A New Calibration Procedure for the Determination of Dimethyl Sulfide in Beer Using Gas Chromatography with a Flame-Photometric Detector[1]

S. S. Lee and K. J Siebert, *The Stroh Brewery Company, 100 River Place, Detroit, MI 48207*

ABSTRACT

Procedures used to calibrate the flame photometric detector for determination of dimethyl sulfide (DMS) in beer headspace by gas chromatography were investigated. Substantial differences in results were attributed to differential quenching responses to DMS and the internal standard caused by nonsulfur-containing compounds eluting at the same times, and to the use of inappropriate calibration methods. A calibration procedure using beer as the medium and iteration techniques at high levels of added DMS was developed. Its applicability was verified with several different beers. Results obtained with this procedure differed from those obtained by the commonly used square root method by as much as 36–206%. The results indicated that a calibration curve constructed for one beer cannot be applied to others. A simplified procedure for obtaining a correction factor for preparing calibration curves in different beers is proposed. The DMS concentrations and correction factors determined for six different beers ranged from 16 to 45 ug/L and from 0.37 to 1.77, respectively. The approach to calibration described should also have application in the determination of other sulfur compounds with the flame photometric detector.

Key words: Correction factor, DMS, Headspace, Iteration, Quenching effect

Dimethyl sulfide (DMS) is recognized as significant to beer flavor, reportedly contributing fullness, overall aroma, and special characteristics to certain types of beer, especially lagers (3,47). Among many sulfur-containing volatile compounds, DMS is the only one that is thought to normally be present in beer at or above its reported flavor threshold (4,22,23,40) of approximately 30 μg/L (8).

DMS has been detected in a range of U.S. lagers (43) and in Canadian (24,40) and British (3,44) ales and lagers. Levels found in lager beers (20–75 μg/L) are significantly higher than those found in ales (1–20 μg/L) (3,51), and this has been used by some authors to distinguish ales from lagers (3,39). Widely differing results for DMS in beer have been reported (in the range of 10–150 μg/L) by many researchers employing various analytical procedures (18).

Direct headspace sampling using a gas chromatograph with a flame photometric detector (FPD) has frequently been employed for the analysis of DMS in beer (4,12,21,24,25,27,31,33,40, 43,44,48–51). Although this method is both selective and sensitive for sulfur-containing compounds, a number of problems have been observed.

1) The sensitivity of the detector is affected by flame conditions, such as the gas flow rates and the oxidant-to-fuel gas ratio (5,15,19,30,32,45). Other conditions, such as the column temperature program used (5), the design of the burner (26,30,35), and the headspace gas sampling technique employed, can also influence the FPD response.

2) The FPD response to sulfur compounds frequently does not obey the theoretical square-law relationship, which states that the detector response is proportional to the square of the sulfur compound concentration (7,9,24,35,41). Exponent (n) values between 1.5 and 2.0 have generally been reported (15,19,32,45).

3) The exponent n varies for different sulfur compounds (17,32,45). In general, values close to 2.0 were obtained for SO_2 and H_2S. For DMS, lower numbers such as 1.59 and 1.70 have been reported (9,45).

4) The FPD response to sulfur compounds is reduced by coeluting compounds that do not contain sulfur (10,11,16,20, 24,30,34,35,36,37,42,46). This is called the quenching effect, and is closely associated with the type of gas chromatography (GC) column and detector employed. The quenching effect in beer has not been quantitatively studied.

5) Substantial errors may result from the calibration procedures generally employed for constructing DMS calibration curves (1,2,9).

Unacceptably large between-lab error was reported when a particular method and calibration procedure (the square root method) (24) were applied to a set of beer samples (1,2). This was mainly attributed to the lack of "an accurate mathematical assessment" to solve for the exponent n in the calibration of each FPD under varying operating conditions (2). Although quantitation problems may be reduced with a dual-flame FPD (34,35) and the use of GC capillary columns with higher resolution (6), an improved calibration procedure that can accurately determine the exponent n under optimized flame conditions (for any individual detector) is required for valid beer DMS measurements. This report describes efforts devoted to develop such a calibration procedure and its application to different beers.

EXPERIMENTAL

Gas Chromatographic Instrumentation and Conditions

DMS analyses were performed using a Varian model 1400 gas chromatograph with an all-Teflon system (Varian Aerograph, Palo Alto, CA) and a sulfur-mode flame photometric detector (FPD 100AT, Meloy Lab., Springfield, VA). The column was ⅛ in. o.d. (0.085 in. i.d.) × 36 ft Teflon tubing packed with 12% polyphenyl ether and 0.5% H_3PO_4 on 40/60 mesh Chromosorb T (Supelco, Bellefonte, PA) as described by Stevens et al (45). The inlet and detector temperatures were 100 and 130° C, respectively. The column temperature was held constant at 105° C. Detector gas flow rates were chosen after an optimization study (see below) to maximize the detector sensitivity for DMS. The carrier gas was nitrogen at a flow rate of 16 ml/min. The detector gas flow rates were 80 ml/min for hydrogen and 16 ml/min for oxygen. The FPD signal was sent to an HP3390A reporting integrator (Hewlett Packard, Avondale, PA).

Optimization of FPD Sensitivity for DMS

The effects of three parameters (H_2 flow, N_2+O_2 flow, and O_2/H_2 ratio) on the FPD response to DMS were each analyzed at three levels. The selection of these parameters and the levels chosen for examination were largely based on the study of the optimization of FPD flame conditions reported by Eckhardt et al (15) and others (32,42). The three levels chosen for each variable were: H_2, 80, 100, or 120 ml/min; $N_2 + O_2$, 60, 80, or 100 ml/min; and O_2/H_2 ratio, 0.20, 0.25, or 0.30. Triplicate samples were analyzed for each combination.

In addition to the 2^3 corner points of a full two-level factorial design, the center points of the six surface planes plus the center of the factor space were tested (for a total of 15 conditions). In each run 10 μl of a 25-μg/L aqueous DMS solution was injected directly into the packed column using a 10-μl Hamilton microsyringe.

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The FPD response for the total area of the DMS peak was employed as the only dependent variable for this study. Data collected from all 45 runs (three replicates each for 15 conditions) were supplied to the BMDP9R All Possible Subsets Regression Program in the Bio-Medical statistical package (14) to select the best subset for predictor variables, including the effects of interactions between variables. The equation obtained was used to select the optimum FPD operating conditions for the detection of DMS.

DMS Assay Method

Twelve ounces (355 ml) of beer in a long-necked bottle was chilled to 0° C, and 127 ml of beer was carefully removed and discarded. Beer samples obtained in other containers were chilled, and a portion (228 ml) was transferred to a long-necked bottle. An aliquot (100 μl) of ethyl methyl sulfide (EMS) internal standard solution (27.3 μl EMS in 100 ml 50% [v/v] aqueous ethanol), 2 ml of 50% (v/v) aqueous ethanol, and five drops of antifoam were added to each beer sample. For calibration, a known amount (from 20 to 2,000 μg/L) of DMS stock solution (27.2 μl DMS in 100 ml 50% [v/v] ethanol solution) was also added, along with sufficient 50% (v/v) aqueous ethanol to maintain the same volume of added ethanol.

