A Colorimetric Procedure for the Measurement of Dimethyl Sulfide in Water, Wort, and Beer[1]

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ABSTRACT

A colorimetric method is presented for the quantitative determination of dimethyl sulfide (DMS) in water, wort, and beer. The method is based on the formation of a complex between DMS and sodium nitroprusside. The DMS-sodium nitroprusside complex which is formed under alkaline conditions turns pink when the solution is acidified. The pink complex has an absorption maximum at 520 nm. Details of the procedure for the determination of DMS are presented in this paper, along with optimum conditions for its extraction from various media as well as for color development and measurement. The method is reproducible (standard deviation = 3.7 μg/l.), accurate (±2%), and rapid (32 analyses may be performed in 8 hr). DMS found in commercial beers ranges from 30 to 160 μg/l., with most beers falling in the 40 to 70 μg/l. range. For a given brand of beer, the DMS concentration appeared to remain fairly constant, indicating that its level is characteristic of the brewing materials and process conditions. Dimethyl sulfide increases in beer upon storage under warm conditions, the extent of increase being dependent on the length of storage and the temperature. Heat treatment of wort brings about an increase in the level of DMS. Microbial contamination of wort also results in appreciable increases in the concentration of DMS.

Key words: *Beer, Colorimetric analysis, Dimethyl sulfide (DMS).*

In recent years, there have been many reports concerning the formation, detection, and measurement of dimethyl sulfide (DMS) in beer. This compound is of considerable importance to beer not only because of its odor, but also because of its low flavor threshold of 25–30 μg/l. (10,14,26,30).

Many investigations have been focused on the role of bacteria and yeast on the formation of dimethyl sulfide. Whereas it is generally agreed that wort spoilage organisms can produce DMS (3,20,21,25,31), there is still some controversy concerning the role of yeast in the production of this compound (2,3,17,20,22,25,31). Other studies on the source of dimethyl sulfide have been directed mainly to the kilning operation and to other areas of the malting process. The presence of DMS in malt has been confirmed (2,4,15,20,22,24,29,31), but there have been conflicting reports regarding the effect of kilning on DMS and the fate of DMS present in malt (2,15,20). It has been found that, in addition to enzymatically produced DMS, malt also contains a heat-labile precursor (2,15,20,22,32) identified as s-methylmethionine sulfonium salt (20). Other workers, however, have not confirmed its identity (2,32).

The techniques used for the quantification of DMS include gas chromatography as well as thin-layer chromatography. The gas chromatography procedures used employed various detection systems, namely: microcoulometric detector (28), thermal conductivity detector (24), and flame photometric detector (2, 4,6,7,11,14,15,19,20,21,25,27). A combination of flash exchange chromatography of mercuric salts (12) and flame ionization detector, as well as the gas chromatography of the sulfone oxidation product of dimethyl sulfide (which uses a flame ionization detector) (8,16) have also been reported. The thin-layer chromatography procedure reported uses the formation of a sulfimidine (18). In spite of the similarity between various methods, widely differing values have been reported for the concentration of DMS in beer. Values from less than 10 μg/l. (11,12) to 140 μg/l. (8) have been reported. More recent investigations have established the range to be 10–30 μg/l. in ales and 20–60 μg/l. in lager beers (20,28,29,30).

Although techniques such as colorimetric, potentiometric, and paper chromatographic methods have been reported for a number of sulfur compounds in beer (8,13,23,29), none have been reported for the measurement of DMS. In this laboratory, it has been found that sodium nitroprusside complexes with DMS to yield a colored product suitable for spectrophotometric determination. Sodium nitroprusside has been used for spot tests for aldehydes, secondary amines, methyl ketones, inorganic sulfides, and thiols(5,9), but not for organic sulfides. In the present application, the complex formed between DMS and sodium nitroprusside has been found to be much more specific than with previously reported applications and it allows accurate measurement of very low levels of DMS.

EXPERIMENTAL

Chemicals
Sodium nitroprusside, $Na_2Fe(CN)_5NO\cdot2H_2O$
Carbon tetrachloride, ACS grade
Dimethyl sulfide (highest purity, Fisher Chemical Co., St. Louis, Mo.)
Tannin (Mallinckrodt Chemicals, St. Louis, Mo.)

