Revised Methods of Diacetyl Analysis

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CONCLUSIONS

1. The specificity of the distillation method (Beer-25,A) could not be determined because of limited collaborator response.
2. The data generated by the broad-spectrum method (Beer-25,B) confirmed its lack of specificity for diacetyl.
3. Specificity of the micro method (Beer-25,C) could not be determined because of limited collaborator response.
4. Temperature, time, and pH were all identified as significant parameters that must be adequately controlled when performing the precursor conversion method.

RECOMMENDATIONS

1. Remove the distillation method (Beer-25,A) from *Methods of Analysis* and place it in the archives.
2. Encourage the membership to provide precursor conversion methods and sample handling procedures. Future subcommittee work could evaluate those methods for inclusion in *Methods of Analysis*.
3. Discharge the subcommittee.

This continuing subcommittee was charged (2) with two tasks: 1) performing ruggedness testing on one precursor conversion method selected for use during sample preparation and 2) conducting studies to determine the specificity of the distillation method (Beer-25,A), the broad-spectrum method (Beer-25,B), and the micro method (Beer-25,C) for the analysis of diacetyl (1).

PROCEDURE

Three samples pairs of packaged beer were prepared representing low, moderate, and high concentrations of diacetyl and pentanedione and were sent to each collaborator. The Youden unit block experimental design (3) was used.

Packaged commercial beer was spiked with standards of diacetyl and pentanedione. Collaborators were requested to use a standard precursor conversion that consisted of aeration by pouring the sample between two containers five times, placing it in a boiling water bath for 20 min, and equilibrating it to 20°C before analysis.

Collaborators were requested to analyze the samples using the distillation method (Beer-25,A), the broad-spectrum method (Beer-25,B), or the micro method (Beer-25,C), as published in *Methods of Analysis*. One of the cochairmen analyzed the samples using the gas chromatographic method (Beer-25,E) (1). Results generated by the collaborators were compared to the cochairman's gas chromatographic data.

RESULTS AND DISCUSSION

Precursor Conversion Method

Ruggedness testing (1) was completed on the precursor conversion method for diacetyl, and the results are listed in Table I. Table II lists the main effects and the confidence intervals for each parameter evaluated.

Increasing the temperature from 92 to 100°C decreased the response by 0.018 mg/L. Raising the residence time in the water bath from 10 to 20 min increased the response by 0.025 mg/

TABLE I
Design and Results of Precursor Conversion Method Ruggedness Test, Diacetyl (mg/L)

	Factors[a]				Test Results			
					Replicates			
Trial	A	B	C	D	1	2	Totals	Differences
1	−	−	−	−	0.023	0.025	0.048	−0.002
2	+	−	−	+	ND[b]	ND	0.000	0.000
3	−	+	−	+	0.034	0.038	0.072	−0.004
4	+	+	−	−	0.017	0.026	0.044	−0.009
5	−	−	+	+	0.052	0.056	0.107	−0.004
6	+	−	+	−	0.027	0.031	0.057	−0.004
7	−	+	+	−	0.080	0.080	0.159	0.000
8	+	+	+	+	0.073	0.068	0.141	0.006

[a]A = temperature, B = time, C = pH, D = aeration.
[b]Below detection limit of gas chromatography; data treated as zero.

TABLE II
Summary of Main Effects of Precursor Conversion Method Ruggedness Test[a]

	Levels Evaluated			
Factors	Low	High	Main Effect	Confidence Interval[c]
A Temperature, °C	92	100	−0.0181[b]	−0.0217 to −0.0145
B Time, min	10	20	0.0254[b]	0.0218 to 0.0290
C pH	4.0	5.0	0.0376[b]	0.0340 to 0.0412
D Aeration, *times*	4	6	0.0014	−0.0022 to 0.0050

[a]All calculations were made based on reference 1.
[b]Significant at the 95% confidence level.
[c]Confidence limits were ± 0.0036 for all factors.

