A Rapid and Sensitive Method for the Determination of 4-Vinyl Guaiacol in Beer by Electron-Capture Gas-Liquid Chromatography[1]

R. Villarreal, J. A. Sierra, and L. Cárdenas, *Cervecería Cuauhtémoc, 64000 Monterrey, N.L., México*

ABSTRACT

A method is described for the determination of amounts as low as 25–50 $\mu g/L$ of 4-vinyl guaiacol in beer as a heptafluorobutyrate derivative. The method involves an extraction of the beer with freon 11, evaporation of the solvent, formation of the derivative, and gas chromatographic analysis. It is rapid (60 min) and takes advantage of sensitive electron-capture detection. The method was tested in several commercial North American beers as well as in experimental beers fermented with brewing yeast strains and with nonbrewing, wild-type yeast strains. The effect of increasing levels of contamination of the pitching yeast with wild yeast on the concentration of 4-vinyl guaiacol in beer was also examined.

Key words: 4-Vinyl guaiacol, Beer, Electron-capture detector, Gas chromatography

Phenolic carboxylic acids (*p*-coumaric, ferulic, and sinapic) present in malt as glycosides are responsible for phenolic off-flavors in beer (10). Under the conditions of wort boiling, these compounds can be thermally fragmented to the corresponding phenols (11). Ferulic acid is found in wort in high concentrations, and is the precursor of 4-vinyl guaiacol, one of the important phenols in beer.

Contamination of the pitching yeast with wild yeasts can produce an undesirable phenolic aroma in the beer as a result of the decarboxylation of these acids to their corresponding phenols. Brewing yeast strains are reported to be unable to decarboxylate these compounds (2,12,15). The phenolic compound whose concentration in beer increases the most as a result of this contamination is 4-vinyl guaiacol. This compound is normally present in beer in concentrations below its flavor threshold (7,8,15); however, higher concentrations in beer cause the presence of a "spicy, clovelike flavor" that strongly undermines the flavor of beer.

There are several reports in the literature on determination of these phenolic compounds in wort and beer using gas chromatography and a flame-ionization detector. Tressl (11) published on the separation and identification of these compounds from an extract of beer; however, the method is lengthy and involves several steps. Wackerbauer (12) also reported the analysis of phenols in wort and beer using the basic method reported by Tressl with some modifications. Thurston and co-workers made determinations of 4-vinyl guaiacol in beer by a rapid method involving extraction and direct injection into a gas chromatograph (10).

The purpose of this work was to develop a rapid and sensitive gas chromatographic method for the determination of 4-vinyl guaiacol using the highly sensitive electron-capture detector.

EXPERIMENTAL

Reagents

4-Vinyl guaiacol (2-methoxy-4-vinyl phenol) was used as reference standard and was prepared according to the procedure of Sovish (9). The compound was kept in an amber flask in the refrigerator before use. A standard solution (1 mg/ml) was prepared in absolute ethanol.

Eugenol (2-methoxy-4-allyl phenol) was obtained from Haarman & Reimer, and used as an internal standard in the gas chromatographic analysis. A solution containing 25 mg of eugenol freshly distilled in 25 ml of absolute ethanol was prepared and kept in an amber flask in the refrigerator before use.

Freon 11 (trichlorofluoromethane, commercial grade); benzene (Mallinckrodt) suitable for pesticide residue analyses, heptafluorobutyric anhydride (HFBA) (Pierce Chemical Co.), triethylamine (TEA) solution (0.1 *M*) in benzene, sodium phosphate buffer solution, pH 6 (1 *M*) SO_4; and anhydrous calcium chloride (J. T. Baker) were all obtained locally.

Sample Preparation

To a 50-ml Erlenmeyer flask immersed in an ice-water bath was added 10 ml of cold beer previously decarbonated by swirling, 10 μl of the internal standard solution, and 10 ml of freon 11. The mixture was magnetically stirred for 10 min. After a 5-min settling period, the mixture was centrifuged to break the emulsion. The organic layer was transferred to a 50-ml flask and evaporated to dryness in a Büchi rotary evaporator with vacuum at ambient temperature. The residue was dissolved in 10 ml of benzene and dried with anhydrous calcium chloride.