Each calibration curve was divided into two ranges, high (500–2,000 μg/L) and low (0–200 μg/L). DMS stock solution and 50% (v/v) aqueous ethanol were added to individual bottles in the following volumes: Low range: DMS, 0, 20, 40, 60, 80, 100, 150, and 200 μl (equivalent to the same number of μg/L DMS added) plus 50% (v/v) aqueous ethanol, 2,000, 1,980, 1,960, 1,940, 1,920, 1,900, 1,850, and 1,800 μl, respectively. High range: DMS, 500, 800, 1,000, 1,200, and 1,400 μl (equivalent to the same number of μg/L DMS added) plus 50% (v/v) aqueous ethanol, 1,500, 1,200, 1,000, 800, 600 μl, respectively.

Both the EMS and DMS stock solutions were prepared just before use and kept at 0° C during sample preparations. The beer bottle was recrowned immediately after the addition of EMS and aqueous ethanol. It was then placed on a rotary shaker at 200 rpm for 30 min and afterwards allowed to stand for at least 1 hr at room temperature. The beer headspace gas was introduced into the gas chromatograph via the six-port gas sampling valve (GSV) depicted in Figure 1. The sample path, including the 20-ml sampling loop, was lined with Teflon. The inlet of the GSV was connected to a needle through Teflon tubing and valve no. 2. With the GSV in the load position (valve no. 1 open and valve no. 2 closed), the sample loop was evacuated to about 30 mmHg, then disconnected from the vacuum pump by closing valve no. 1. The needle was inserted through the septum of a sample bottle into the headspace, and valve no. 2 was opened to fill the sample loop. The sample was then injected into the GC.

Two bottles were prepared and analyzed for each sample. Beer DMS concentrations in micrograms per liter were calculated by multiplying the nth root of the peak height ratio h_D/h_E (i.e., the height of the DMS peak divided by the height of the EMS peak), by the response factor (F) obtained from the calibration curve using the proposed calibration procedure. Each calibration curve for beer was accompanied by one obtained with 4% (v/v) ethanol under the same conditions.

RESULTS AND DISCUSSION

Optimization of FPD Sensitivity to DMS

Multiple regression analysis of the results of the optimization study showed that the effects of the three parameters studied, namely H_2 flow (P_1), $N_2 + O_2$ flow (P_2), and O_2/H_2 ratio (P_3) on detector response could be described by the following regression equation:

$$Z = 13.9827 - 0.0438582 \cdot P_1 - 0.131878 \cdot P_2 - 14.6248 \cdot P_3 + 2.97987 \times 10^{-4} \cdot P_1 \cdot P_2 + 2.04164 \times 10^{-4} \cdot (P_2)^2 + 0.144735 \cdot P_2 \cdot P_3 \quad (1)$$

where Z is the FPD response expressed as the total area of the DMS peak. The observed effects of gas flow rates on FPD sensitivity to DMS are shown in Figure 2. The detector response increased as the hydrogen or oxygen flow rate decreased for a fixed nitrogen flow rate. This result indicated that the optimum condition was at the lower end of the flow rate ranges investigated. In order to maintain a stable flame in the FPD, however, there are physical limits for the gas flows. A satisfactory combination of operating conditions was chosen: H_2, 80 ml/min, O_2, 16 ml/min; and N_2, 16 ml/min. The operating conditions chosen influence the exponent n of the correlation between FPD response and total DMS concentrations (15,17,19,32,38,45).

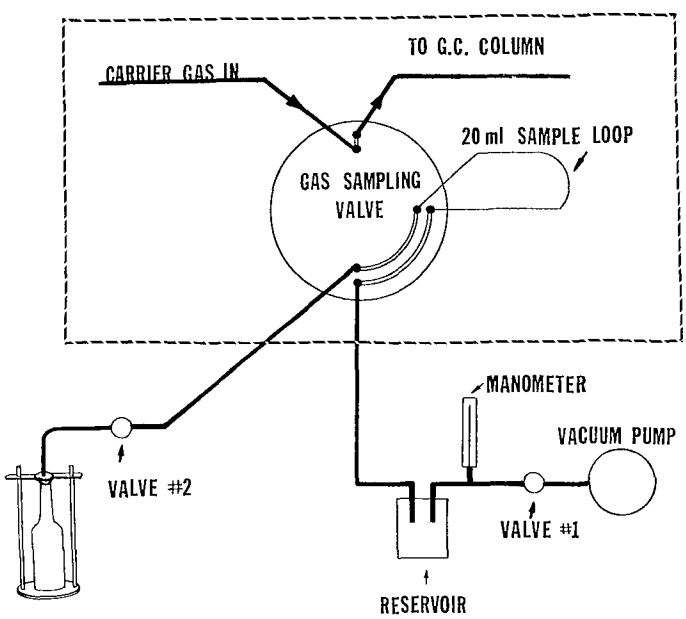

Fig. 1. Beer headspace sampling system. The six-port sampling valve was equipped with a 20-ml sampling loop.

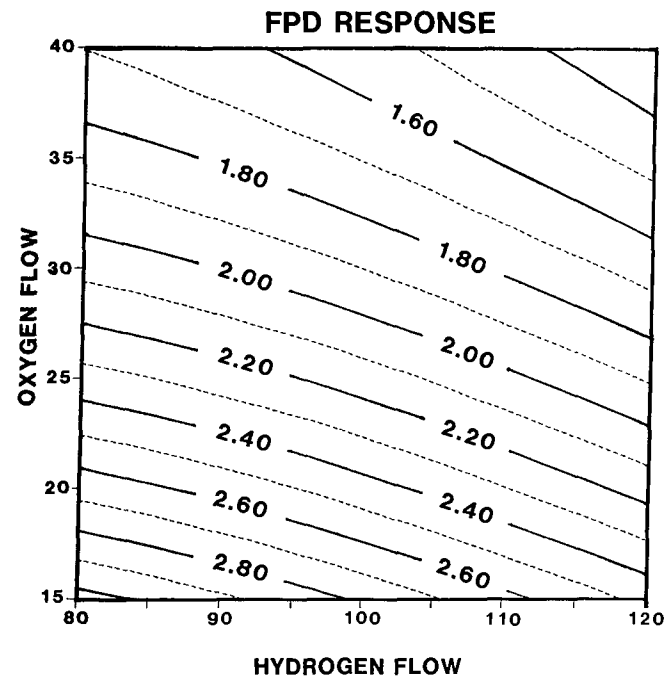

Fig. 2. The observed effects of detector gas flow rates (ml/min) on flame photometric detector (FPD) response for dimethyl sulfide.

Nonlinearity of the FPD Response

The well-known nonlinear correlation between FPD response and total DMS concentration, shown in Figure 3, was obtained by plotting the peak height ratio (h_D/h_E) on a linear scale against the total DMS concentration in a 4% ethanol solution. For a series of calibration curves prepared in this way, the exponent (n) values were reproducible and varied within the range of 1.77–1.83. This was different from the theoretical value of 2.0 but similar to some values previously reported for DMS (9).

Derivation of the Proposed DMS Calibration Procedure

The major problem with calibration of the FPD for sulfur compounds stems from its nonlinear response. The commonly employed known-addition methods for constructing calibration curves do not apply in this case. Using DMS calibration curves in beer based on the theoretical square response that do not consider the endogenous DMS present will lead to erroneous results. The same comments apply to other approaches that use a matrix different from beer whether they use a more realistic exponent value or not. A new procedure is thus proposed and explained as follows.

