

Modified Method for the Determination of Sulfur Dioxide in Malt¹

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ABSTRACT

A method suitable for the routine determination of residual sulfur dioxide in large numbers of malt samples is described. The technique is a modification of an existing method and is rapid and simple in that it involves no distillation or nitrogen stripping. Sulfur dioxide is extracted from ground malt with a dilute solution of sodium tetrachloromercurate and a colored complex is formed with para-rosaniline hydrochloride. The technique is sensitive and offers repeatability that is comparable to that of existing methods. Coefficients of variation on malts with mean sulfur dioxide levels of 5.8, 18.4, and 27.2 mg/kg were 5.9, 6.6, and 5.7%, respectively. The mean coefficient of variation calculated from data on three similar malts accumulated over a several month period averaged 9.5%. Data obtained were comparable and correlated very highly with that obtained by three more elaborate techniques.

Key words: *Analysis, Malt, Method, Sulfur dioxide*

Treatment of green malt with sulfur dioxide (SO₂) in the early stages of kilning has a useful bactericidal and bleaching effect on the malt. However, high residual levels of SO₂ in the malt may impart off-flavors and undesirable physical characteristics to beer (5,7,15). For this reason, it is desirable to monitor levels of residual SO₂ in the finished malt to insure that it is at a level compatible with the intended use of the malt. Recently, residual levels of SO₂ have been correlated with the *N*-nitrosodimethylamine (NDMA) content in malt and proposed as a simple means for NDMA estimation (10).

A number of colorimetric and titrimetric methods are available for the estimation of SO₂ in beer. The present recommended methods (8) of the European Brewery Convention (EBC) include the ASBC BEER-21 method and a modified Monier-Williams method (11). The Institute of Brewing (IoB) also recommends (9) a modification of the Monier-Williams method. An ASBC collaborative study (2) comparing the Monier-Williams method with the colorimetric procedures of Brenner et al (6) and Stone and Laschiver (12) led to the adoption of the latter colorimetric procedure as the recommended ASBC method (BEER-21) for beer (1). The Association of Official Analytical Chemists (3) has also adopted this method. The volumetric and gravimetric methods of Monier-Williams and the colorimetric procedure of Brenner et al involve distillation and/or nitrogen stripping. In the Stone and Laschiver method, total SO₂ is determined by decomplexing bound SO₂ with alkali in the presence of sodium tetrachloromercurate followed by the formation of a colored complex with para-rosaniline hydrochloride and formaldehyde. Sodium tetrachloromercurate was introduced as an absorber of SO₂ by West and Gaeke (14) as an improvement over previously used absorbers such as 5% glycerol in 0.1*N* NaOH (13). They also substituted para-rosaniline hydrochloride for basic fuchsin in the preparation of the chromogen.

There is no recommended ASBC, EBC, or IoB method for the determination of SO₂ in malt. The reaction between evolved SO₂ absorbed in sodium tetrachloromercurate and para-rosaniline hydrochloride has been used by Beetch and Oetzel (4) and Lukes et al (10). Others use methods based on the Monier-Williams method. The method employed in the authors' laboratory for a number of years was an adaptation of the Brenner et al (6) method. Assays involving distillation and nitrogen stripping are, however, generally time-consuming, unwieldy, and undesirable for routine work (2). Recent increases in sulfuring rates as an interim measure

to reduce NDMA levels in malt have emphasized the need for a rapid and precise method for the routine determination of SO₂ in large numbers of malt samples. An evaluation of some of the existing methods has led to the development of the present technique. It bears similarity to the Stone and Laschiver method (12) for beer, and to ASBC BEER-21 in that the SO₂ is extracted directly into sodium tetrachloromercurate, thus eliminating distillation and nitrogen stripping. This approach provided the desired simplicity and speed without sacrificing sensitivity or precision.

EXPERIMENTAL

Reagents

All chemicals were of an analytical reagent grade and demineralized water was used. Five solutions were used:

Extracting Stock Solution. Mercuric chloride (54.4 g), sodium chloride (23.4 g), and sodium azide (0.06 g) were weighed into a 2-L volumetric flask, dissolved, and made to volume with 5% glycerol.

Dilute Extracting Solution. One volume of the extracting stock solution was diluted with 14 volumes of water.

Formaldehyde Solution (0.2%, v/v). This was made by diluting 2.7 ml of 37% (w/w) formaldehyde to 500 ml with water. A fresh solution was prepared daily.

