Residual Sulfur Dioxide in Finished Malt: Colorimetric Determination and Relation to *N*-Nitrosodimethylamine[1,2]

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ABSTRACT

Residual SO_2 concentrations in production malts from direct-fire, fossil fuel kilns in which SO_2 was applied to control *N*-nitrosodimethylamine (NDMA) formation were determined by a modified colorimetric procedure and correlated to malt NDMA content from analysis by gas chromatography-thermal energy analysis. Malt samples containing residual SO_2 greater than 30 mg/kg had less than 10 μg/kg of NDMA. Samples with SO_2 residual less than 30 mg/kg were correlated to NDMA levels. Determination of residual SO_2 values can therefore be used as a rapid screening procedure to detect production malts that may contain NDMA in excess of 10 μg/kg.

Key words: *Analysis, Correlation, Malt, Nitrosamine, Sulfur dioxide*

Currently available methods of analysis for *N*-nitrosodimethylamine (NDMA) in malt are time-consuming and costly, limiting their applicability for routine screening of production malts. To inhibit NDMA formation, sulfur dioxide (SO_2) is now applied to malt as it is dried in direct-fire, fossil fuel kilns. Application is in the form of SO_2 gas, either applied directly or from combustion of elemental sulfur. The search for an alternative to direct NDMA analysis as a routine quality control procedure for malt production has centered on measurement of residual SO_2 and surface pH. In our experience, measurement of residual SO_2 (mg/kg) is more significant as an indicator of malt NDMA level than is measurement of surface pH. Residual SO_2 analysis is specific, and the procedure can be closely controlled (2,3,8,9). The colorimetric analysis of residual SO_2 by a modification of the method of Beetch and Oetzel (3) has proven to be relatively fast, accurate, and requires minimal investment in reagents and equipment.

Production malts (March 1979 through February 1980) of eight varieties were analyzed for residual SO_2 and NDMA by a gas chromatograph-thermal energy analyzer system (CG-TEAS) (4). Many of these malts were produced and analyzed during the developmental stage of sulfuring, when a wide range of rates, exposure times, and drying schedules in single, double, and triple level kilns was examined.

The relationship between the level of residual SO_2 and the corresponding NDMA in finished malt was statistically evaluated and graphed.

EXPERIMENTAL

SO_2 Determination

Reagents. Basic fuchsin (0.10 g) dissolved in 100 ml of distilled water and filtered to remove particulates.

Acid-bleached basic fuchsin solution was prepared from exactly 8.0 ml of basic fuchsin solution and approximately 100 ml of distilled water, mixed in a 200-ml volumetric flask. Reagent grade hydrochloric acid (12.0 ml) was added, followed by dilution to volume with distilled water. The solution was allowed to bleach to a pale yellow color and was prepared daily.

Reagent grade formaldehyde (36%; 5.5 ml) was diluted to 100 ml with distilled water.

Sodium tetrachloromercurate (II) solution was prepared from reagent grade sodium chloride (11.7 g) and reagent grade mercuric chloride (27.2 g), dissolved in distilled water (1.0 L).

Pyrogallic acid (5.0 g) was dissolved in 25 ml of hot distilled water, cooled and mixed with 100 ml of 80% (w/v) aqueous potassium hydroxide. Each preparation of this solution was used for a maximum of 30 analyses and was stored in the dark when not in use.

A particular lot of reagent grade sodium hydrogen sulfite was assayed for SO_2 content (1) before preparation of an aqueous solution containing 500 mg/L of SO_2. A 5.0-ml aliquot of this solution was immediately diluted to 100 ml with sodium tetrachloromercurate solution. This standard was stable for 30 days at 5° C.

Apparatus. A Bausch and Lomb Spectronic® 100 with 20-mm cuvets was used for all colorimetric analyses. An all-glass distillation apparatus with tygon connectors (Fig. 1) was used for recovery of SO_2.