The basic assumption made here is that the power function relationship (7) applies not only for the 4% ethanol solution but also for beer.

$$Y = C \cdot X^n. \tag{2}$$

The FPD response, Y, equals a constant, C, times the total DMS concentration, X, raised to the power n. Because the total DMS concentration is the sum of the endogenous DMS, k, and that added, x, substitution in equation 2 results in:

$$Y = C \cdot (x+k)^n. \tag{3}$$

This is not simple to use because it has three unknowns, n, C, and k. However, if large amounts of DMS (i.e., 500–2,000 μg/L) are added relative to the endogenous DMS level, then X can be approximated by the added DMS, x, as k tends toward insignificance. Equation 2 can be used to estimate the correlation and solve for n and C by fitting the high-range DMS additions to a power function. The resulting n and C can then be used with the data for low levels of added DMS (i.e., 20–200 μg/L) to estimate k.

As it has been reported that the FPD response for sulfur compounds changes to an approximately linear relationship at higher concentrations (e.g., 2,000 μg/L for DMS [41]), the basic assumption was first tested by adding DMS levels up to 2,000 μg/L in a 4% (v/v) ethanol solution. Figure 4 shows a straight line was obtained by plotting peak height ratios against added DMS concentrations on a log-log scale. The slope of this line, 1.74, represents the exponent n of the power function. The power function correlation clearly holds up to 2,000 μg/L DMS, which was not exceeded in this study.

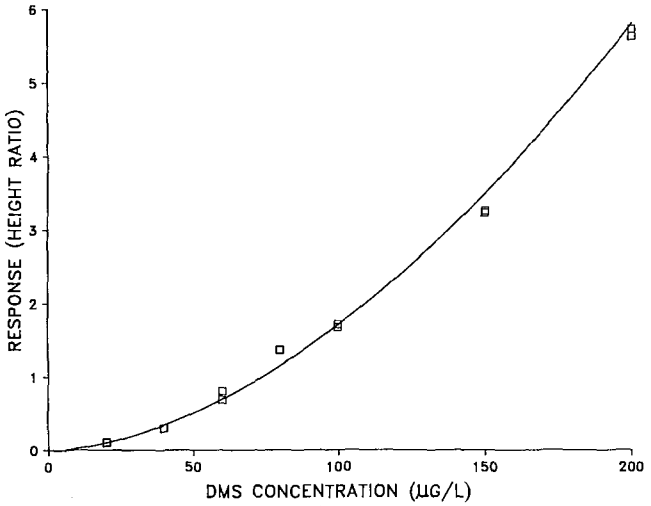

Fig. 3. Dimethyl sulfide (DMS) calibration curve constructed employing 4% (v/v) aqueous ethanol with 0–200 μg/L added DMS.

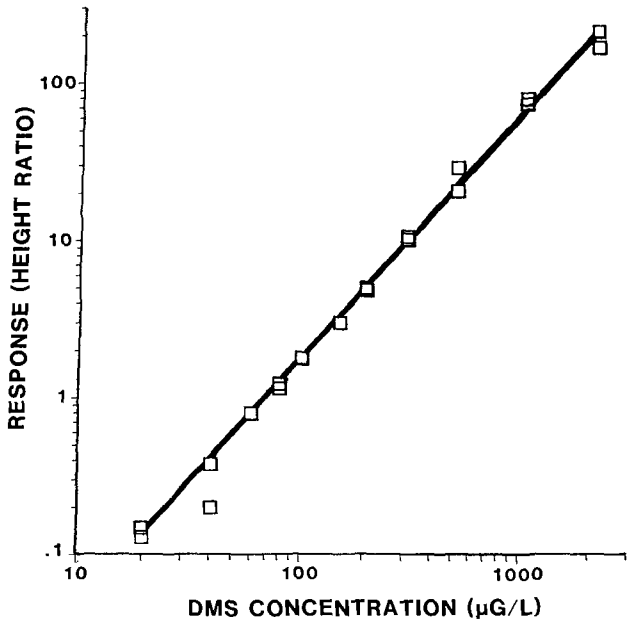

Fig. 4. Dimethyl sulfide (DMS) calibration curve constructed in 4% (v/v) aqueous ethanol with DMS up to 2,000 μg/L added DMS.

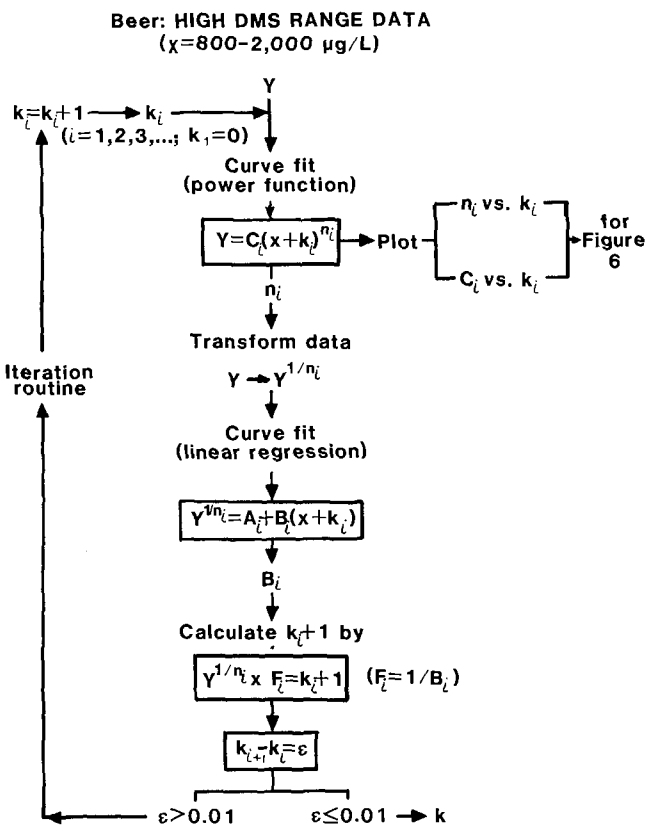

Fig. 5. Treatment of the high-range dimethyl sulfide (DMS) data (800–2,000 μg/L) obtained with beer using the proposed DMS calibration procedure.

The approach was then tried with a beer, designated beer A. A flow chart of the proposed procedure is illustrated in Figures 5 and 6. Various levels (10–2,000 μg/L) of DMS were added and the beer headspace samples were analyzed. As shown in Figure 5, the high-range (800–2,000 μg/L) data were first fit to a power function assuming that the endogenous DMS concentration in the beer (k) was zero ($k_1 = 0$). The values n (n_i) and C (C_i) were determined by the curve fitting procedure. The FPD responses were then transformed by finding their n_ith roots and fit to the formula:

$$Y^{(1/n_i)} = A_i + B_i \cdot (x + k_i). \quad (4)$$

with linear regression. The slope, B_i, is the reciprocal of the response factor, F, as defined by Hysert et al (24). The endogenous DMS concentration (k_i) was calculated by multiplying the nth root of Y_0 (the FPD response to DMS in beer without any added DMS) by F:

$$k_i = Y_o^{(1/n_i)} \cdot F. \quad (5)$$

The new k value (k_{i+1}) was then employed and the same curve fitting procedures were repeated. This iteration routine was performed until the difference between k input (k_i) and k output (k_{i+1}) was less than 0.01; usually four or five iterations. Results after each iteration for beer A are shown in Table I. The criterion was satisfied after five iterations, resulting in the following equation:

$$Y^{(1/1.700)} = 0.0512 + 0.00844 \cdot X. \quad (6)$$

During the iterations the n_i values increased from 1.631 to 1.700 as the calculated k_i rose from 0.0 μg/L to 33.5 μg/L. The C_i values declined from 0.000514 and settled near 0.000302. The intercept value (A_i) decreased from 0.0527 to 0.0512, and the slope results (B_i) dropped from 0.00957 to 0.00844. The calculated factor F settled at 118.5.