Equipment
Reciprocating box shaker (265 oscillations/min)
Wrist-action shaker
Centrifuge (International)
Centrifuge (Sorvall RC2B, refrigerated)
Spectrophotometer (visible)
Centrifuge tubes (350-ml capacity)
Centrifuge tubes with plastic screw cap and cap liner (50-ml capacity)
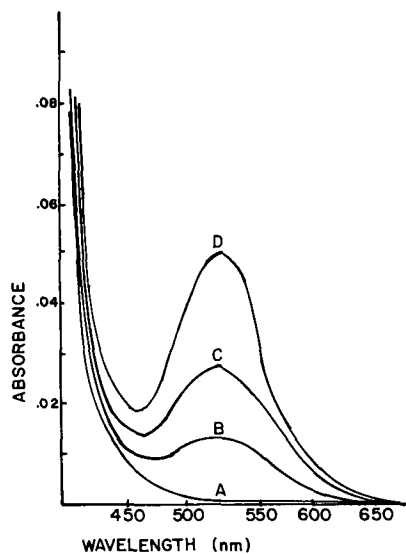Whatman Filter Paper (1 PS)

Fig. 1. Visible spectra of the DMS-sodium nitroprusside complex. A = Reagent blank, B = 25 μg DMS/l., C = 50 μg DMS/l., and D = 100 μg DMS/l.

Reagents (% Concentrations are w/v)

Stock Solution: 0.33% sodium nitroprusside in distilled water. Note: The stock solution is subject to degradation and should be prepared fresh weekly and protected from light.

Complexing Reagent: 10 ml of stock solution diluted to 100 ml with distilled water and made alkaline by addition of 10 ml of 10% sodium hydroxide. Prepare this just prior to reaction.

5 and 10% sodium hydroxide
2 and 6N hydrochloric acid
4% tannin in distilled water

DMS-Sodium Nitroprusside Complex: Measurement of Color

Sodium nitroprusside reacts with DMS in an alkaline medium to yield a complex which possesses a pink color following acidification. The reaction product has maximum absorbance at 520 nm (Fig. 1) and can be used to measure the concentration of DMS in a given system. This complex, however, is somewhat unstable and slowly decomposes with time.

To optimize sensitivity, 5-cm cells and a spectrophotometer with a readout to 0.001 absorbance units should be used. In order to make the method accurate to 5 μg DMS/l., the color determination must be accurate to 0.002 absorbance units; therefore, minor differences in turbidity and cell characteristics must be taken into consideration. In addition to the pink DMS complex, the reagent reacts with beer extract to produce a yellow complex. The color contribution of this interfering product must also be taken into account.

To establish turbidity corrections, readings are taken at 700 nm. Absorbance is read at 460 nm as an arbitrary indication of interference from the yellow complex (Fig. 2). Sources of these interferences are not completely understood. However, it is known that these interferences are variable, are more prevalent in hopped samples, and that some of the interfering species are soluble in 5% NaOH.

The following steps should be taken to determine the absorbance of the DMS-sodium nitroprusside complex:

1. The reference solution for the color determinations is prepared by mixing 1.2 ml 6N HCl with 22 ml of the complexing reagent. Fill both 5-cm cells with this reagent blank and adjust the absorbance to 0.000 at 700 nm. Measure the absorbances at 520 and at 460 nm.
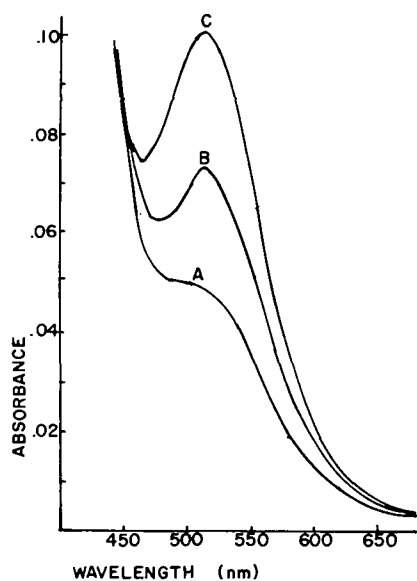
2. Read the sample against the blank at 700, 520, and 460 nm. If the absorbance at 700 nm is greater than 0.010, the sample should be clarified further.

3. Correct the values for absorbance at 520 and 460 nm obtained in step 2 by subtracting the 700 nm reading from both and then subtracting the respective values obtained in step 1. The corrected 520 and 460 nm absorbances are then used in the calculation of the absorbance of the DMS complex.