Preparation of the Derivative

In a 2-ml graduated test tube equipped with a glass stopper were added 0.5 ml of the benzene extract, 100 μl of the 0.1 *M* solution of TEA, and 10 μl of HFBA. The mixture was stirred for 1 min and incubated for 10 min at ambient temperature to complete the reaction. At the end of this time 0.5 ml of the buffer solution was added, and the mixture was stirred for 30 sec.

The layers formed were separated by centrifugation, and the organic layer was used for gas chromatographic analysis.

Gas Chromatographic Analysis

The apparatus used was a Varian-3700 gas chromatograph equipped with a ^{63}Ni electron-capture detector and a CDS-111 data system. A 3.6 m $\times$ 2 mm glass column packed with 5% SE-30 on Chromosorb W (HP) mesh 80/100 was used. Temperatures were: detector, 250°C; injection port, 200°C; and oven, 8 min at 100°C raised to 140°C (10°C/min) followed by 12 min at 140°C. The flow rate of nitrogen (carrier gas) was 30 ml/min. The range was 32×10^{-11}, and the sample injected was 1 μl.

Calibration Curve

A calibration curve was constructed from analyses of beers containing 4-vinyl guaiacol in the range of 0–500 $\mu g/L$.

RESULTS AND DISCUSSION

All methods reported in the literature for the analysis of phenols in beer involve an extraction step, and different solvents have been used (3,6,10–12). In this work, freon 11 was selected as the extracting solvent because of its low boiling point. The lower temperature prevents possible thermal degradation of the 4-vinyl guaiacol during concentration of the extract. In spite of its weak polarity, this solvent was reported as a good extracting solvent for phenols in wine (5). The recovery of 4-vinyl guaiacol under the conditions of the method was of the order of 90 $\pm$ 5%.

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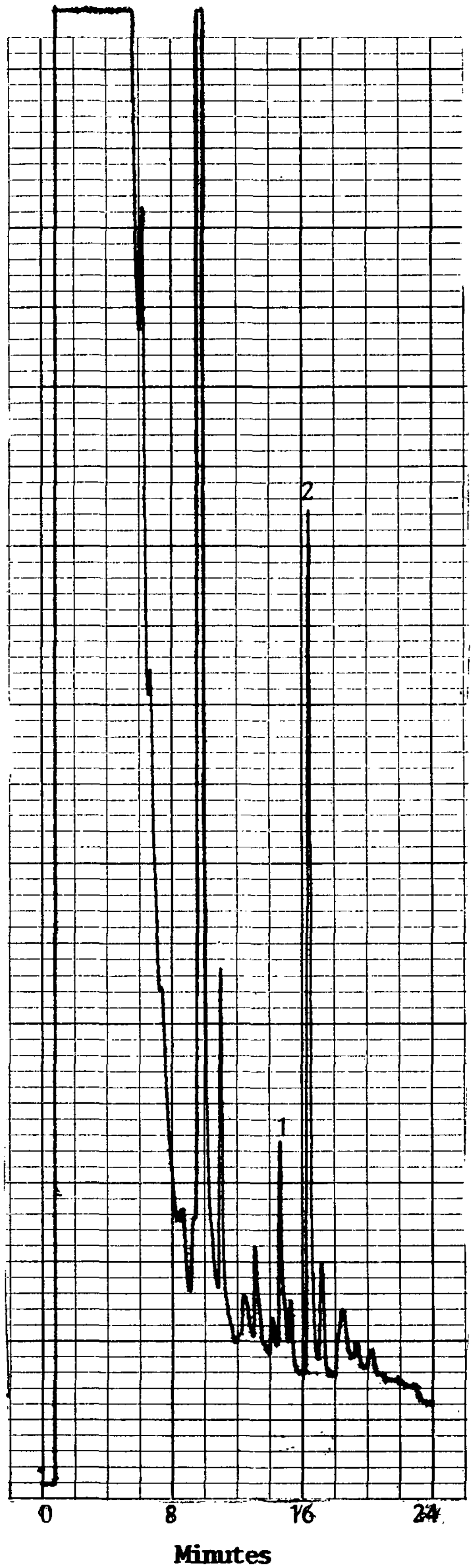

Fig. 1. Gas chromatogram of an extract from normal beer after reaction with heptafluorobutyric anhydride. Peak **1,** 4-vinyl guaiacol; peak **2,** eugenol (internal standard).