If equation 2 is modified by taking the nth root of each side of the equation, the resulting equation is:

$$Y^{(1/n)} = C^{(1/n)} \cdot X. \quad (7)$$

The slope of 0.00844 in equation 6 is equivalent to the nth root of C, and theoretically the intercept, 0.0512, should be close to zero (compare equations 6 and 7).

For the low-range DMS data (Fig. 6), a curve-fitting procedure to a power function (equation 2) was performed for a number of assumed k values (k_i = 10, 20, . . . and so on through 100 μg/L, as long as the resultant n_i did not exceed 2.0). The n (n_i) and C (C_i) values were found for each assumed k (k_i). The results for beer A are shown in Table II. The values found for n varied from 1.36 to 2.0 over a range of k values from 20 to 50 μg/L. Small changes in k resulted in large changes in n and C. These were attributed to the greater relative importance of the k values at low added DMS concentrations.

Because both the high- and low-range data points should fall on the same calibration line, the two curves intersect when n is plotted

TABLE I
Curve-Fitting Results for High-Range Dimethyl Sulfide Data for Beer A Using Equations 3[a] and 4

i	k_i	n_i	C_i	F_i	A_i	B_i
1	0.0	1.631	0.000514	104.5	0.0527	0.00957
2	28.0	1.688	0.000330	116.2	0.0516	0.00861
3	32.6	1.698	0.000307	118.1	0.0510	0.00846
4	33.4	1.699	0.000303	118.5	0.0511	0.00844
5	33.5	1.700	0.000302	118.5	0.0512	0.00844

[a] k_i, n_i, and C_i represent the values obtained for k, n, and C in equation 3 after i iterations.

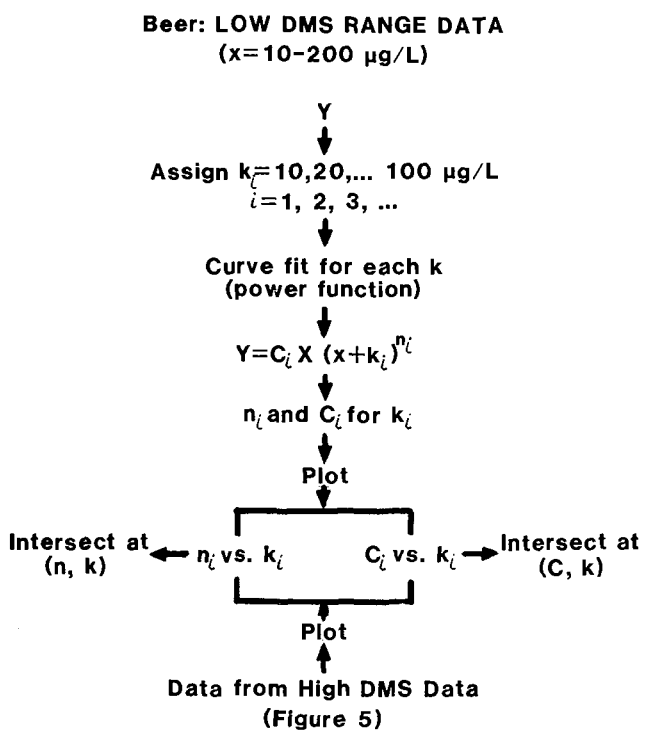

Fig. 6. Treatment of the low-range dimethyl sulfide (DMS) data (0–200 μg/L) obtained with beer using the proposed calibration procedure and estimation of endogenous DMS levels.

TABLE II
Curve Fitting Results for Low-Range Dimethyl Sulfide Data for Beer A Using Equation 3[a]

i	k_i	n_i	C_i
1	20.0	1.361	0.001612
2	30.0	1.597	0.000452
3	35.0	1.673	0.000297
4	40.0	1.810	0.000138
5	50.0	2.008	0.000045

[a] k_i, n_i, and C_i represent the values obtained for k, n, and C in equation 3 after i iterations.

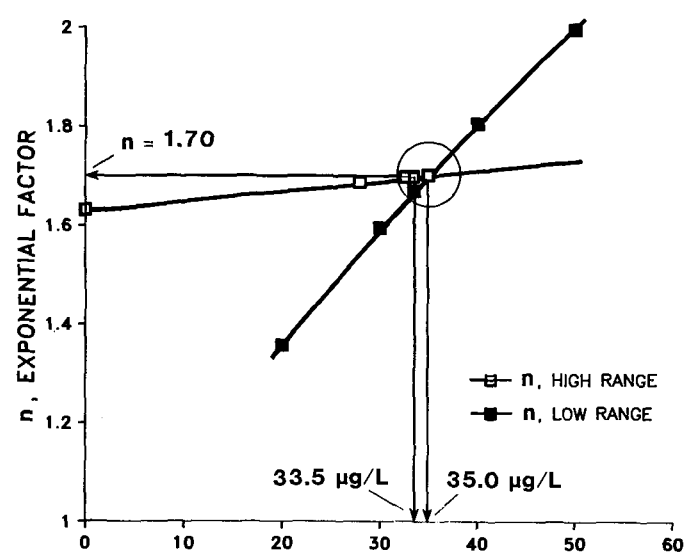

Fig. 7. Result of the proposed calibration procedure: the exponent n vs. endogeneous dimethyl sulfide concentration (k) plot for beer A.

against k for both cases (Fig. 7). The two curves meet at a point where $k = 35.0\ \mu g/L$ and $n = 1.70$. A similar plot of C values versus k provided a second estimate of the k value (see Fig. 8). C was about 0.0003 at $k = 35.0\ \mu g/L$. The k value obtained by iteration (33.5 $\mu g/L$) using the high-range data was close to that determined graphically (35.0 $\mu g/L$). The low-range DMS data were refitted using $k = 35.0\ \mu g/L$, and the final calibration line obtained was

$$Y^{(1/1.700)} = -0.00155 + 0.00788 \cdot X. \quad (8)$$

The F calculated from this equation is 126.9 $\mu g/L$.

Comparison of equations 6 and 8 shows a slight difference in their n and F values. Application of this procedure to another beer (beer B) showed a larger discrepancy between the k values assessed by iteration and those determined graphically. The k determined graphically was 42 $\mu g/L$, whereas that found with the iteration routine was 36 $\mu g/L$. Although the difference was only 6 $\mu g/L$, the resulting final calibration curves would differ significantly. The same phenomenon occurred to varying degrees when the calibration procedure was applied to four other beers. The k values measured using high-range data were from 2 to 18% lower than those found graphically (Table III). This points out that the results from the high-range data should only be used in the first stage, for estimation of the calibration line coefficients. The low DMS-added range, which includes the endogenous DMS level of an unknown beer, is more important for determination of the actual beer DMS level.

