**	**O**	**P**
1	17	16	19	21	30	30	64	62	85	85	73	72	110	108	143	141
2	14	14	20	21	24	25	57	57	75	75	66	64	102	106	126	125
3	13	14	22	22	26	25	59	55	67	66	78	76	106	104	131	136
4	14	14	19	19	23	23	50	48	64	65	57	57	87	90	109	117
5	21	21	28	28	35	35	73	75	99	99	87	89	138	136	169	171
6	18	16	25	25	30	28	62	62	85	86	79	74	110	114	142	140
7	22[a]	20[a]	32[a]	32[a]	34[a]	37[a]	89[a]	89[a]	119[a]	105[a]	107[a]	105[a]	173[a]	177[a]	228[a]	230[a]
8	20	18	31[b]	27[b]	30	28	55	60	82[b]	74[b]	81[b]	69[b]	108	105	174[b]	155[b]
9	20	21	26	27	33	32	66	65	85	87	76	77	122	120	156	157
10	17	17	26	26	30	30	64	64	87	87	76	76	117	118	142	138
Mean	17.1	16.8	23.1	23.6	29.0	28.4	61.1	60.9	80.9	81.3	74.0	73.1	111.1	111.2	139.8	140.6
Grand mean	16.9		23.4		28.7		61.0		81.1		73.6		111.2		140.2	

[a] Because collaborator 7 could not separate the guanine peak completely, all data sets from collaborator 7 were excluded from the statistical analysis.
[b] Outliers identified by outlier tests and excluded from the statistical analysis.

TABLE II
Statistical Summary of Results of the HPLC-UV Method

	Sample pair A/B	Sample pair C/D	Sample pair E/F	Sample pair G/H	Sample pair I/J	Sample pair K/L	Sample pair M/N	Sample pair O/P
Number of laboratories	9	8	9	9	8	8	9	8
Grand mean (m)	16.9	23.4	28.7	61.0	81.1	73.6	111.2	140.2
Repeatability standard deviation (S_r)	0.8	0.6	0.8	1.7	0.7	1.6	1.9	2.7
Relative repeatability standard deviation (RSD_r, %)	4.6	2.6	2.7	2.8	0.8	2.1	1.7	1.9
Repeatability limit (r_{95})	2.2	1.7	2.2	4.8	1.9	4.4	5.4	7.6
Predicted relative repeatability standard deviation ($PRSD_r$, %)	7.0	6.6	6.4	5.7	5.5	5.6	5.2	5.1
$HORRAT_r$ ($RSD_r/PRSD_r$)[a]	0.7	0.4[b]	0.4[b]	0.5	0.1[b]	0.4[b]	0.3[b]	0.4[b]
Reproducibility standard deviation (S_R)	2.9	3.4	3.9	7.1	11.6	9.3	13.5	17.7
Relative reproducibility standard deviation (RSD_R, %)	16.8	14.7	13.5	11.6	14.3	12.6	12.1	12.6
Reproducibility limit (R_{95})	8.0	9.6	10.9	19.9	32.5	25.9	37.7	49.5
Predicted relative reproducibility standard deviation ($PRSD_R$, %)	10.4	10.0	9.7	8.6	8.3	8.4	7.9	7.6
$HORRAT_R$ ($RSD_R/PRSD_R$)[a]	1.6	1.5	1.4	1.4	1.7	1.5	1.5	1.7

[a] According to AOAC International Guidelines, HORRAT values should be between 0.5 and 2.0 (1).
[b] Accurate results although the $HORRAT_r$ values were under 0.5.

sodium dihydrogenphosphate (Nacalai tesque, 98% purity) aqueous solution to pH 2.5 with phosphoric acid); flow rate, 0.6 mL/min; column temperature, 35°C; detector wavelength, 260 nm; injection volume, 20 µL. Measurement of adenine, guanine, hypoxanthine, and xanthine was performed in duplicate.

The subcommittee recommended each collaborator to check any peak that was separated completely in the pretest. If a peak was not separated completely, the collaborator was requested to optimize pH of the mobile phase from 2.3 to 2.8.

The results were processed according to JIS Z 8401 guidelines (3), and statistical analysis of the processed data was performed according to JIS Z 8402-2 guidelines (4) and AOAC International Guidelines (1).

RESULTS AND DISCUSSION

Results from 10 collaborators who performed the HPLC-UV method were received for the eight sample pairs (A/B, C/D, E/F, G/H, I/J, K/L, M/N, and O/P). The results for total purine content are shown in Table I. Because collaborator 7 could not separate the guanine peak completely, all data sets of collaborator 7 were excluded from the statistical analysis.

All of the remaining samples were checked for outliers using Mandel's *h* and *k* statistics, and Cochran's and Grubb's outlier test, and outliers were excluded from the statistical analysis (4). The statistical summary of results is shown in Table II.

Each of the calculated analytical values ranged as follows: RSD_r ranged from 0.8 to 4.6%; r_{95} ranged 1.7 to 7.6 mg/L, respectively, and were judged acceptable.

RSD_R ranged from 11.6 to 16.8%; R_{95} ranged 8.0 to 49.5 mg/L, respectively, and were judged acceptable.

It was concluded that the HPLC-UV method is capable of determining total purine content in beer, low-malt beer, and third-category beer containing a total purine content of approximately 20–140 mg/L. The subcommittee recommends that the HPLC-UV method should be adopted for inclusion in the *Methods of Analysis of the BCOJ.*

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