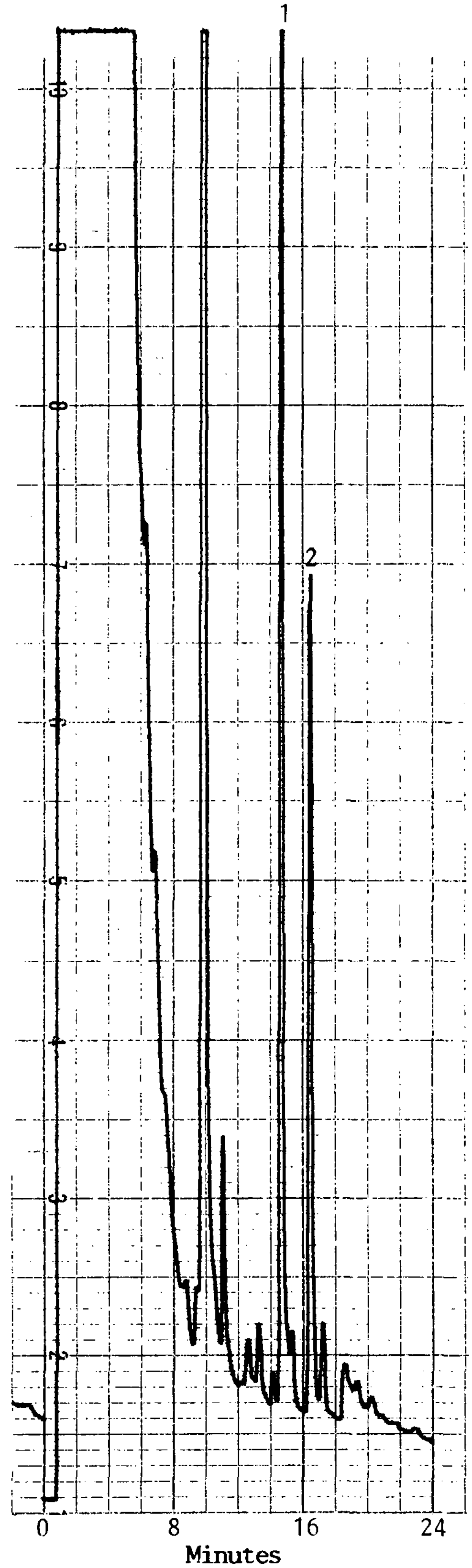

Fig. 2. Gas chromatogram of an extract from beer with 500 μg/L of 4-vinyl guaiacol added, after reaction with heptafluorobutyric anhydride. Peak **1,** 4-vinyl guaiacol; peak **2,** eugenol (internal standard).

Formation of a stable and volatile derivative to facilitate the gas chromatographic analysis was accomplished by reacting the 4-vinyl guaiacol with HFBA to form its corresponding heptafluorobutyrate. The presence of seven fluorine atoms in the derivative increases the sensitivity of the analysis by electron capture.

The reaction conditions used in the formation of the derivative, using an amine as catalyst and benzene as solvent, have been widely studied for the quantitative determination of phenols. The procedure is rapid and requires an anhydrous medium. The use of any Teflon material should be avoided because this material absorbs fluorine-containing compounds such as HFBA (1,4).

A typical chromatogram of the product of the reaction of a beer extract with HFBA is shown in Figure 1. The retention time of the derivative of 4-vinyl guaiacol identified in beer is identical to the retention time of the derivative made from the pure compound. This was corroborated by the method of standard addition, as observed in Figure 2. Both chromatograms show, besides the solvent peak, some peaks caused by other compounds present in the extract that reacted with the HFBA. However, none of these peaks interfere in the quantification of the 4-vinyl guaiacol.