Verification of the Proposed Calibration Procedure

The applicability of the proposed calibration procedure has been further tested using the six beers shown in Table III. The DMS contents of beers A and C determined graphically were close to the flavor threshold of 30–35 $\mu g/L$, whereas the DMS levels of beers D and F were much lower than the flavor threshold. Beers B and E were found to contain more than 40 $\mu g/L$ of DMS. Although 30 $\mu g/L$ is the most frequently cited flavor threshold for DMS, much higher values (over 60 $\mu g/L$) have been reported (21,24). The thresholds found are likely to vary with different beers because of different endogenous DMS contents and different methods of threshold determination (24). It is interesting to note that the six beers examined here, all produced in the United States, contained DMS levels below the range reported for U.S. beers by the square root method (60–150 $\mu g/L$)(24) but were in good agreement with results obtained with an independent method using a microcoulometric detector (43).

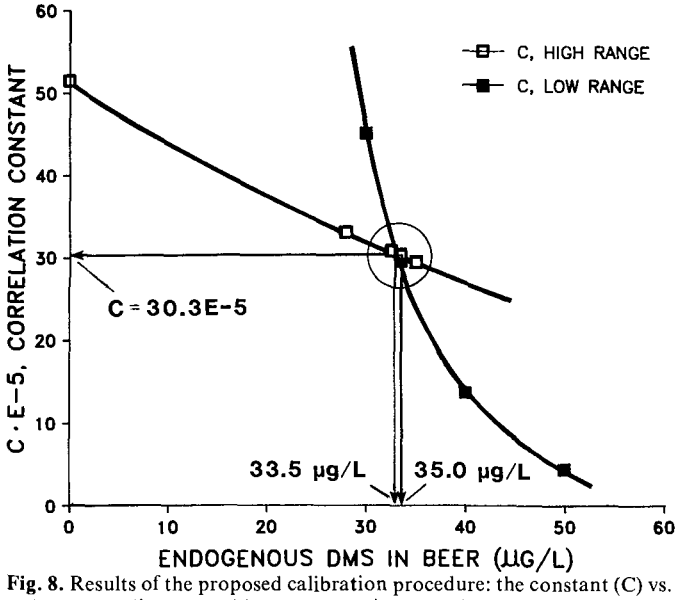

Fig. 8. Results of the proposed calibration procedure: the constant (C) vs. endogeneous dimethyl sulfide concentrations (k) plot for beer A.

The n and F values found in all six beers are also shown in Table III. The n values varied from 1.27 to 1.71, and all were lower than the n found for the 4% ethanol control curve (1.80). The F value for beer C (78.2) was close to that found for 4% ethanol (75.0), but F values for the other beers ranged from 41.9 to 126.9. These results indicate that a separate calibration curve applies for each beer (Fig. 9). This occurs because there are different degrees of quenching of the DMS and EMS responses in different beers (discussed later). Because the calibration curve for beer C falls nearly on the line for 4% ethanol, the DMS content of beer C can reasonably be estimated using the 4% ethanol control curve. The slopes of the calibration lines for beers A and B are fairly close to each other. This may have arisen because the same yeast is used to produce these beers, and the yeast may have produced similar quantities of the quenching substances in both cases.

Monthly calibration curves were constructed for production samples of two commercial beers for several months; the results for each beer were found to be quite consistent in both cases.

The results of a recovery study for DMS added to beer B are shown in Table IV. The values found were in good agreement with the amounts added to beer.

Comparison of DMS Calibration Methods

Several different DMS calibration methods have been reported; these can be grouped into three approaches: 1) plotting peak height or area or their response ratios (DMS/internal standard) for DMS added to beer on a linear or logarithmic scale (13,25,40,48,49,51); 2) plotting the square root of peak height ratios for DMS added to beer, the square root method (24,29,52,53), or use of an FPD response linearizer with a fixed exponent, n, of 2.0 (9); and 3) applying the techniques of method 1 to DMS added to 3–10% (w/w) aqueous ethanol or carbonated water (25,28) and using the resulting calibration curves for beer results.

Method 1 has the deficiency that the FPD response is expressed as a function of added DMS concentration (x) instead of total

TABLE III
Dimethyl Sulfide (DMS) Levels, Exponents, and Factors Calculated for a Number of Beers

	Endogenous DMS Concentration ($\mu g/L$)				
Beer	Graphs[a]	Iterations[b]	% Difference	n	F
A	35	33.5	4	1.70	125.6
B	42	36.0	14	1.71	122.8
C	31	25.3	18	1.51	78.2
D	19	18.7	2	1.61	59.3
E	45	42.5	6	1.50	50.8
F	16	15.0	6	1.27	41.9
4% Aqueous ethanol	...	...	...	1.80	75.0

[a] DMS concentration determined graphically employing plots of n vs. k and C vs. k from both high- and low-range DMS data.
[b] DMS concentration determined by iteration of high-range DMS data.

TABLE IV
Results of Recovery Study for Dimethyl Sulfide (DMS) Added to Beer B

DMS Added ($\mu g/L$)	Total DMS Found ($\mu g/L$)	Apparent Added DMS ($\mu g/L$)	% Recovery
0	42	0	...
20	65	23	115
40	83	41	103
60	103	61	102
80	117	75	94
100	141	99	99
150	187	143	95
200	247	205	103

DMS concentration ($x + k$). Because the endogenous DMS concentration (k) in a beer is a substantial part of the total DMS present during calibration with the concentration ranges generally used, this can lead to significant errors. With method 3, no endogenous DMS (k=0) is originally present in the carbonated water or ethanol solution, hence the coefficients of the power function (equation 2) can be correctly determined. However, a calibration curve established in this way can lead to substantial errors if it is used to determine DMS in beer samples (Fig. 9).

Method 2 has frequently been employed and was used by the ASBC Subcommittee on Gas Chromatography for a collaborative study of DMS in beer (1,2). The basic assumption of this approach is that the FPD response is proportional to the square of "added" DMS concentration. In addition to the error of neglecting k when calibrating the FPD, the square law assumption is not valid. The original DMS level, k, calculated using such calibration curves can lead to errors of 40–200%, depending on how much the true n value deviates from 2.0 (9).

The results for beer A were calculated in three different ways: the proposed procedure, the square root method, and a 4% ethanol calibration line. The square roots of the low-range data for beer A ($Y^{1/2}$) fitted to a straight line against added DMS concentration (the square root method) gave the following function:

$$Y^{(1/2)} = 0.346 + 0.00677 \cdot x, \quad (9$$

which is designated as line n = 2.0 in Figure 10. The k value calculated from equation 9 was 50.0 μg/L, which corresponds to a DMS concentration 43% higher than that obtained with the proposed method. The DMS concentration determined using the 4% ethanol calibration line (Fig. 10, n = 1.77) was 22 μg/L or 37% less than the result of the proposed method, 35 μg/L. The calibration lines obtained using the square root and 4% ethanol solution methods diverged significantly from the calibration line constructed with the proposed calibration procedure (Fig. 10, n = 1.70).