4. Calculate the absorbance of the DMS complex (A_{DMS}) by subtracting the contribution of the yellow complex at 520 nm from the measured 520 nm absorbance. For this step, determine the relative contributions of the yellow complex at 520 nm and the DMS complex at 460 nm.

R_1 = Absorbance yellow complex (520 nm)/Absorbance yellow complex (460 nm)

R_2 = Absorbance DMS complex (460 nm)/Absorbance DMS complex (520 nm)

Determine R_1 by analyzing beer which has been purged with N_2 for five hr. This procedure removes DMS, but retains the less volatile interfering species.

Determine R_2 by analyzing a standard solution of DMS. Once the R_1 and R_2 values have been established, they may be used in all subsequent calculations.

The formula for A_{DMS} is derived as follows:

a) $A_{DMS} = A_{520} - A_{yellow\ complex}\ (520)$

b) $A_{yellow\ complex}\ (520) = R_1\ A_{yellow\ complex}\ (460)$

c) $A_{yellow\ complex}\ (460) = A_{460} - A_{DMS}\ (460)$

d) $A_{DMS}\ (460) = R_2\ A_{DMS}$

e) $A_{DMS} = A_{520} - R_1\ A_{460} + R_1\ R_2\ A_{DMS}$ or $A_{DMS} = \dfrac{A_{520} - R_1\ A_{460}}{1 - R_1\ R_2}$

Average values for R_1 and R_2 were found to be 0.26 and 0.21, respectively. The following formula may then be used for all calculations:

$$A_{DMS} = [\text{corrected}\ A_{520} - 0.26 \times \text{corrected}\ A_{460}] \times 1.06$$

Methods for the Determination of DMS in Various Samples

A. Aqueous and Ethanolic Samples

Mix 9 ml of sample with 1 ml of 0.33% sodium nitroprusside stock solution and 1 ml 10% NaOH in a stoppered tube. Acidify

Fig. 2. Visible spectra of beer extract-sodium nitroprusside complex. A = Beer. B = beer + 25 μg DMS/l., C = beer + 50 μg DMS/l.
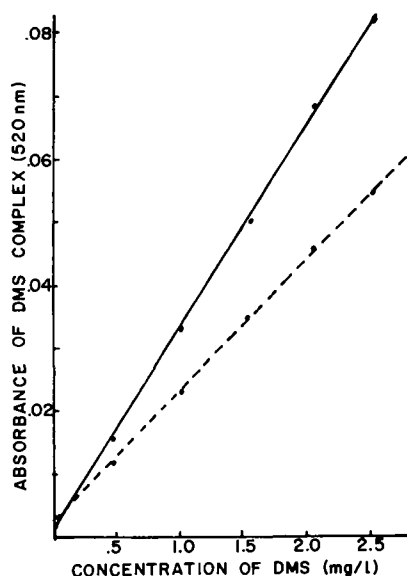

Fig. 3. Calibration curves of DMS additions to water and CCl_4. Water analysis by method A, dashed line. CCl_4 analysis by method B, solid line.

the mixture after 30 min with 0.6 ml of 6N HCl. Calculate the A_{DMS} from the color readings. (Note: ethanol concentrations of greater than 30% will cause interference.) The concentration of DMS is calculated from a calibration curve (Fig. 3) prepared by analyzing samples of known DMS concentration. The slope is 0.021 A units/mg DMS/l.; the inverse of the slope (the response factor) is 48 µg/l. of DMS/0.001 A units. The concentration of DMS in µg/l. is equal to 1000 × A_{DMS} × 48. The lower limit of detection is approximately 250 µg/l.