Para-Rosaniline Hydrochloride Solution. Para-rosaniline hydrochloride (Sigma, 0.4 g) was weighed into a 1-L volumetric flask and dissolved in about 700 ml of water by heating the mixture in a water bath (70°C) for 20 min with occasional swirling. The solution was then cooled to 20°C. After 60 ml of concentrated hydrochloric acid was added, the acidic solution was made to volume with water. The solution was stable for at least one month when stored in an amber bottle in a refrigerator.

Para-Rosaniline Hydrochloride-Formaldehyde (Chromogen). Equal volumes of the formaldehyde and para-rosaniline solutions were mixed as required. Two milliliters of this solution were used for each test sample.

Procedure for Malt Analysis

After thorough screening to remove rootlets and separated husk, the malt (25 g) was ground in a Moulinex coffee grinder for 20 sec. Grist (2 g) was weighed into a 250-ml ground-glass stoppered flask and 100 ml of the dilute extracting solution was added. The flask contents were shaken for 30 min on a wrist action shaker, then filtered through Whatman No. 1 filter paper, returning the first few milliliters of filtrate. The filtrate (10 ml) was transferred to a second test tube and 2 ml of the chromogen was added. Mixing was achieved by inverting the tube twice; the sample was allowed to incubate for 35 min at 25°C. Absorbance was determined at 560 nm after zeroing the spectrophotometer with an aliquot of a mixture of 10 ml of the dilute extraction solution and 2 ml of the chromogen. The SO₂ content of the malt was then determined from a standard curve.

For comparison, several malts were also analyzed by adaptations of the Brenner et al, Monier-Williams volumetric, and the Beetch and Oetzel methods.

Calibration

A 10 mg/L SO₂ solution was prepared by diluting 10 ml of a sodium bisulfite stock solution (1.54 g dissolved in one L of dilute extracting solution) to one L with the dilute extracting solution. Standard solutions ranging in concentration from 0 to 0.2 mg/100 ml were prepared by diluting appropriate volumes to 100 ml with the dilute extracting solution and adding these to 250-ml ground-

¹Presented at the 47th Annual Meeting, Miami, FL, May 1981.

glass stoppered flasks containing 2.0 g unsulfured ground malt. The dilute extracting solution (100 ml) was added, the SO₂ extracted as described above, and a standard curve prepared.

RESULTS AND DISCUSSION

Application of dilute sodium tetrachloromercurate as a medium for the direct extraction of SO₂ from ground malt was found to be a useful alternative to its traditional role as an absorber to trap the gas liberated by distillation. Sulfur dioxide was reported by West and Gaeke (14) to react with sodium tetrachloromercurate to form a stable and nonoxidizable disulfomercurate ion; presumably, the same complex is formed under the present conditions. Interference from other malt extractables was not great (Fig. 1) and in any event was at least partially corrected for by preparing the standard curve in the manner described. A slight inherent error in the test arises from the fact that absorbance readings on unsulfured malts may vary. Therefore, for calibration purposes, it is important to use a composite of several unsulfured malts.

The test was linear over the concentration range tested (0–0.2 mg/100 ml; Fig. 1) which corresponded to malts with SO₂ contents ranging from 0 to 100 mg/kg. Hull, rootlet, and malt extracts that had higher levels of SO₂ were diluted as required to give absorbance readings on scale. Recoveries of SO₂ added to three malts are shown in Table I and were within the precision limits of the test.

Repeatability data on three malts with mean SO₂ contents of 5.8, 18.4, and 27.2 mg/kg gave coefficients of variation of 5.9, 6.6, and 5.7%, respectively (Table II). The coefficient of variation calculated from data on a second set of three similar malts accumulated over a several month period averaged 9.5%. In this latter set of data, error was greatest for the malt with the lowest level of SO₂ (range 4.7 to 7.2 mg/kg; n = 11). The presence of rootlets in the sample was probably the most important factor adversely affecting the precision of the test. Levels of SO₂ in rootlets have been reported to