Similar comparisons were made for beer B and the four other beers. For each beer investigated, all three calibration lines had different slopes, which led to different results. As indicated in Table V, the difference could be as large as 206% (beer F). For beers A and B, the differences were less than 50% because the n values for both beers did not differ greatly from 2.0 (1.70 for beer A and 1.71 for beer B). It is likely that much larger errors will occur with greater deviations of the exponent from the theoretical value (9). The DMS contents estimated by the square root method were all substantially higher (36–206%) than with the proposed method. This presumably explains why reported DMS levels of U.S. beers analyzed this way were much higher (24). On the other hand, some DMS levels estimated with a 4% aqueous ethanol calibration curve were higher (beers A–D) and others lower (beers E and F) than those determined by the proposed procedure. The result depends on the differences in the slopes of their calibration curves (Fig. 9). This may also account in part for the wide range of DMS levels reported for various beers.

When the final power function for beer A obtained with the

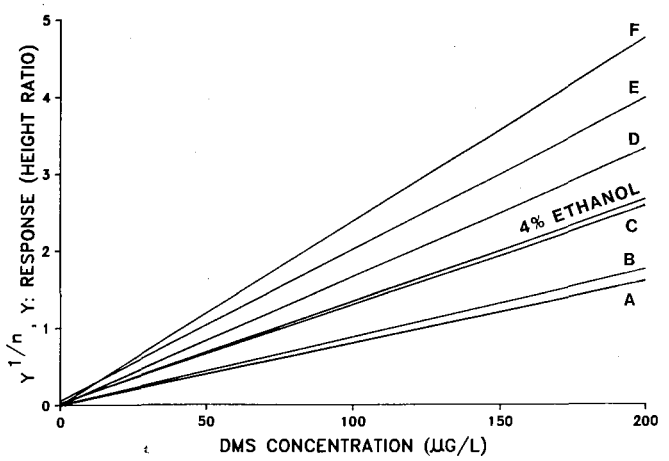

Fig. 9. Calibration curves for six different beers and 4% aqueous ethanol constructed using the proposed calibration procedure. DMS = dimethyl sulfide.

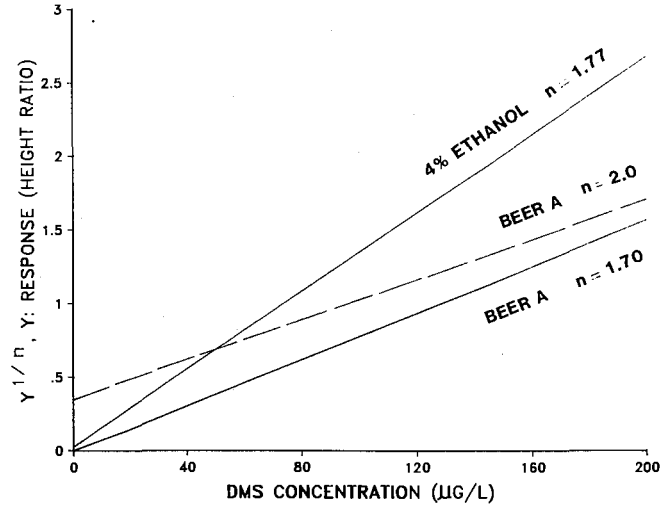

Fig. 10. Comparison of dimethyl sulfide (DMS) calibration curves constructed using different calibration methods for beer A. For the square root method, n = 2.0; for the proposed procedure, n = 1.70; and for the 4% (v/v) aqueous ethanol curve, n = 1.77.

TABLE V
Comparison of Dimethyl Sulfide (DMS) Results Calculated by Various Methods

Beer	Proposed Procedure DMS (μg/L)	Square Root Method DMS	Square Root Method % Difference	4% Aqueous Ethanol Calibration Curve DMS	4% Aqueous Ethanol Calibration Curve % Difference
A	35	50	43	22	−37
B	42	57	36	26	−38
C	31	56	81	27	−13
D	19	33	74	26	−37
E	45	78	73	63	40
F	16	49	206	31	94

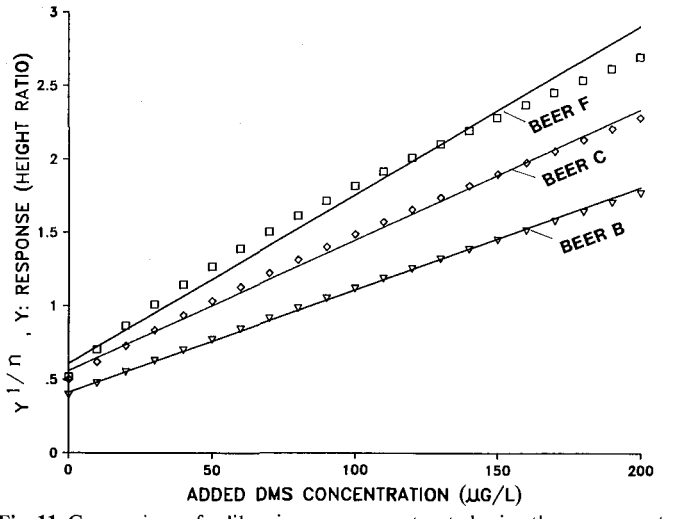

Fig. 11. Comparison of calibration curves constructed using the square root method and data generated by the proposed calibration curves treated with the square root method (▽, beer B; ◇, beer C; □, beer F).

proposed procedure (presumably the true correlation between FPD response and DMS concentration),

$$Y = 0.000261 \cdot (x+k)^{1.700} \quad (10)$$

is transformed into the form described by equation 7 adopting $n = 2.0$, the following formula results:

$$Y^{(1/2)} = 0.0158 \cdot (x+k)^{0.850}, \quad (11)$$

which possesses an exponent value (0.850) close to 1.0 (i.e., nearly linear). If k is purposely neglected, the data points generated by equation 11 will fall exactly on the calibration line ($n = 2.0$) constructed using the square root method (Fig. 11 and equation 9). This means that experimental results treated with the square root method can always be fitted to a straight line through linear regression, even when the exponent n is much lower than 2.0 (beer F), as demonstrated in Figure 11 and Table VI. The magnitudes of $n/2$ ranged from 0.635 to 0.855, but all data points generated by the transformed formulas coincided with the ones found by the square root method.

Improvement of DMS Calibration

The improvement of DMS measurements in beer with this calibration technique can be demonstrated by plotting treated and untreated data points on a logarithmic scale, as shown in Figure 12. The proposed calibration curve for beer A is a straight line whereas the original data deviate from this line significantly at low DMS levels 20–200 μg/L). In the high DMS range (500–2,000 μg/L) the data points fell on the calibration line. This result confirms that using the high-range DMS data with the iteration technique to predict the calibration line reduces error in finding the DMS levels in beer.

Quenching Effect

It has been reported (11,37,42,46) that FPD response is adversely affected by the presence of hydrocarbons (methane, ethane, pentane, butane, and 2-methyl-pentane), alcohols (methanol, ethanol, isobutanol, and 3-pentanol), acetone, and carbon dioxide. Although quenching has been attributed to the presence of large amounts of hydrocarbons (30), it has been shown that as little as 10^{-6} g of nonsulfur compounds in the carrier gas can exert a significant quenching effect (46). Most of the reported quenching investigations were done by mixing the interfering compounds into the carrier gas; no references showing a quantitative investigation of the quenching effect in beer were found.