B. Organic Solvent Solutions of DMS

The method is applicable to solutions of DMS in solvents which are immiscible with water. In this investigation, carbon tetrachloride (CCl_4) solutions were used. Extract 20 ml of sample with 11 ml of complexing reagent by shaking in a closed tube for 30 min on a wrist-action shaker. Acidify the aqueous phase with 0.6 ml of 6N HCl and calculate the absorbance of DMS (A_{DMS}) from the colorimetric readings. Prepare the calibration curve (Fig. 3) by making known additions of DMS
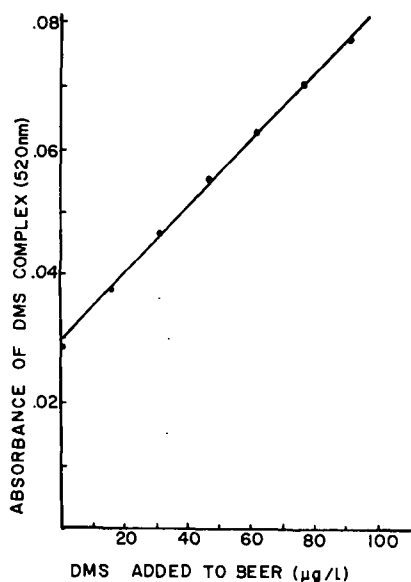
to CCl_4. The slope of 0.033 A units/mg DMS/l. gives a response factor of 30 µg/l. DMS/0.001 A unit. The concentration of DMS in µg/l. is equal to 1000 × A_{DMS} × 30. The lower limit of detection is approximately 150 µg/l.

C. Beer, Wort, and Aqueous Samples with Less Than 250 µg of DMS/l.

1. Sample Preparation
   a. Packaged beer, filtered beer: No treatment is required.
   b. In-process fermentation samples: Centrifuge until the sample is clear (20 min at 12,000 rpm, i.e., 23,000 relative centrifugal force).
   c. Wort samples: Mix 350 ml of the sample with 10 ml of 4% tannin solution, centrifuge until clear. For wort samples with high specific gravities (17° to 20° Plato), dilute the sample 1:1 with distilled water.

2. Extraction
   Add 330-ml sample and 20 ml CCl_4 to a 12-oz returnable beer bottle, crown, and shake for 4 hr in a reciprocating shaker.

3. Recovery of Extract
   Discard the aqueous phase and decant the CCl_4 phase (along with residual sample) into a 60-ml separatory funnel. Wash the extract with 10 ml 5% NaOH by inverting the funnel gently 15 times. Remove the aqueous layer by vacuum aspiration and repeat the wash with a second 10-ml portion of 5% NaOH. (In rare cases, an emulsion may persist after these treatments. When this occurs, a third washing using 10 ml 2N HCl breaks the emulsion.) Pass the CCl_4 phase through a

Fig. 4. Standard additions of DMS to beer.
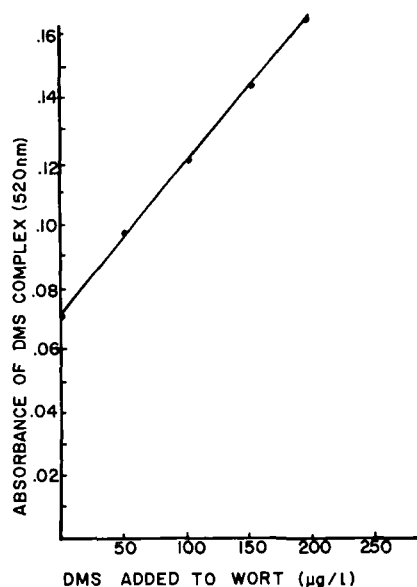
Fig. 5. Standard additions of DMS to wort.
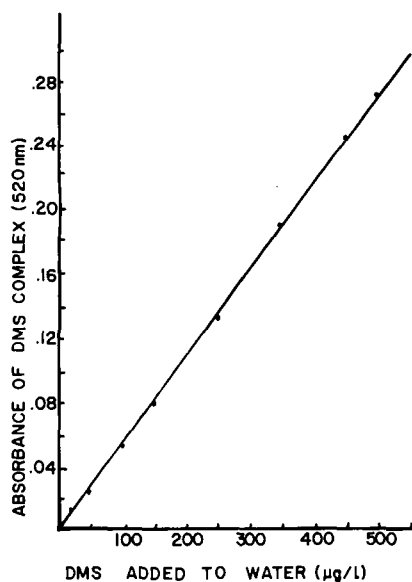
Fig. 6. Standard additions of DMS to water.

TABLE I
Slopes and Response Factors Obtained from
Calibration Curves of Known DMS
Additions to Beer, Wort, and Water

Sample	Slope × 1000 A_{DMS}/µg DMS/l.	Response Factor µg DMS/l./0.001A_{DMS}
Beer 1	0.53	1.9
Beer 2	0.47	2.1
Wort 1	0.48	2.1
Wort 2	0.51	2.0
Water 1	0.56	1.8
Water 2	0.47	2.1
Average	0.50	2.0

phase separation filter paper (1 PS) into a 50-ml Corex centrifuge tube (with screw cap) containing the complexing reagent.