Preparation of the Standard Curve. Aliquots (0.5–5.0 ml) of standard sulfite solution were adjusted to a final volume of 5.0 ml with sodium tetrachloromercurate solution before addition to individual 100-ml volumetric flasks containing 25 ml of acid-bleached basic fuchsin solution and 2.0 ml of formaldehyde solution. Color was developed for 8 min before dilution to volume with distilled water. Absorbance was determined immediately at 560 nm and was stable for approximately 5 min. In color development, 1.0 ml of standard sulfite solution was equivalent to a residual SO_2 level of 10 mg/kg of finished malt for each 50 g of malt used in distillation. The standard curve is shown in Fig. 2.

Malt Analysis. Fifty grams of whole malt, cleaned manually over a precision sieve (slotted, 4 7/8/64 in. × ¾ in.) to remove rootlets and chaff, and 300 ml of distilled water were placed in a three-neck 1-L boiling flask. Sodium tetrachloromercurate solution (100 ml) was pipeted into the 250-ml graduated cylinder, and 20 ml of reagent grade hydrochloric acid was placed in the 125-ml pressure

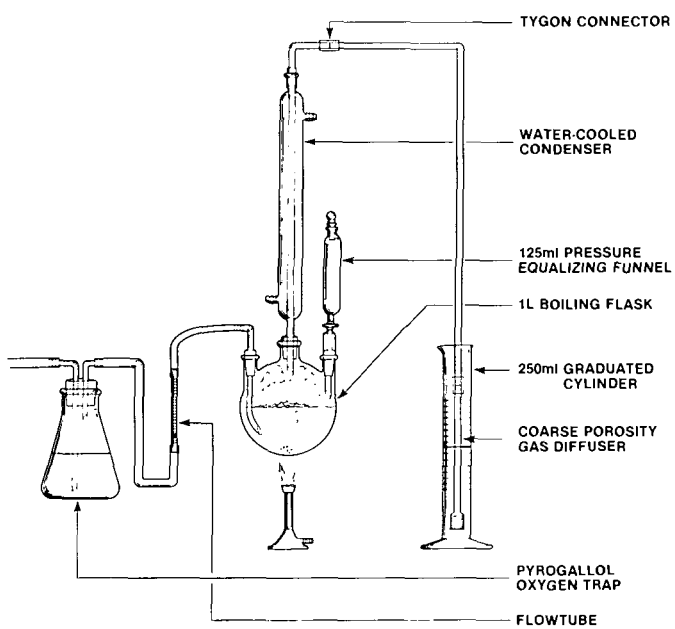

Fig. 1. Distillation apparatus for SO_2 recovery.

[1] Presented at the 46th Annual Meeting, Minneapolis, MN, May 1980.
[2] Presented in part to ASBC Local Section No. 7 (Northwest), Yakima, WA, August 1979.

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equalizing funnel with the stopcock closed. Nitrogen gas flow was initiated at 75 ml/min, 10 psi, and the distillation apparatus purged for 5 min. Hydrochloric acid was introduced into the pressure-equalizing funnel, and the contents of the flask were brought to boiling and refluxed for 45 min. A 5.0-ml aliquot from the graduated cylinder was used for color development, as in preparation of the standard curve. Malt SO_2 concentration was obtained from the standard curve.

We found certain precautions to be necessary: preparation of a separate standard curve for each lot of basic fuchsin, protection of the sodium tetrachloromercurate (II) solution from heat during distillation (1), replacement or cleaning of gas diffusers after several distillations, and uniform cleaning of and rootlet removal from the malt sample. Rootlets and chaff normally carry 10–50 times more residual SO_2 by weight (Table I) than does cleaned whole malt. Glass tubing joints were butted under tygon connectors to avoid SO_2 adsorption.

Standard sulfite additions to malt in distillation were not attempted. Previous studies[3] have shown that malt has the ability to bind a portion of the sulfite applied in a nondistillable form.