TABLE III
Comparison of Broad Spectrum and Gas Chromatography (GC) for Total Vicinal Diketones (VDK) in Beer (mg/L)

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F
1	0.14	0.14	0.17	0.18	0.21[a]	0.21[a]
2	0.20	0.19	0.22	0.25	0.26[a]	0.24[a]
3	0.07	0.10	0.04	0.04	0.04[a]	0.04[a]
4	0.05	0.05	0.08	0.07	0.12	0.12
5	0.08	0.09	0.12	0.09	0.13	0.15
6	0.05	0.04	0.08	0.09	0.14	0.14
7	0.08	0.08	0.11	0.11	0.15	0.15
Mean[b]	0.094	0.098	0.116	0.117	0.134	0.140
Grand mean[b]	0.096		0.116		0.138	
Diacetyl by GC	0.041		0.062		0.111	
Pentanedione by GC	0.013		0.029		0.083	
Total VDK by GC	0.054		0.091		0.194	

[a]Outlier at $P \leq 0.01$ based on totals and/or differences (1).
[b]Calculated excluding outliers.

L, and raising the pH from 4.0 to 5.0 increased the diacetyl by 0.038 mg/L. The degree of aeration had no significant effect at the 95% confidence level.

Specificity Studies

Only one collaborator agreed to analyze the beer samples using the distillation method (Beer-25,A); therefore, no determination of its specificity was possible.

TABLE IV
Statistical Summary of Results for the Broad-Spectrum Method for Total Vicinal Diketones in Beer[a]

Sample Pair	No. of Labs	Grand Mean	Repeatability			Reproducibility		
			s_r	cv_r	r_{95}	s_R	cv_R	R_{95}
A/B	7	0.096	0.010	10.2	0.028	0.055	56.9	0.153
C/D	7	0.116	0.014	11.5	0.038	0.068	58.2	0.189
E/F	4	0.138	0.003	2.2	0.009	0.013	9.5	0.036

[a]All calculations were made based on reference 3.

TABLE V
Comparison of Micro Method and Gas Chromatography (GC) for Diacetyl in Beer (mg/L)

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	A	B	C	D	E	F
1	0.06	0.07	0.10	0.08	0.14	0.14
2	0.11	0.05	0.07	0.06	0.09	0.14
3	0.02	0.02	0.02	0.05	0.08	0.07
4	0.13	0.07	0.08	0.04	<0.02[a]	<0.02[a]
5	3.69[a]	10.39[a]	7.93[a]	7.11[a]	5.88[a]	5.33[a]
Mean[b]	0.080	0.053	0.068	0.058	0.103	0.117
Grand mean[b]	0.067		0.063		0.110	
Diacetyl by GC	0.041		0.062		0.111	
Pentanedione by GC	0.013		0.029		0.083	
Total vicinal diketones by GC	0.054		0.091		0.194	

[a]Outliers at $P \leq 0.01$ based on totals and/or differences (1).
[b]Calculated excluding outliers.

Data from seven collaborators were received for the broad-spectrum method (Beer-25,B) and are listed in Table III. Outliers were identified using Dixon's outlier test (1). The Youden unit block statistics are listed in Table IV. The repeatability coefficients of variation ranged from 2.2% for the high-concentration pair to 10.2% for the low-concentration pair, indicating acceptable precision. The gas chromatographic method (Beer-25,E) generated a range of total vicinal diketones for the three sample pairs of 0.14 mg/L, whereas the broad-spectrum method (Beer-25,B) could detect only a 0.042-mg/L range. The reproducibility coefficients of variation were unacceptable and ranged from 9.5 to 58.2%. The broad-spectrum method (Beer-25,B) did not show specificity for diacetyl.

The number of results from collaborators for the micro method (Beer-25,C) was insufficient to generate reliable statistical comparisons, but the data are listed in Table V. Outliers were identified using Dixon's outlier test (1). Because of insufficient data, the specificity of the micro method (Beer-25,C) for diacetyl could not be substantiated.

LITERATURE CITED

1. American Society of Brewing Chemists. *Methods of Analysis*, 7th ed. Beer-25A Distillation method, Beer-25B Broad-spectrum method, Beer-25C Micro method, Beer-25E Gas chromatography method, Statistical Analysis-3 Ruggedness testing, Statistical Analysis-4 Youden unit block collaborative testing procedure. The Society, St. Paul, MN, 1976.
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