4. Reaction

Shake the capped tube containing 20 ml CCl₄ extract and 11 ml of complexing reagent for 30 min on an automatic wrist-action shaker.

5. Color Development and Clarification

Acidify the sample by mixing in 0.6 ml of 6N HCl, then centrifuge until the aqueous phase is clear (20 min at 1100 relative centrifugal force is sufficient).

6. Calculation

Determine the color on the clear supernatant and calculate the A_{DMS}. Figures 4, 5, and 6 show typical calibration curves for beer, wort, and water, respectively. These curves were obtained by making standard additions of DMS to the samples. Table 1 lists some typical values of slopes and response factors determined using this method. The average value of the response factor is 2 μg DMS/l./0.001 A unit. Concentration of DMS in μg/l. is equal to $1000 \times A_{DMS} \times 2$.

Discussion of Methodology

Whenever samples are to be treated or transferred, they should be chilled to prevent loss of DMS. Water samples should be free of any residual chlorine so as to prevent loss of DMS due to oxidation.

Selection of 11 ml of complexing reagent was based on the volume required to fill the 5-cm cells. If cells of the same path length but less volume were used, a smaller amount of reagent could be used in the reaction, giving increased sensitivity for the analysis.

Figure 7 shows that 30-min shaking time was sufficient for reaction of the CCl₄ extract with the complexing reagent. Beer and beer with added DMS were extracted for varying lengths of time to establish the optimum extraction time (Fig. 8). Based on these results, a 4-hr extraction was selected. Slight variations in fill volume, solvent-to-sample ratio, and presence of air in the head space gave no significant differences in the reproducibility of extraction. Slight variations in the alkalinity of the reaction medium and acidity of the reading solution likewise gave no significant differences in reproducibility.

The CCl₄ extraction procedure was applied to beers not only as a means of concentrating DMS to measurable levels but also to eliminate interfering substances present in beer. If this step is not taken, the interfering substances react with the complexing reagent, producing an intensely dark product. A distillation procedure was examined as the concentration step, but the extraction procedure gave more reproducible results.

Centrifugation and tannin treatment steps used on some samples were necessary to prevent the formation of emulsions during the extraction.

Recovery of the extract was the most time-consuming step. Chilling the beer samples after extraction improved the separation of phases, but wort samples had to be left at room temperature, as chilling increased the emulsion. The 5% NaOH washes served to break the residual emulsion (thus facilitating quantitative recovery of the extract), and to remove residual beer, CO₂, and a large amount of material that contributed to the interfering yellow complex. Loss of DMS from the extract during these washings was found to be insignificant. By filtering the extract through phase separation filter paper, any residual aqueous phase was eliminated.

The acidification step was required to develop the pink color characteristic of the complex. Acidification not only reduced the intensity of the yellow color of the complexing reagent but also precipitated acid-insoluble species which caused high turbidity, especially in beer samples. The acid conditions used were found to destroy the complexes formed by thiols, sulfide ion, and hydrogen sulfide, thus removing interferences from these sources. Clarification, when necessary, was effected either by forcing the aqueous layer through fine filter paper or by centrifugation. The latter was found to be more convenient and reproducible.

Turbidity of final solutions had to be less than 0.010 A units at 700 nm for making accurate corrections.

In preparing calibration curves, a solution of 1000 mg of DMS/l. in 50% ethanol was used. The stock solution had to be kept cold and tightly stoppered under an atmosphere of N₂ to prevent loss by volatilization and oxidation.

For optimum efficiency, 8 samples were analyzed concurrently, since the capacity of the wrist-action shaker and the centrifuge used was 8 tubes. Eight 60-ml separatory funnels were supported on funnel racks directly over eight 7-cm funnels (with 1 PS filter paper) which, in turn, were supported by funnel racks directly over the eight centrifuge tubes. In this manner, a set of eight extracts could be recovered in a group. In a typical analysis, as one set was reacting, another set was being recovered, and as one set was being centrifuged, the other was being reacted. Thirty-two samples could be analyzed per day by this arrangement.
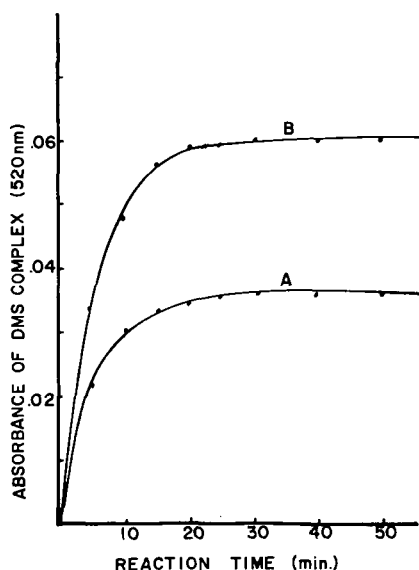

