Sulfur Dioxide in Malt

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Key words: *Colorimetric, Distillation method, Extraction method.*

CONCLUSIONS

1. Statistical evaluation of data obtained from the direct extraction procedure for estimation of residual SO_2 in malt (5) found the method to be within acceptable limits of within-laboratory and between-laboratory error. Combined-laboratory coefficient of variation (c.v.) was acceptable for this type of analysis.
2. Systematically low results were reported by some collaborators again this year for the distillation procedure (2,4). The associated between-laboratory error was not related to standardization but was the result of variables in the distillation process.
3. The direct extraction procedure was generally preferred by the subcommittee for its simplicity and speed.

RECOMMENDATIONS

1. The direct-extraction method is recommended for inclusion in the ASBC "Methods of Analysis" as a reference method.
2. The subcommittee should be discharged.

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PROCEDURE

Two pairs of commercial malt samples were sent to each collaborator. One pair consisted of two-row malts processed similarly and containing approximately 25 mg/kg residual sulfur dioxide; the other pair consisted of six-row malts processed similarly and containing approximately 8 mg/kg residual sulfur dioxide. Collaborators were requested to store the samples at room temperature and avoid prolonged exposure of the samples to light.

Collaborators were previously sent instructions for the distillation and extraction procedures, in addition to basic fuchsin, para-rosaniline hydrochloride, and assayed sodium metabisulfite. They were requested to familiarize themselves with both methods before analyzing test samples.

Collaborators were requested to perform a single analysis of each test sample by distillation and extraction methods within 60 days after samples arrived at their laboratory. An assay procedure for available SO_2 in sulfite solution was included (1), and the sodium metabisulfite was assayed by each collaborator when SO_2 standard solutions were prepared. Collaborators were requested to include a 20 mg/kg standard with test samples analyzed by each method and to specify extraction device and grist preparation for the extraction method.

RESULTS AND DISCUSSION

Ruggedness Testing

The ruggedness of the extraction procedure was examined with a 2^3 factorial design replicated twice. Time constraints precluded ruggedness testing of the distillation method.

The factorial design presented in Table I was used to examine the effects of changes in: the temperature at which color was developed; the concentration of para-rosaniline hydrochloride; and the

extraction time. Analysis of each of the eight different factor combinations was performed in duplicate to give an estimate of the mean squared error (MSE) with 8 degrees of freedom (d.f.).

TABLE I
Ruggedness Test: 2^3 Factorial Design (Replicated Twice)

	Factor[a]			Response (Residual SO_2)[b]	
Run	1	2	3	1	2
1	−	−	−	22.0	21.7
2	−	−	+	25.5	25.1
3	−	+	−	24.5	24.9
4	−	+	+	27.7	27.6
5	+	−	−	27.5	28.0
6	+	−	+	30.2	29.5
7	+	+	−	30.3	29.2
8	+	+	+	32.6	31.3

Analysis of Variance of Main Effects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Treatments	(7)	(149.790)		
Temperature	1		98.01	385.866[c]
Para-rosaniline	1		21.623	85.130[c]
Extraction time	1		28.623	112.687[c]
Error	8	2.030	0.254	
Total	15	151.820		
Critical $F_{0.05\,(1,8)}$ = 5.32				

[a] Factors: 1, temperature (°C), − = 20, + = 30; 2, para-rosaniline concentration (mg/L, − = 350, + = 450; 3, extraction time (min), − = 20, + = 40.
[b] Values are mg/kg malt.
[c] Significant effects.

A standard analysis of variance was performed for the three contrasts representing the main effects as presented in Table I. The F-statistic derived from comparison of the mean square of each effect with the MSE establishes the significance of the treatment effect. The effects for each factor were large and significant.

The temperature for color treatment is the most critical of the factors tested. A temperature range specification of 25 ± 2°C should be included in the method.

Para-rosaniline concentration had a major effect at the levels indicated. Although the difference in concentration is somewhat extreme, the specification in the method for 0.4 g of para-rosaniline in preparation of the para-rosaniline hydrochloride solution should be changed to 400 mg. The effect of time on color development was investigated. An additional 15 min of color development resulted in an average decrease of 0.004 absorbance for ruggedness tests at 20° C and 0.019 absorbance at 30° C. This effect was judged minor because color development prolonged to this extent is highly unlikely.

The effect of extraction time was major and indicates a flaw in the method, leaving it highly susceptible to error from such changes. The extraction procedure does not fully recover SO_2 in the specified time; therefore, samples must be extracted under exactly the same conditions and time constraints observed when preparing the calibration. Filtration of samples should begin immediately upon completion of the extraction. Grist preparation may also affect extraction and should be specified in the method.