That compounds in beer cause quenching can be demonstrated by mixing beer with 4% ethanol solution in several ratios (Fig. 13). The expected responses to the calculated amounts of DMS present are shown for 4% aqueous ethanol and for beer A. The actual values found for the mixtures fall on or near the aqueous ethanol or beer lines where each is predominant in the mixture but are intermediate in between. This must have been caused by progressively greater quenching of the DMS response as the percentage of beer increased.

TABLE VI
The Proposed Calibration Curves Transformed to Fit the Square Root Form[a]

Beer	$C^{1/2}$	$n/2$
A	0.0158	0.850
B	0.0162	0.855
C	0.0375	0.755
D	0.0364	0.805
E	0.0532	0.750
F	0.0888	0.635

[a] $Y^{1/2} = C^{1/2} \cdot (x+k)^{n/2}$

It is possible to evaluate the degree of reduced response (quenching) for both the DMS and EMS (the internal standard) peaks. Quenching was quantified by comparing the DMS peak height, the EMS peak height, and the ratio, h_D/h_E in beer to their counterparts found in 4% ethanol solution. A quantity called the quenched response (Q%) is expressed as the percentage of the response seen in a quenching medium to the same amount of DMS in aqueous ethanol:

$$Q\% = \frac{\text{peak height or ratio in beer}}{\text{peak height or ratio in 4\% aqueous ethanol}} \times 100. \quad (12)$$

This definition is similar to the intensity ratio defined by Sugiyama et al (46), which has been used to quantify quenching effects exerted on FPD response for sulfur compounds by

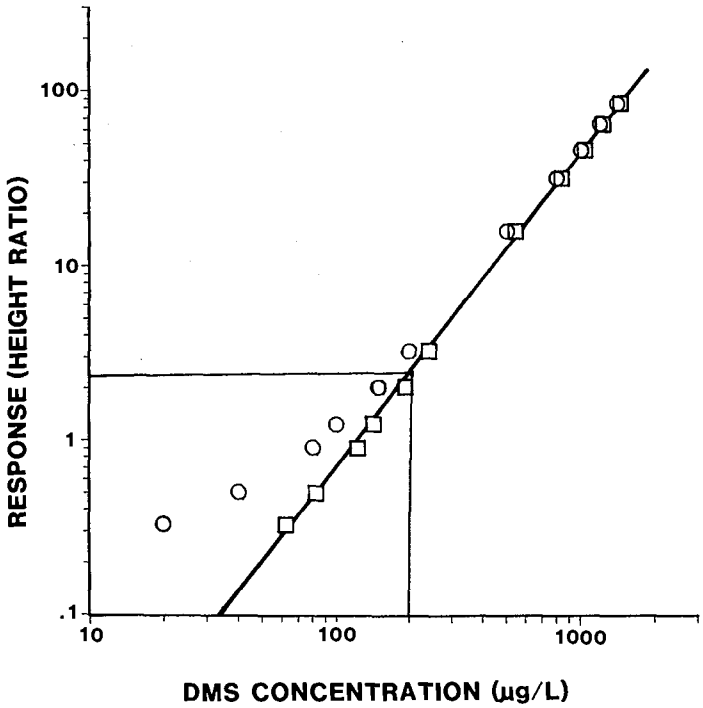

Fig. 12. Comparison of the proposed dimethyl sulfide (DMS) calibration curve (□) and original data points (o).

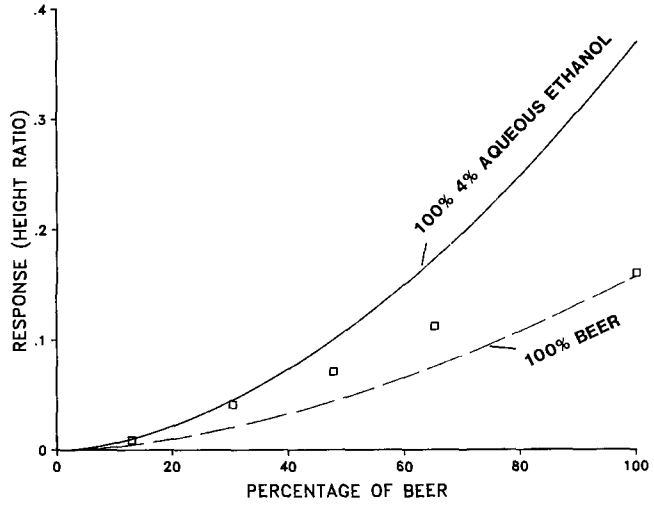

Fig 13. The quenching effect in beer. Flame photometric detector (FPD) responses (□) to dimethyl sulfide in mixtures of beer (0–100%) and 4% ethanol solution (100–0%) fall between the theoretical curves for aqueous ethanol (——) and beer (— —).

measuring the ratio of FPD responses with the quenching compounds present and absent. Estimates of the quenched response in beer A are shown in Figure 14. A value of 100% indicates no quenching, whereas lower percentages indicate quenching effects of varying degrees. The EMS peak heights in beer A were only 55% as large as those in the 4% ethanol solution (i.e., the EMS peak height was reduced by 45%). DMS peak heights were reduced more severely, to only 26% of the level in aqueous ethanol, and the Q% for the peak height ratios was about 41%. The Q% of the peak height ratio (Y), which was employed for the DMS calibration, is a ratio of Q% for DMS peak height to Q% for EMS peak height, as derived from the definition of Q% (equation 12):

$$Q\%_{\text{height ratio}} = \frac{(h_D/h_E)\ \text{beer}}{(h_D'/h_E')\ 4\%\ \text{aqueous ethanol}} \times 100$$

$$= \frac{h_D/h_D'}{h_E/h_E'} \times 100$$

$$= \frac{Q\%\ \text{of}\ h_D}{Q\%\ \text{of}\ h_E'} \times 100. \quad (13)$$

It is interesting to note that the degree of quenching is independent of the DMS concentration, as reported elsewhere (46). This indicates that the effect is mainly caused by other constituents in the beer coeluting with the DMS or EMS peaks and resulting in a constant percentage quenching effect.

The same evaluation was applied to other beers and the results are summarized in Table VII. The quenching effects for DMS and EMS clearly varied for different beers. For DMS, the quenching effects in beers D and E were much less than in the other four beers. The greater quenching of the EMS peak heights (i.e. lower Q%) resulted in larger peak height ratios (over 100%) (equation 13). These results indicate quenching may contribute to the widely differing levels of DMS reported in the literature for various beers by different methods. Some of the differences between beers likely arise from differing proportions of the compounds that quench the responses to DMS and the internal standard used.

The interference has been shown to be proportional to the concentration of coeluting nonsulfur organic compounds (42,46), and the quenching effect reportedly increases exponentially with the concentration of the interfering compounds (46). Ethanol and carbon dioxide are abundant in beer headspace, and other organic compounds present at lower concentrations may also play a part.