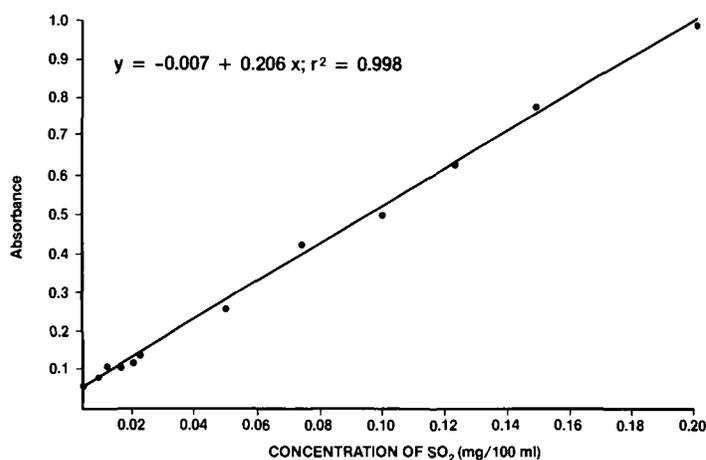


Fig. 1. Calibration curve for sulfur dioxide (SO₂) assay. Absorbance (560 nm) plotted against SO₂ in mg/100 ml.

TABLE I
Recovery of Various Amounts of Sulfur Dioxide (SO₂)
Added to Three Malts

SO ₂ Added (mg/kg)	SO ₂ Content (mg/kg) ^a		
	Malt A	Malt B	Malt C
None	6.0	28.3	54.7
10	15.7 (16.0) ^b	37.0 (38.3)	63.8 (64.7)
20	26.0 (26.0)	49.4 (48.3)	80.7 (74.7)
40	47.6 (46.0)	74.3 (68.3)	96.8 (94.7)

^a Values reported are means of two determinations.

^b Values in parentheses are expected values.

be as high as 2,800 mg/kg (10). For this reason, malts should be thoroughly screened before grinding. A brief evaluation of two additional grinders (hammer-mill type and cone grinder) was made. Neither offered improved repeatability over the coffee grinder which was adopted for use. Sampling and grinding 50 g of malt (30 sec) appeared to improve the precision of the test; for routine work only 25 g were used.

Table III shows data comparing residual levels of SO₂ in several malts as determined by the Brenner et al, Beetch and Oetzel, and the present technique. The mean value obtained with the Beetch and Oetzel method was considerably lower than the mean for the other two methods. Data by all three methods correlated very highly with each other, largely because of the wide range in SO₂ content of the samples. The present method appeared to be the most sensitive of

TABLE II
Repeatability Data on Three Malts Obtained
with the Present Sulfur Dioxide Assay

	Malt A	Malt B	Malt C
Number	6	6	10
Range	5.5–6.2	16.8–20.2	24.9–29.2
Mean (mg/kg)	5.8	18.4	27.2
Standard deviation	0.34	1.21	1.54
Coefficient of variation (%)	5.9	6.6	5.7

TABLE III
Sulfur Dioxide (SO₂) Content of Nine Malt Samples as Determined by
Brenner et al, Beetch and Oetzel, and Present Method

Malt	Residual SO ₂ (mg/kg) ^a		
	Brenner et al	Beetch and Oetzel	Present Method
1	0.2	0.0	0.3
2	5.3	5.8	8.3
3	14.2	12.2	16.5
4	25.2	22.1	24.5
5	29.8	19.5	27.0
6	40.1	41.2	39.1
7	82.8	65.6	85.0
8	98.4	74.4	110.0
9	127.2	96.2	139.4
Mean	47.0	37.4	50.0

Correlation: Brenner et al (6) vs Beetch and Oetzel (4) = 0.994
Brenner et al vs Present Method = 0.998
Beetch and Oetzel vs Present Method = 0.989

^a Values reported are means of two determinations.

TABLE IV
Sulfur Dioxide (SO₂) Content of Nine Malt Samples as Determined
by the Monier-Williams and Present Method

Malt	Residual SO ₂ (mg/kg) ^a	
	Monier-Williams	Present Method
1	2.8	1.7
2	4.8	9.3
3	16.8	14.9
4	22.4	23.0
5	22.4	28.9
6	30.4	33.0
7	40.4	37.2
8	62.4	79.8
9	72.0	98.8
Mean	30.5	36.3

Correlation coefficient = 0.982

^a Values reported are means of two determinations.

the three as it gave the widest range for the malts. Comparison of data obtained on another set of malts with the Monier-Williams volumetric method and the present method is shown in Table IV. While the latter method showed greater sensitivity at the higher levels and gave a higher mean value for the malts, ranking agreement between the two methods was good ($r = 0.982$).

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[Received May 12, 1981]