Collaborative Testing

Results from collaborative testing of the extraction and distillation methods are presented in Table II. One outlier was identified in results for the extraction method by Dixon's test at $P \leq 0.05$. Statistical treatment of the results is presented in Table III.

Several collaborators experienced difficulty in obtaining a linear

TABLE II
Residual SO_2 in Malt[a]

	Extraction Method					Distillation Method				
	Sample Pair I		Sample Pair II		Standard	Sample Pair I		Sample Pair II		Standard
Collaborator	A	B	A	B	20 mg/kg	A	B	A	B	20 mg/kg
1	28.0	27.8	9.0	7.9	20.7	29.8	29.4	6.0	6.7	20.4
2	30.3	24.9	8.0	8.0	11.4	12.9	17.8	1.5	0.8	19.9
3	32.8	26.5	8.8	9.0	21.0	13.2	13.7	3.0	3.3	20.3
4	34.2	26.2	8.3	8.6	17.9	27.4	26.1	6.4	5.8	20.1
5	31.0	24.3	7.2	8.2	20.1	28.4	22.0	7.6	8.5	19.6
6	28.9	27.1	7.6	8.4	19.6	27.6	27.4	6.7	6.2	21.1
7	29.6	25.8	8.3	7.7	20.8	28.1	28.7	8.5	8.1	18.9
8	20.4[b]	25.4	9.3	7.0	...	...	...	...	...	...
Mean	30.7	26.1	8.3	8.1		23.8	23.6	5.7	5.6	
Grand mean	28.4		8.2			23.7		5.7		

[a] Values are mg/kg malt; data not adjusted for standard.
[b] Outlier according to Dixon's test, $P \leq 0.05$ (3).

TABLE III
Residual SO_2 in Malt: Statistical Summary

Method	Sample Pair	No. of Laboratory	Grand Mean[a]	Error			c.v.[d] (%)	Calculated F[b]	Critical F[e]
				Within-Laboratory[b]	Between-Laboratory[b]	Combined[c]			
Extraction	I	8	27.7	1.99	0.00	1.99	7.00	0.58	4.284
	II	8	8.2	0.77	0.00	0.77	9.37	0.48	3.787
Distillation	I	7	23.8	2.41	6.27	6.72	28.36	14.56	4.284
	II	7	5.7	0.52	2.53	2.59	46.07	49.05	4.284

[a] Grand mean = GM = $(\bar{A} + \bar{B})/2$.
[b] Calculated per Youden and Steiner (6).
[c] Combined error (S_c) calculated from within-laboratory error (S_r) and between-laboratory error (S_b); $S_c = \sqrt{S_r^2 + S_b^2}$.
[d] Coefficient of variation of S_c = c.v. = 100 (S_c/GM).
[e] Criticial F from tables of F distribution (3) at $P \leq 0.05$.

calibration for the distillation procedure. The granular structure of the basic fuchsin used in collaborative testing did not allow complete solubilization at room temperature, which resulted in insufficient basic fuchsin available for color development.

All collaborators used the ASBC fine-grind grist in the extraction procedure and various devices for extraction, including stir plates, wrist-action, orbital and horizontal platform shakers, and a food blender (Waring Blendor or equivalent).

An SO_2 standard equivalent to 20 mg/kg residual SO_2 in malt was included with test samples in each method. Values obtained for the standard were close to expected values in nearly all cases. This indicates that the deviation experienced by two of the collaborators in the distillation method occurred in the distillation step and was not related to standardization.

LITERATURE CITED

1. American Public Health Association, Inc. Method of Air Sampling and Analysis. 2nd ed.: Washington, DC, 1977.
2. American Society of Brewing Chemists. Report of Subcommittee on Estimation of Residual Sulfur Dioxide in Malt. *Journal* 39:98, 1981.
3. Dixon, W. J. *Biometrics* 9:74, 1953.
4. Lukes, B., O'Brien, T. J., and Scanlan, R. A. *J. Am. Soc. Brew. Chem.* 38:146, 1980.
5. Pitz, W. J., and Lubert, D. J. *J. Am. Soc. Brew. Chem.* 39:112, 1981.
6. Youden, W. J., and Steiner, E. H. Statistical Manual. Assoc. Off. Anal. Chem.: Washington, DC, 1975.

APPENDIX
SULFUR DIOXIDE IN MALT
EXTRACTION-COLORIMETRIC PROCEDURE

Reagents

(a) *Stock extracting solution.* Dissolve mercuric chloride (54.4 g), sodium chloride (23.4 g), and sodium azide (0.06 g) in 5% glycerol and bring to 2 L in a volumetric flask.

