Vicinal Diketones and Precursors

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CONCLUSIONS

Results from the EBC method for vicinal diketones (VDK) in beer were similar in precision to those from the modified broad spectrum method but appear to overestimate the quantity of VDK present.

RECOMMENDATIONS

1. Discontinue further testing of the EBC method.
2. Test the improved micro method of Inoue (7).

In 1978–1979, the subcommittee tested for a second time the modified broad spectrum procedure (6); poor results were obtained (2). A gas chromatographic method (6) was also tested, but too few results were submitted to permit evaluation of this method. The EBC method (5) was selected for testing in 1979–1980.

PROCEDURE

Three pairs of beer samples were analyzed by the EBC method. One pair of these, designated A and B, had low VDK levels. The second pair, C and D, were prepared as follows. Measured quantities of freshly distilled diacetyl (calculated to add 0.05 and 0.10 mg/L) were placed in two 5-gal tanks. The same finished beer was added to both. The tanks were shaken and allowed to stand for over 48 hr. The beer was then bottled. The third sample pair, E and F, consisted of an imported beer obtained from two local suppliers.

The method used is the official EBC method (5) as revised to permit the use of a conventional still rather than the jacketed Markham still. In each case the paired samples were treated statistically by the Youden unit block design (8).

RESULTS AND DISCUSSION

Ten collaborators submitted results (Table I). The results of collaborator 6 are obvious outliers. This appears to have been a result of the very low value for the diacetyl standard, a difference of only 0.025 absorbance (A) units from the blank. All other collaborators found differences between standard and blank of

TABLE I
Results[a] for VDK in Beer by EBC Method

Lab.	A_{335}		Sample					
No.	Blank	Standard	A	B	C[b]	D[b]	E	F
1	0.008	0.250	0.13	0.28[c]	0.28[d]	0.23	0.29	0.24
2	0.012	0.250	0.14	0.09	0.13	0.14	0.10	0.12
3	0.011	0.266	0.17	0.13	0.18	0.17	0.19	0.30
4	0.010	0.230	0.13	0.14	0.17	0.23	0.09	0.10
5	0.088[e]	0.337	0.06	0.08	0.09	0.14	0.27	0.27
6	0.020	0.045	3.95[c]	4.50[c]	4.48[c]	3.38[c]	3.10[c]	3.35[c]
7	0.00	0.221	0.17	0.13	0.11	0.21	0.13	0.14
8	0.138[e]	0.414	0.06	0.07	0.08	0.11	0.07	0.05
9	0.15[e]	0.359	0.10	0.12	0.10	0.14	0.09	0.08
10	0.002	0.236	0.08	0.10	0.16	0.12	0.09	0.12
Mean	...	...	0.116	0.108	0.128	0.166	0.147	0.158

[a] mg/L, as diacetyl.
[b] Supplemented with diacetyl.
[c] Outliers by Dixon's test at 99% confidence level, not included in mean.
[d] Outlier by Dixon's test at 95% confidence level, not included in mean.
[e] Water rather than ethanol used for blank preparation.

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TABLE II
Statistical Summary of Results[a] for VDK in Beer by EBC Method

Beers	No. of Labs.	Grand Mean[b]	Error			Calculated F[c]	Critical F[e]	c.v., %[f]		
			Within-Lab.[c]	Between-Lab.[c]	Combined[d]			S_c	S_r	S_b
A, B	8	0.11	0.0220	0.0297	0.0370	4.64	3.79	33.4	19.9	26.9
C, D	8	0.14	0.0307	0.0266	0.0406	2.50	3.79	28.5	21.6	18.7
E, F	9	0.15	0.0311	0.0806	0.0864	14.4	3.44	56.7	20.4	52.9

[a] mg/L.
[b] Grand mean = GM = $(\overline{X} + \overline{Y})/2$.
[c] Calculated per Youden and Steiner (8).
[d] Combined error (S_c) calculated from within-lab. error (S_r) and between- lab. error (S_b); $S_c = \sqrt{S_r^2 + S_b^2}$.
[e] Critical F from tables of F distribution (4) at $P = 0.05$.
[f] Coefficient of variation.