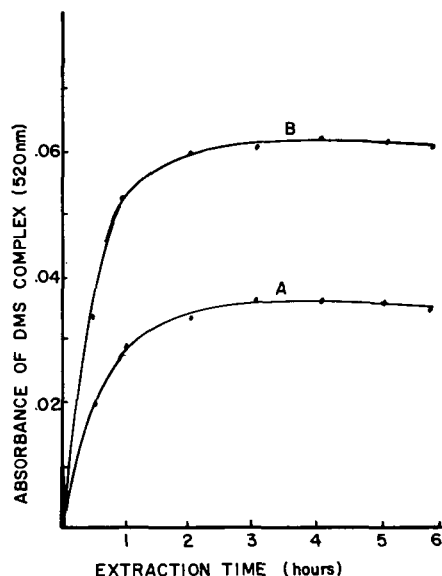Fig. 7. Effect of reaction time on the degree of complexation: A = Beer, B = beer + 50 μg DMS/l.

Fig. 8. Relationship of extraction duration to color developed: A = Beer, B = beer + 50 μg DMS/l.

RESULTS AND DISCUSSION

Addition of known concentrations of several sulfur-containing compounds to beer did not contribute to the absolute value of DMS (Table II). Dimethyl and diethyl disulfides (DMDS and DEDS) gave no apparent reaction. Methanethiol (MeSH) and Na$_2$S gave colored products, but the complexes were destroyed during the acidification step. Diethyl sulfide additions of up to 150 μg/l. did not interfere in DMS determinations. Methyl ketones examined gave no interference even at a 5 mg/l. level.

The results of analyses of beer, wort, and water samples for DMS showed good agreement between colorimetric and GC methods.

The colorimetric method described here gave results which allowed determination of DMS concentration as low as 5 μg/l. Table III shows the results of multiple analyses of ostensibly identical beer samples. Treatment of data obtained on replicate samples gave a standard deviation of 3.7 μg/l. It is also apparent from this table that recoveries of added DMS averaged 98%.

Application of the colorimetric method to various market beers over a 5-month period gave the results shown in Table IV. Although the data indicate some fluctuation in DMS concentration throughout the test period, it would appear that the DMS level remains relatively constant for a given beer and is therefore a function of materials and process conditions used in brewing. The lowest level of DMS found in 25 competitive lager beers was 30 μg/l. and the highest was 160 μg/l. Twenty-one of the samples fell in the range of 40–90 μg/l.

Beer subjected to warm storage shows an increase in DMS. The increase is a function of temperature and duration of storage (Table V). Ahrenst-Larsen and Hansen (1) also reported an increase in DMS in stale beer when they analyzed an extract of beer distillate

TABLE II
Effect of Various Sulfur-Containing Test
Compounds on A_{DMS} Complex at 520 nm[a]

Level Added μg/l.	Compound Added					
	DMS	DES	DMDS	DEDS	MESH	Na$_2$S
0	22	24	21	23	22	21
50	45	26	21	21	22	22
100	71	28	23	18	20	21
150	94	29	22	20	20	23

[a]($A_{DMS} \times 1000$).