(b) *Dilute extracting solution.* Dilute one volume of reagent (a) with 15 volumes of distilled water.

(c) *Formaldehyde solution (0.2% v/v).* Dilute 2.7 ml of 37% (w/w) reagent grade formaldehyde to 500 ml with distilled water. Prepare a fresh solution daily.

(d) *Para-rosaniline hydrochloride solution.* Dissolve 400 mg of para-rosaniline hydrochloride in about 700 ml of distilled water in a 1-L volumetric flask by heating the mixture in a water bath (70° C) for 20 min with occasional swirling. Cool to 20° C and add 60 ml of reagent-grade hydrochloric acid and bring to volume with distilled water. Solution is stable for at least one month if stored in amber glass at 5° C.

(e) *Para-rosaniline hydrochloride—formaldehyde solution.* Mix equal volumes of reagent (c) and reagent (d) sufficient for the number of samples tested (2 ml of this mixture required per sample). Prepare a fresh solution daily.

(f) *Stock SO_2 solution (25 mg/L).* Dilute 5.0 ml of a freshly prepared 500 mg/L aqueous SO_2 solution (approximately 742 mg sodium metabisulfite or 812 mg sodium bisulfite in 1 L of distilled water) to 100 ml with reagent (a). The above weights are based on theoretical yields of SO_2. The sodium metabisulfite or sodium bisulfite must be assayed for actual SO_2 content to prepare the 500 mg/L SO_2 solution (1,2).

(g) *Standard SO_2 solution (10 mg/L).* Dilute 20.0 ml of reagent (f) to 50.0 ml with reagent (b).

Apparatus

(a) *Pipettes,* volumetric, assorted.
(b) *Test tubes,* 20 ml.
(c) *Filter funnels.*
(d) *Filter paper,* Whatman No. 1.
(e) *Volumetric flasks,* 50, 100, 500, and 1,000 ml.
(f) *Extraction vessel,* 500-ml glass-stoppered.
(g) *Extraction device,* shaker, stir plate, or blender capable of repetitive duplication of extraction conditions.
(h) *Spectrophotometer,* at 560 nm.

Calibration

To a series of 100-ml volumetric flasks, add 0.0 ml, 2.0 ml, 4.0 ml, 6.0 ml, 8.0 ml, and 10.0 ml of reagent (g) and dilute each flask to volume with reagent (b) to give SO_2 solutions ranging from 0 to 1.0 mg/L. Add the contents of each flask to individual 250-ml glass-stoppered flasks containing 2.0 g of unsulfured malt grist prepared in accordance with ASBC fine grind (MALT-4). Shake or stir the contents for 30 min or blend for 5 min, then filter immediately through a Whatman No. 1 filter paper, returning the first few milliliters of filtrate. Transfer 10.0 ml of the filtrate to a test tube and add 2.0 ml of reagent (e). Mix by inverting each tube twice and incubate at 25 ± 2° C for 35 min. Use an aliquot from the test tube that received filtrate from the flask with no addition of reagent (g) to establish zero absorbance for the spectrophotometer at 560 nm. Determine the absorbance values for the calibration samples.

Method

Screen malt thoroughly to remove rootlets, then grind 25 g of the clean malt according to ASBC fine grind (MALT-4). Mix the grist thoroughly and transfer 2.0 g into 250-ml glass-stoppered flask for extraction. Add 100 ml of reagent (b) and proceed with the extraction and color development exactly as in the calibration. It is important that extraction conditions remain constant from calibration. Zero the spectrophotometer with a reagent blank from unsulfured malt subjected to the entire analysis. Concentration of residual SO_2 in the malt sample is determined from a regression equation or curve of the calibration. The mg/L SO_2 from the curve multiplied by 50 gives the mg/L SO_2 in the malt on an "as-is" moisture content basis. Report results to one decimal place.

LITERATURE CITED

1. American Society of Brewing Chemists. Methods of Analysis, 7th ed. BEER-21. The Society: St. Paul, MN, 1976.
2. American Public Health Association, Inc. Methods of Air Sampling and Analysis, 2nd ed. The Association: Washington, DC, 1977.

REFERENCES

1. Association of Official Analytical Chemists. Official Methods of Analysis, 13th ed. 10.079. The Association: Washington, DC, 1980.
2. Beetch, E. B., and Oetzel, L. I. *J. Agric. Food Chem.* 5:951, 1957.
3. Lukes, B., O'Brien, T. J., and Scanlan, R. A. *J. Am. Soc. Brew. Chem.* 38:146, 1980.
4. Pitz, W. J., and Lubert, D. J. *J. Am. Soc. Brew. Chem.* 39:112, 1981.