NDMA Determination

Malt Extraction. For the analysis of NDMA, ground malt was vacuum distilled from mineral oil by a modification (6,7) of the procedure of Fine et al (4,5). Fifty grams of whole malt, cleaned manually by sieving, as above, was ground in liquid nitrogen. The ground malt, 5 g of ammonium sulfamate dissolved in 40 ml of 1 *N* sulfuric acid, and 50 ml of pharmaceutical grade mineral oil were placed in a 1-L round-bottomed distillation flask fitted with a thermometer well. A vacuum of more than 100 μ was applied and the distillate trapped in two vacuum vapor traps connected in series and cooled in liquid nitrogen. The flask was heated to 100° C over a 1-hr period. The distillate was thawed and each trap rinsed with distilled water (3 × 40 ml) followed by dichloromethane (DCM) (1 × 40 ml). The aqueous distillate and the combined water rinses were acidified (with sulfuric acid, pH 2), saturated with sodium sulfate, and extracted with the combined DCM rinses. The aqueous phase was further extracted with DCM (2 × 40 ml), and the combined DCM extracts were washed with 1.5 *N* sodium hydroxide (1 × 40 ml) and dried by being passed through anhydrous sodium sulfate.

[3] E. B. Beetch. Analytical Chemistry Progress Report. Rahr Malting Co.: Manitowoc, WI. June 1956.

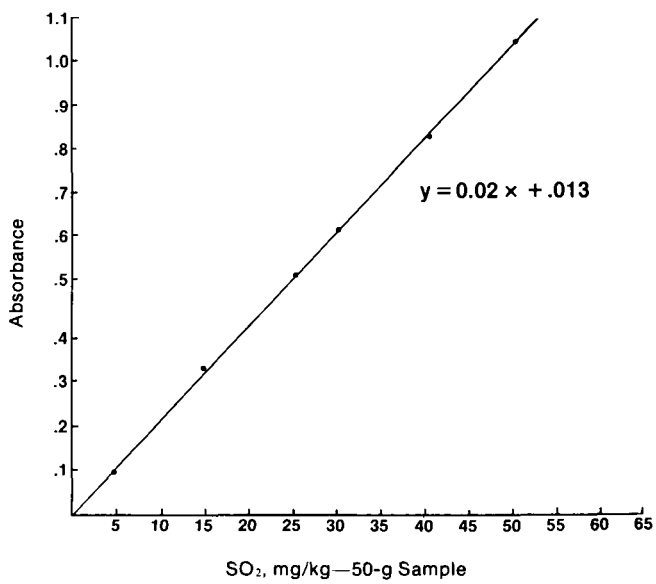

Fig. 2. Bisulfite calibration curve for modified colorimetric SO_2 analysis: absorbance vs SO_2 concentration.

The DCM fractions were first concentrated to about 3 ml in a Kuderna-Danish concentrator and then further concentrated to 1 ml under a stream of nitrogen, using a micro-Snyder column.

Analysis by GC-TEAS. Samples were quantitatively analyzed against external standards by injecting portions of 4–8 μl into the GC-TEAS. Although the NDMA values were not corrected for recovery, percent recovery was determined by adding 3 μg of NDMA in 1 ml of hexane to 50 g of ground malt that had been dried in an all-electric kiln and did not contain NDMA above 0.5 μg/kg. The recovery for NDMA was 83%. The GC-TEAS parameters were: injector, 180° C; column, 3.7 m × 3.18 mm od stainless steel packed with 10% Carbowax 20M on 60/120 Chromosorb G-AW, 180° C isothermal; furnace, 400° C; vacuum, 5 mm; trap, −160° C.

RESULTS AND DISCUSSION

Results of the analysis of 57 production malts for residual SO_2 and NDMA are summarized in Fig. 3. Production malts with residual SO_2 levels greater than 30 mg/kg did not exceed 10 μg/kg of NDMA, and 89% of these samples contained less than 5 μg/kg. The nature of the SO_2-NDMA relationship and the limitations inherent to both analytical procedures (3,7) prevented correlation of data points exceeding 30 mg/kg residual SO_2. Nineteen of the 57 malt samples contained residual SO_2 levels less than 30 mg/kg and were correlated to NDMA levels ($r = 0.800$) at 99.9% significance. The slope of linear regression (−0.205), accurately defined for 64% of these samples, indicates the rapid increase in NDMA as residual SO_2 falls below 30 mg/kg. The residual SO_2 of blended malts cannot be correlated to NDMA; inclusion of a small percentage of malts with very high NDMA content would not appreciably alter the SO_2 concentration of the blend.

For the purpose of relating residual SO_2 to NDMA, malt samples should be from recent production. A loss of approximately 10% of the original SO_2 residue has been observed in samples stored for six months in closed nonporous containers.