TABLE III
Results for VDK in Beer by Other Methods[a]

Lab. No.	Method	Sample					
		A	B	C	D	E	F
3	EBC modification	0.10	0.08	0.12	0.14	0.09	0.11
	Gas chromatography (free VDK)	0.021	0.025	0.064	0.118	0.037	0.038
4	Gas chromatography						
	(free VDK)	0.08	0.09	0.15	0.21	0.04	0.04
	(VDK + precursors)	0.07	0.09	0.14	0.20	0.05	0.06
5	ASBC broad spectrum (1)	0.02	0.03	0.08	0.09	0.45	0.57
8	ASBC broad spectrum (1)	0.03	0.03	0.06	0.10	0.31	0.35
10	Improved micro method (7)	0.06	0.07	0.09	0.12	0.06	0.10
	EBC modification	0.09	0.04	0.12	0.14	0.05	0.06

[a] mg/L.

TABLE IV
Results of Modified EBC Method by Collaborator 3

Sample	A		VDK (ppm)
	Sample	Blank	
A	0.068	0.026	0.10
B	0.054	0.023	0.08
C	0.075	0.026	0.12
D	0.127	0.069	0.14
E	0.074	0.036	0.09
F	0.163	0.120	0.11

0.21–0.28 *A* units. Of the remainder of the results, those from Collaborators 5, 8, and 9 tended to be lower than the others. In these cases, distilled water instead of 10% ethanol was used to prepare the blank and standard diacetyl solutions. This result accorded with the note in the EBC method, which indicates that some reagent grade ethanol preparations may contain substances that form colored products with *o*-phenylenediamine. This in turn causes a high blank result, which when inserted in the formula:

$$\frac{A \text{ Sample} - A \text{ Blank}}{A \text{ Standard} - A \text{ Blank}} \times 0.625$$

results in a low value for the sample. The three laboratories that substituted distilled water for ethanol, however, all found very high blank absorbances with water as well, which presumably accounts for their low results. The results for these laboratories for samples A–D are in fairly good agreement.

Dixon's test for outliers (3) caused the rejection of some data points. The Youden unit block statistical treatments (Table II) showed large coefficients of variation. Results with the A-B and C-D sample pairs were comparable to those of the modified broad spectrum method in the previous year's test. The results with the European beer, sample pair E-F, were much poorer, in spite of their higher VDK level.

Several of the collaborators analyzed portions of the same samples by other methods (Table III.) In most cases, these results were lower than the averages obtained with the EBC method. The results obtained for samples E and F by both collaborators who used the broad spectrum method were quite high. This was also seen in the previous year's testing and is apparently caused by the response of the broad spectrum method to acetoin, which tends to be at higher levels in European beers (2). Collaborator 3 prepared an individual blank for each sample by substituting 0.5 ml of 4*N* HCl for the 0.5 ml of 1% *o*-phenylenediamine in 4*N* HCl. The blank absorbances were fairly substantial (Table IV), indicating the presence of UV-absorbing volatiles in the beer distillates. This would lead to falsely high results. The "EBC modification" in Table III subtracts the blank value obtained for each sample in this manner from the result obtained with the *o*-phenylenediamine reagent. Collaborator 10 made some further trials, which showed UV-absorbing compounds and some non-VDK compounds that react with *o*-phenylenediamine in the distillate; these substances appear to be pyrolysis products formed on the walls of the distillation flask.

The EBC method appears to be capable of giving fairly reproducible results, but the results are higher than those obtained with other methods. This appears to be caused by the presence of both UV-absorbing compounds and non-VDK substances that react with *o*-phenylenediamine in the distillate.

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