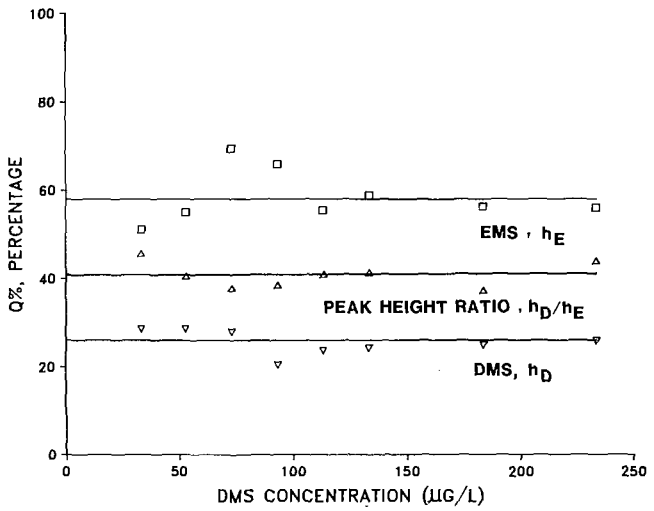

Fig 14. The quenching effect on dimethyl sulfide peak height (▽), EMS peak height (□), and their ratio (△), expressed as the quenched response, Q% (equation 14).

The differences seen between different beers seem unlikely to be solely caused by the more prominent compounds, as the amounts of ethanol and CO_2 would not be expected to vary greatly between different beers.

Simplifying the Proposed DMS Calibration Procedure for Routine Use

The proposed calibration procedure is complicated and time-consuming for a routine assay. Because the calibration curve is unique for each beer and exhibits little brew-to-brew variation, a possible simplification of the procedure would be to compare the

TABLE VII
Quenched Response (Q%) of Dimethyl Sulfide (DMS) and Ethyl Methyl Sulfide (EMS) Peak Heights and Their Ratios in Various Beers

	Q%		
Beer	DMS Peak Heights	EMS Peak Height	Height Ratio
A	26	55	41
B	31	56	49
C	55	50	115
D	90	80	108
E	100	70	138
F	60	100	57

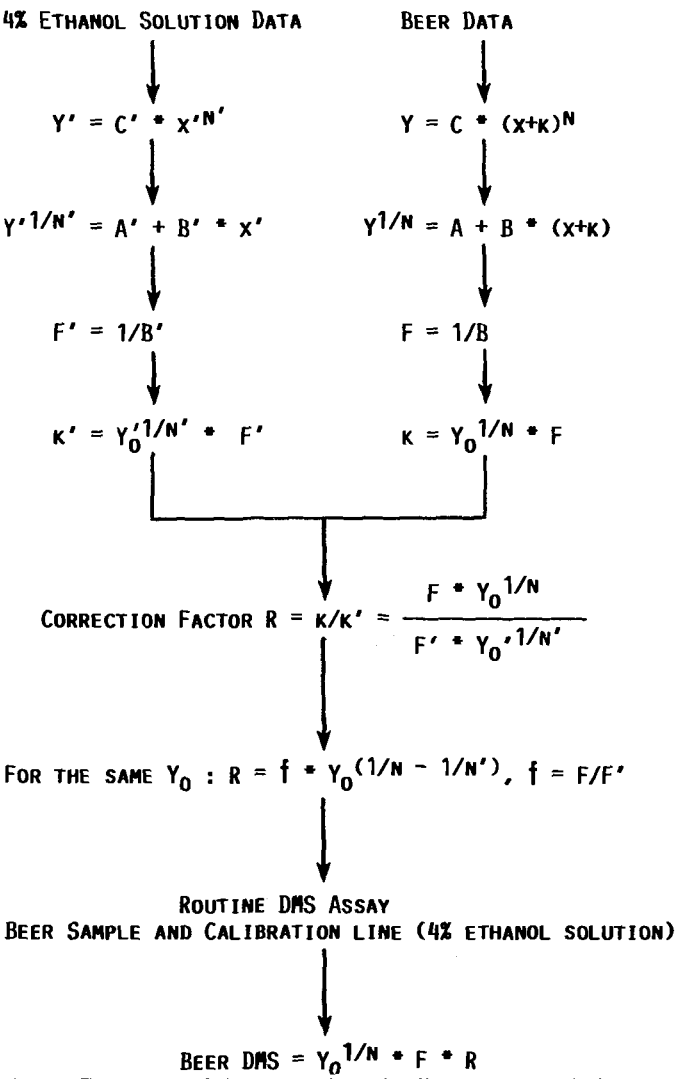

Fig. 15. Flow chart of the approach to simplify the proposed dimethyl sulfide (DMS) calibration procedure.

calibration line, once constructed via the proposed procedure, to that obtained with a 4% ethanol solution. A correction factor, R, could be defined as:

$$R = \frac{F \cdot Y_o^{1/n}}{F' \cdot Y'^{1/n'}_o} \quad (14)$$

where F, Y_0, and n are for data collected with beer, and F', Y'_0, and n' are results from a 4% ethanol solution. For the same response, Y_0, the correction factor could be expressed as a power function of Y:

$$R = f \cdot Y_o^{(1/n - 1/n')}, \quad (15)$$

where f equals F/F'. For a given beer sample, the DMS concentration could be calculated with the following equation:

$$\text{DMS} = Y_o^{(1/n)} \cdot F \cdot \text{R}. \quad (16)$$

After the calibration curve for a beer is established by employing the proposed calibration procedure (i.e., n and F are found), the R value can be calculated. For a routine assay, only a few points in the 4% ethanol solution would have to be checked with the beer sample to insure the reproducibility of the control standard curve. The complete calibration procedure would only have to be performed once for each beer to define its particular correction formula. The simplified procedure is shown in Figure 15.

R is determined by three major parameters, f, n, and n', and is also a function of the value Y. The f value has the strongest influence on the R value. The major correction factor parameters calculated for six different beers are shown in Table VIII. The f values for beers D, E, and F are close to 1.0, whereas those for beers A, B, and C are greater than 1.0 ($R = 1.0$ means no need for correction). This can be explained by varying degrees of quenching in the different beers (Table VII). For beers A, B, and C, the quenching effects on the DMS peak height were greater than on the EMS peak height, which caused the Q% of the height ratios to be smaller than 100% (equation 13). This means that for the same response Y_0, the k determined using a 4% ethanol calibration curve would be smaller than the level actually present in the beer. The resulting correction factors are higher than 1.0 (1.006–1.769). For beers D, E, and F, the quenching of the EMS peak height is greater than that of the DMS peak height. As a result, the Q% for the peak height ratio is greater than 100% and the resultant R is less than 1.0.

CONCLUSIONS

Several conclusions can be drawn from the above results. The proposed DMS calibration technique correctly determines DMS concentrations in various beer samples. Each beer has a unique calibration curve, which cannot be used to determine DMS concentration in other beers. The quenching effects on the DMS and EMS peaks are different in different beers. As a result, the peak height ratios obtained in beer may be different from those found in 4% ethanol solution. The results obtained from the square root method for six different beers were 36–206% higher than the values determined by the proposed procedure, whereas those obtained with a 4% ethanol calibration curve were lower in four cases (by 13–37%) and higher in two cases (by 40 and 94%). The proposed new calibration procedure is complicated for a routine DMS assay in beer. It can be simplified by defining a correction factor for each beer.

TABLE VIII
Correction Factors Determined for a Number of Commercial Beers Using Equation 15

Beer	R[a] Y_o = 0.05–10.0	f	$1/n-1/n'$
A	1.529–1.769	1.660	0.0275
B	1.536–1.696	1.625	0.0186
C	1.006–1.658	1.334	0.0943
D	0.669–1.003	0.841	0.0764
E	0.505–0.948	0.721	0.1190
F	0.371–1.181	0.714	0.2186

[a] As R is a function of the detector response (Y_0), it exhibits a range of values; those shown were calculated at a low and a high level of Y.

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