TABLE I
Levels of 4-Vinyl Guaiacol in Mexican Beers

Sample	4-Vinyl Guaiacol (μg/L)
1	121
2	138
3	128
4	152
5	89
6	60
7	75
8	140
9	93
10	56
11	39
12	131
13	51
14	72
15	152
16	170
17	117
18	98
19	118

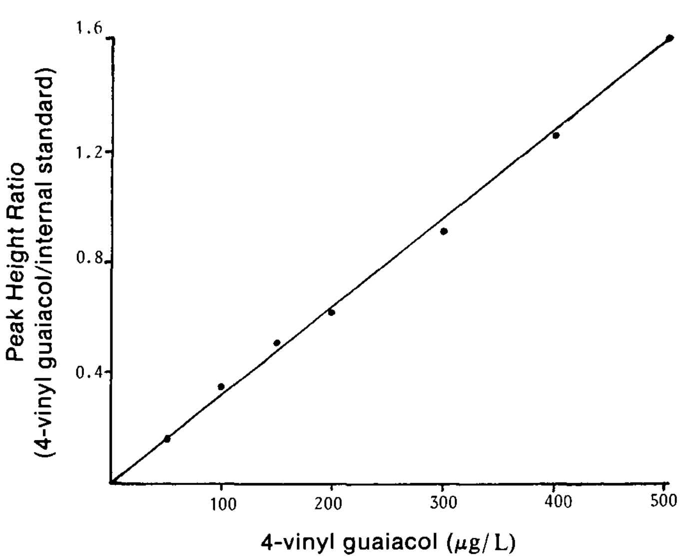

Fig. 3. Calibration curve of the gas chromatographic method for the determination of 4-vinyl guaiacol in beer.

A calibration curve was constructed by analyzing beers with 4-vinyl guaiacol added in the range of 0–500 μg/L using eugenol as the internal standard. Even though limitations to work in a linear range using the electron capture detector are well known, the curve obtained (plotting the ratio of 4-vinyl guaiacol and internal standard peak heights) was a straight line, as can be observed in Figure 3. In the case of some "special" beers with a high content of eugenol, it would be necessary to select a different internal standard (11,13,14).

This method had a sensitivity of 25–50 μg/L for 4-vinyl guaiacol; this permitted the evaluation of normal beers as well as beers with a suspected phenolic deviation, because this sensitivity is below the flavor threshold values reported in the literature for 4-vinyl guaiacol (7,8,15). The reproducibility of the method was obtained by analyzing six samples of beer. The coefficient of variation found was 6.3%.

Total time for the analysis was 60 min, which is short compared with other methods that employ long extraction times prior to derivatization and gas chromatographic analysis.

The method was also used to determine the levels of 4-vinyl guaiacol in Mexican beers, and the results are shown in Table I. A wide range in the levels of 4-vinyl guaiacol in Mexican beers was found. Lower levels were observed in beers from breweries in México City and Toluca. In Toluca and México City wort boils at 91 and 93°C, respectively, and if we consider that all the 4-vinyl guaiacol present in beer arises from the thermal decomposition of ferulic acid during wort boiling, then (all other factors being equal) breweries located at high altitudes should have lower levels of 4-vinyl guaiacol.

The results obtained in the determination of 4-vinyl guaiacol in a small number of American beers ranged from 86 to 147 μg/L. The individual sample scores were beer A, 86; beer B, 127; beer C, 140; beer D, 147; and beer E, 135 μg/L, respectively. The method was also used to determine the level of 4-vinyl guaiacol in beers fermented with brewing yeasts and also with wild-type strains. These results are presented in Table II. Clearly, levels of 4-vinyl guaiacol 10 times higher were found when wild yeasts were picked and allowed to ferment worts.