TABLE III
Reproducibility of DMS Determinations and
Recovery Determinations in Beers from the Same Lot

Sample	As-Is DMS μg/l.	μg/l. DMS Added	DMS μg/l.	% Recovery
1	45	50	95	100
2	50	50	95	90
3	45	50	90	90
4	45	50	100	110
5	50	100	145	95
6	55	100	150	95
7	50	100	150	100
8	45	100	150	105
Av.	48			98
Std. Dev.	3.7			

TABLE IV
Dimethyl Sulfide Levels Measured in Market Beers
over a 5-Month Test Period

Beer	μg DMS/l.					
	Month 1	Month 2	Month 3	Month 4	Month 5	Av.
A	35	45	35	40	40	40
B	30	75	45	45	35	45
C	45	45	50	45	40	45
D	55	80	40	45	55	55
E	60	60	65	60	60	60
F	55	60	65	60	60	60
G	115	120	135	125	110	120
H	125	170	125	110	100	125

TABLE V
Effect of Warm Storage on the DMS Level in Beer

Storage Conditions		Test 1	Test 2 μg DMS/l.	Test 3
1 week	40° F	45	60	55
2 weeks	85° F	50	70	60
1 week	100° F	60	70	70
2 weeks	100° F	65	80	75
1 week	120° F	95	105	100

TABLE VI
Effect of Microorganisms, Heat, and pH on
Formation of DMS

Sample	Treatment	μg DMS/l.
Beer	As-is	55
Beer	Heated at 80°C, 1 hr	65
Fermenting wort	As-is	70
Fermenting wort	Heated at 80°C, 1 hr	115
Sweet wort	As-is	995
Sweet wort	Heated at 80°C, 1 hr, pH 2.0	1145
Sweet wort	Heated at 80°C, 1 hr, pH 5.8	1240
Sweet wort	Heated at 80°C, 1 hr, pH 10.0	1650
Sweet wort	Sterile, 1 day at 30°C	1020
Sweet wort	Nonsterile, 1 day at 30°C	1625
Sweet wort	Nonsterile, 3 days at 30°C	3155

CONCENTRATION OF DMS (μg/l)

EARLY MASHING
MIDDLE MASHING
SWEET WORT
DURING KETTLE BOIL
AFTER KETTLE BOIL
FERMENTING WORT
EARLY FERMENTATION
AFTER FERMENTATION
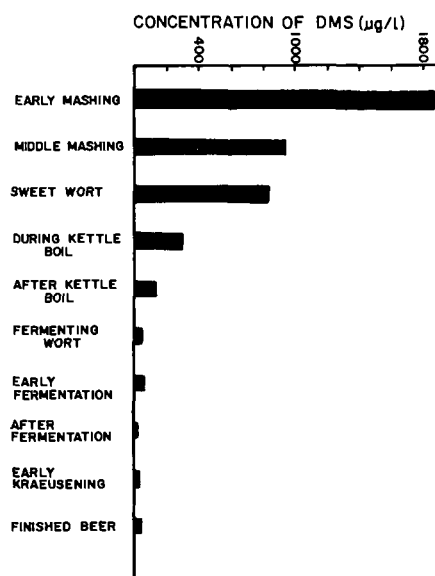EARLY KRAEUSENING
FINISHED BEER

Fig. 9. Levels of DMS measured at different stages of the brewing process.

by gas chromatography. These findings as well as our own results seem to suggest that DMS is probably involved in the phenomenon of beer staling. Also, as this increase in the concentration of DMS proceeds in pasteurized beer, the origin of this compound in such samples is presumed to be a heat-labile precursor. Heating samples of sweet wort, fermenting wort, and beer generates increased levels of DMS, these increases being further enhanced when the pH of the reaction medium is raised. Similarly, bacterial contamination of the sample produced increased levels of DMS. A small selection of results is presented in Table VI.

A summary of results obtained in a tracking study wherein samples were taken throughout the brewing process is presented in Fig. 9. The DMS level is initially very high, but decreases through the mashing and kettle boiling operations. During the kettle boiling operation, both the formation of DMS from the heat-labile precursor and the loss of DMS due to volatilization take place. By the time boiling is completed and the wort is cooled, the level of DMS falls well below 100 μg/l. In the early fermentation of wort, the DMS concentration increases briefly, then decreases to its lowest level by the end of fermentation. The kraeusening process results in a slight increase in the DMS level of fermented wort.

Early reports place the threshold level of DMS at around 30 μg/l. Tests in our laboratory, however, indicate that this level is about 60 μg/l.

SUMMARY

A colorimetric method has been presented for the measurement of dimethyl sulfide in water, wort, and beer.

The method uses the reaction between dimethyl sulfide and sodium nitroprusside under specific conditions to give a pink-colored complex with a λ_{max} of 520 nm.

The method is reproducible, accurate, and rapid.

Dimethyl sulfide concentrations in various market beers and in beer samples subjected to different treatments have been presented and discussed.

Acknowledgments

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