TABLE I
SO_2 Residual and *N*-Nitrosodiethylamine (NDMA) in Fractional Components of a Finished Production Malt

	SO_2 Residual (mg/kg)	NDMA (μg/kg)
Rootlets	2,800.0	7.8
Husk	860.0	2.9
Endosperm	16.0	1.0

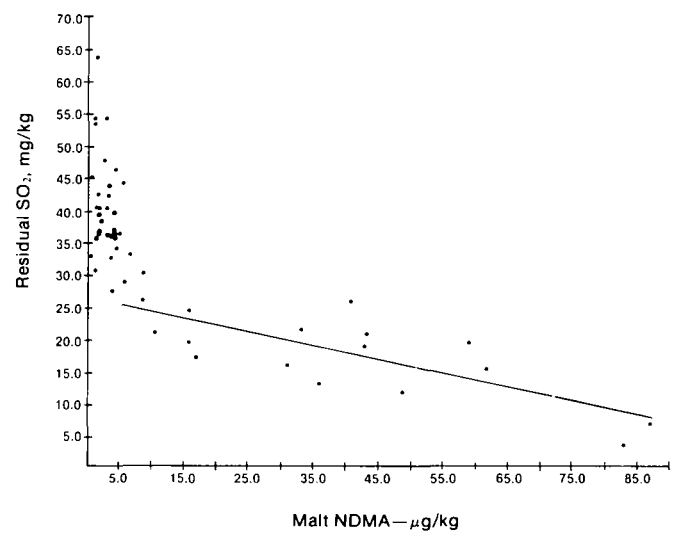

Fig. 3. Relationship between residual SO_2 and *N*-nitrosodiethylamine content of production malts. $n = 19$, $Y = -0.205 + 26.4$, $r = 0.800$.

Analysis for residual SO_2 has proven useful in examination and improvement of sulfuring techniques during kilning and as an aid in studying the mechanisms of NDMA formation and inhibition during sulfuring. An interesting finding was that residual SO_2 of the rootlet, husk, and endosperm fractions of a finished malt from our production paralleled NDMA concentration (Table I).

In view of the effectiveness of current sulfuring techniques in controlling NDMA formation, an important application for residual SO_2 in malt is the routine screening of malts from direct-fossil fuel kilns when NDMA analysis of each production piece is not possible. This study has shown that residual SO_2 values can be used as a rapid screening procedure to detect most malts containing relatively high amounts of NDMA. Malt with a residual SO_2 level less than an established critical value, 30 mg/kg in this case, must be suspected of containing unacceptable levels of NDMA and be submitted for NDMA analysis before final disposition.

ACKNOWLEDGEMENTS

This investigation was supported in part by Grant No. 5R01 CA 25002, awarded by the National Cancer Institute, Department of Health and Human Services. We also thank the National Cancer Institute, DHHS, for the loan of the thermal energy analyzer under Contract No. N0 1-CP-8-5660.

LITERATURE CITED

1. American Public Health Association, Inc. Methods of Air Sampling and Analysis (2nd ed.). The Association: Washington, DC, 1977. p. 696.
2. Anderson, R. J., Howard, G. A., and Hough, J. S. *Eur. Brew. Conv., Proc. Congr. 13th, Estoril, 1971,* p. 253.
3. Beetch, E. B., and Oetzel, L. I. *J. Agric. Food Chem.* 5:951, 1957.
4. Fine, D. H., Rounbehler, D. P., and Oettinger, P. E. *Anal. Chim. Acta* 78:383, 1975.
5. Fine, D. H., and Rounbehler, D. P. *J. Chromatogr.* 109:271, 1975.
6. Hotchkiss, J. H.., Libbey, L. M., and Scanlan, R. A. *J. Assoc. Off. Anal. Chem.* 63:74, 1980a.
7. Hotchkiss, J. H., Barbour, J. F., and Scanlan, R. A. *J. Agric. Food Chem.* 28:678, 1980.
8. Scaringelli, F. P., Saltzman, B. E., and Frey, S. A. *Anal. Chem.* 39:1709, 1967.
9. West, P. W., and Gaeke, G. C. *Anal. Chem.* 28:1816, 1956.

[Received May 12, 1980]