We also looked at the effect that increasing levels of contamination of the pitching yeast with wild yeast had on the content of 4-vinyl guaiacol in beer. The wild yeast selected was

TABLE II
Levels of 4-Vinyl Guaiacol in Beers Fermented with Brewing Yeasts and with Wild Type Strains

Yeast Strain	4-Vinyl Guaiacol (μg/L)
Wild Yeast	1,154
Saccharomyces diastaticus	1,914
S. pastorianus	1,034
S. willianus	1,333
S. uvarum, J-3015	132
S. uvarum, J-2270	150
S. uvarum, J-2036	115
S. uvarum, W-28C4	132

TABLE III
Effect of Level of Wild Yeast Contamination on the Content of 4-Vinyl Guaiacol in Beer

Level of Contamination with *S. diastaticus*	4-Vinyl Guaiacol (μg/L)
0.0001%	151
0.001%	144
0.01%	131
Control	147

TABLE IV
Taste Panel Evaluation of Beers with Different 4-Vinyl Guaiacol Contents

4-Vinyl Guaiacol in Beer (μg/L)	No. of Tasters that Identified Phenolic Off-Flavor[a]
Control	None
200	3
300	7
400	8
500	8

[a]Taste panel formed by eight members.

Saccharomyces diastaticus, which had given the beer with the highest concentration of 4-vinyl guaiacol. The results obtained are summarized in Table III, where it can be seen that a contamination of up to 100 cells of wild yeast per 10^6 cells of pitching yeast did not appreciably affect the final level of 4-vinyl guaiacol. A higher level of contamination seriously affected the content of 4-vinyl guaiacol in the final beer: levels present were, in wort 162 μg/L; in the control beer, 155 μg/L; and in the beer contaminated with 5% *S. diastaticus*, 346 μg/L.

Our results agree with those previously reported by Wackerbauer, who found a concentration of 277 μg/L of 4-vinyl guaiacol in a beer produced with a brewing yeast contaminated with 5% of a wild yeast (14).

There are differences in the literature as to the flavor threshold for 4-vinyl guaiacol in beer. Russell and Stewart (8) report a flavor threshold of 170 μg/L, whereas Wackerbauer (15) reports a flavor threshold of 250 μg/L, and Meilgaard (7) of 300 μg/L. We conducted some informal taste tests to have an idea of the tentative flavor threshold of 4-vinyl guaiacol in our beer, and the results are presented in Table IV. Our results suggest a flavor threshold for 4-vinyl guaiacol of the order of 200–250 μg/L, although more extensive and systematic taste tests should be conducted to have a reliable value.

LITERATURE CITED

1. Blau, K., and King, G. S. Acylation. Page 104 in: Handbook of Derivatives for Chromatography. K. Blau and G. S. King, eds. Heyden & Son Ltd., London, 1977.
2. Dadic, M. *Brew. Dig.* 55(4):30, 1980.
3. Dallos, F. C., Lautenbach, A. F., and West, A. B. *Am. Soc. Brew. Chem. Proc., 1967*, p. 103.
4. Ehrsson, H., Walle, T., and Brotell, H. *Acta Pharm. Suecica.* 8:319, 1971.
5. Etievant, P. X. *J. Agric. Food Chem.* 29:65, 1981.
6. Kieninger, H. *Brauwissenschaft* 30:357, 1977.
7. Meilgaard, M. C. *Tech. Q. Master Brew. Assoc. Am.* 12:151, 1975.
8. Russell, I., Hancock, I. F., and Stewart, G. G. *J. Am. Soc. Brew. Chem.* 41:45, 1983.
9. Sovish, R. C. *J. Org. Chem.* 24:1354, 1959.
10. Thurston, P. A., and Tubb, R. S. *J. Inst. Brew.* 87:177, 1981.
11. Tressl, R., Kossa, T., and Renner, R. *Eur. Brew. Conv. Proc. Congr. 15th, Nice, 1975*, p. 737.
12. Wackerbauer, K., Kossa, T., and Tressl, R. *Eur. Brew. Conv. Proc. Congr. 16th, Amsterdam, 1977*, p. 495.
13. Wackerbauer, K., and Krämer, P. *Brauwelt* 122:758, 1982.
14. Wackerbauer, K., and Krämer, P. *Monatsschr. Brauerei* 36:242, 1983.
15. Wackerbauer, K., Krämer, P., and Siepert, J. *Brauwelt* 122:618, 1982.

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