Iron in Beer by Ferrozine Method

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CONCLUSIONS

1. Judgment of the applicability of the method to low-iron beers cannot be made until the determination limit is established.
2. Recovery of inorganic iron spiked into one of the samples averaged 73.1%. The reason for the incomplete recovery is not clear.
3. Ruggedness testing indicated that only beer pH was a sensitive parameter.

RECOMMENDATIONS

1. Repeat the collaborative study to determine if reproducibility can be improved.
2. Establish the determination limit of the method.
3. Compare the results of the Ferrozine Method with those obtained by an existing method in *Methods of Analysis*.
4. Determine the cause of the incomplete recovery of added iron.

The purpose of this subcommittee was to evaluate the Ferrozine Method for iron in beer (2,3,5). The method is more rapid than Beer-18A Iron Analysis by Colorimetry (1).

PROCEDURE

Three sample pairs representing relatively low, moderate, and high native iron levels in commercial beer were sent to each subcommittee participant. The Youden unit block design (4) was the basis of the collaborative experiment. Also, one of the samples of the low-iron pair was spiked with additional iron (0.20 mg/L) as a recovery check. Collaborators were also requested to analyze the samples by one of the official ASBC iron methods (1), Beer-18A (colorimetry with bipyridine or orthophenanthroline) or Beer-18B (atomic absorption spectrophotometry). This part of the subcommittee evaluation was optional and for informational purposes only.

RESULTS AND DISCUSSION

Before the collaborative study, ruggedness testing was undertaken according to Statistical Analysis-3 (1). Parameters evaluated were: beer color, turbidity, beer pH, ascorbic acid concentration, ferrozine concentration, time before reading the sample, and wavelength. Table I lists the results; Table II shows main effects and confidence intervals for each parameter. Only beer pH was found to be a sensitive parameter. An increase in beer pH of 1.0 was predicted to reduce the iron result by 0.020 mg/L. The results of the collaborative study indicate that such an increase is not significant when compared to the precision of the method. Age of the ferrozine solution (i.e., one month old vs. fresh) was also examined and was found not to be a significant parameter.

Results from the collaborative experiment are shown in Table III. Collaborator 8 reported slow color development, and the

TABLE I
Design and Results of Ferrozine Method Ruggedness Test (Iron, mg/L)

	Factors[a]							Test Results			
Trial	A	B	C	D	E	F	G	Replicates		Totals	Differences
1	−	−	−	−	+	+	+	0.043	0.048	0.091	−0.005
2	+	−	−	+	+	−	−	0.035	0.040	0.075	−0.005
3	−	+	−	+	−	+	−	0.043	0.037	0.080	0.006
4	+	+	−	−	−	−	+	0.040	0.050	0.090	−0.010
5	−	−	+	+	−	−	+	−0.013	0.020	0.007	−0.033
6	+	−	+	−	−	+	−	0.035	0.038	0.073	−0.003
7	−	+	+	−	+	−	−	0.035	−0.010	0.025	0.045
8	+	+	+	+	+	+	+	0.038	0.035	0.073	0.003

[a] A = color, B = turbidity, C = beer pH, D = ascorbic acid concentration, E = ferrozine concentration, F = time before spectrophotometric determination, G = wavelength.

TABLE II
Summary of Main Effects of Ferrozine Method Ruggedness Testing[a]

Factor	Levels Evaluated		Main Effect[b]	Confidence Interval[c]
	Low	High		
A Color	2 SRM	4 SRM	.014	−0.003 to 0.031
B Turbidity	0.5 NTU	5.0 NTU	0.002	−0.015 to 0.019
C Beer pH	4.0	5.0	−0.020*	−0.037 to −0.003
D Ascorbic acid	0.8 ml	1.2 ml	−0.006	−0.022 to 0.011
E Ferrozine	0.8 ml	1.2 ml	0.002	−0.015 to 0.019
F Time before reading	2 min	10 min	0.015	−0.002 to 0.032
G Wavelength	557 nm	567 nm	0.000	−0.017 to 0.017

[a] All calculations were made according to Statistical Analysis-3 (1).
[b] * = Significant at the 95% confidence level.
[c] Confidence limits were ± 0.016 for all factors.

TABLE III
Iron in Beer by Ferrozine Method (mg/L)

Collaborator	Sample Pair A	B	Sample Pair C	D	Sample Pair E	F
1	0.030	0.015	0.111	0.076	0.184	0.164
2	0.049	0.037	0.142	0.088	0.191	0.183
3	0.034	0.037	0.109	0.095	0.183	0.180
4	0.034	0.042	0.118	0.103	0.147	0.164
5	0.040	0.052	0.126	0.109	0.205	0.198
6	0.048	0.072	0.093	0.087	0.192	0.174
7	0.025	0.033	0.093	0.071	0.095[a]	0.090[a]
8	0.025[a]	0.032[a]	0.079[a]	0.071[a]	0.103[a]	0.092[a]
9	0.038	0.038	0.123	0.093	0.188	0.193
10	0.037	0.034	0.124	0.093	0.200	0.187
11	0.053	0.064	0.139	0.120	0.203	0.208
12	0.043	0.043	0.109	0.081	0.193	0.183
13	0.034	0.035	0.115	0.082	0.200	0.201
Mean[b]	0.0388	0.0418	0.1168	0.0915	0.1896	0.1850
Grand mean[b]	0.0403		0.1042		0.1873	

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

results were identified as outliers. Reaction time was not found to be a sensitive parameter in the ruggedness testing.

Youden unit block statistics are given in Table IV. The repeatability values were similar for all sample pairs, with coefficients of variation ranging from 4.2 in the high-iron pair to 18.7 in the low-iron pair. The reproducibility values were also similar for all sample pairs. Reproducibility coefficients of variation ranged from 8.1 to 30.0. The relatively high coefficients for the low-iron pair reflect the low mean iron concentration.

The number of results from collaborators also testing alternate methods was insufficient to generate reliable statistical comparisons. Generally, results were higher by the ferrozine method.

TABLE IV
Statistical Summary of Results[a]

Sample Pair	No. of Labs	Grand Mean	Repeatability s_r	cv_r	r_{95}	Reproducibility s_R	cv_R	R_{95}
A/B	12	0.0403	0.0075	18.7	0.0211	0.0121	30.0	0.0339
C/D	12	0.1042	0.0090	8.6	0.0251	0.0147	14.1	0.0412
E/F	11	0.1873	0.0078	4.2	0.0219	0.0152	8.1	0.0425

[a]All calculations were made based on reference 4.

Recovery values from the spiked samples ranged from 55 to 93% and averaged 73.1%. The reason for the incomplete recovery is not clear. Differences in preparations of standards do not account for this; no correlation was seen between recovery and slope of the calibration curve or between recovery and time elapsed between sample shipment and analysis.

LITERATURE CITED

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