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Note

Simple and sensitive technique for detecting trace flavour components in beer

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Many flavour components in foodstuffs are sensorily detectable at the sub-ppb* level. However, at present, no simple mild technique exists for detecting these trace components.

A method has been developed which is particularly applicable to the gas chromatographic detection of trace volatile flavour compounds in beer. Beer was vacuum-steam distilled at 25° and 0.02 mmHg pressure, and the distillate was passed through Amberlite XAD-2 porous polymer beads. The resin was eluted with diethyl ether and the extract analysed by gas-liquid chromatography (GLC). Using this technique, sulphur-containing compounds are detectable with a flame photometric detector at the sub-ppb level, and with a flame ionisation detector compounds which do not contain sulphur, are detectable at the ppm level.

EXPERIMENTAL

Instruments

A Pye GCV gas chromatograph, equipped with a linear temperature programmer and synchronous flame ionisation and flame photometric detection (394 nm filter), was used. The GLC column was 2.8 m × 4 mm I.D. glass packed with 10% Carbowax 20M on Chromosorb W AW DMCS (80–100 mesh).

The column was operated with a nitrogen carrier gas flow-rate of 52 ml/min and a temperature programme of 50–200° at 3°/min.

GLC-mass spectrometry (MS) was carried out with the Carbowax 20M column linked to an MS-12 mass spectrometer (Associated Electrical Industries, Manchester, Great Britain) using a membrane separator. Helium was used as a carrier gas and mass spectra were measured with an ionisation energy of 70 eV.

Reagents

Amberlite XAD-2 resin¹⁻⁵ (BDH, Poole, Great Britain) was purified by washing sequentially with water, methanol, and diethyl ether just before use, using a procedure similar to that described by Van Rossum and Webb¹.

* Throughout this article, the American billion (10⁹) is meant.

Diethyl ether was AnalaR grade (BDH) and was used without further purification.

Sulphur-containing flavour compounds were prepared by known methods⁶⁻⁸ and were homogeneous according to GLC analysis.

Non-sulphur-containing flavour compounds were commercial samples and were used without further purification.

Nitrogen was OFN grade (oxygen free), (British Oxygen, Wembley, Great Britain) and was dried by passage through calcium chloride.

Antifoam was a solution of 10% silicone DC antifoam RD emulsion (Hopkin & Williams, Chadwick Heath, Great Britain) in water.

No interfering sulphur compounds were detected in any of the reagents described above.

Experimental procedure

Beer (2 l) containing antifoam solution (5 ml) is vacuum-steam distilled at 25° and 0.02 mmHg pressure, using the method of Pickett *et al.*⁹. For a period of 5 h, the vacuum distillate is passed through a column of Amberlite XAD-2 beads (60 mm × 7 mm, 2 g dry weight), which is then washed with deionised water (100 ml). To extract flavour components from the resin beads, they are then eluted with diethyl ether by successive equilibration with five portions of ether (total volume 10 ml) for a period of 10 min each. The combined extract is separated from excess water, dried over sodium sulphate (BDH) and reduced to a volume of 10 μ l with a slow stream of nitrogen. The extract (5 μ l) is analysed by GLC.

Compounds were identified by comparison of the MS and GLC retention times with those of standard samples.

RESULTS AND DISCUSSION

Beer is a complex mixture of flavour compounds¹⁰, many of which are sensitive to both heat and traces of oxygen. Therefore any technique for extracting the volatile flavour components should be carried out at a temperature below 25° with minimal contact with air. This note describes an extraction method fulfilling these requirements.

Vacuum distillation at 25° effects a separation of volatile flavour components from involatile material which interferes with GLC analysis. Flavour components are then separated from water, ethanol, and most of the lower fusel alcohols present in the vacuum distillate by passage through a column of Amberlite XAD-2 beads. The colourless extract, prepared by extracting the beads with diethyl ether, is suitable for direct analysis by GLC.

In work concerned with flavour chemistry it is important to be able to detect compounds in complex mixtures at concentrations at or below their flavour threshold levels, so as to determine whether the compounds contribute individually to the overall flavour. Several sulphur-containing compounds are easily detected in beer at their flavour threshold levels, in the ppb range, by analysing the extracts prepared as described above using a flame photometric detector. These compounds and their flavour thresholds are shown in Table I. In contrast to the present technique, a head-space sampling procedure¹⁴ does not detect dimethyl trisulphide below 100 ppb, *i.e.* 1000 × its flavour threshold concentration.

TABLE I
SULPHUR COMPOUNDS DETECTED IN BEER

Compound	Flavour threshold (ppb)
Dimethyl trisulphide ¹¹	0.1
S-Methyl hexanethioate ¹²	1
S-Methyl 4-methylpentanethioate ¹³	15
4-(4-Methylpent-3-enyl)-3,6-dihydro-1,2-dithiine ¹³	10

A typical gas chromatogram (obtained using a flame photometric detector), showing a profile of the sulphur compounds present in an extract of beer previously treated with dry hops, is given in Fig. 1a.

The method is also capable of detecting many non-sulphur-containing compounds present in beer at the ppm level. A typical gas chromatogram (obtained using a flame ionisation detector) of an extract of a commercial beer is shown in Fig. 1b, together with the identities of a number of the principal components. Decanoic acid, shown to be present in a commercial beer at a level of 1.7 ppm by the method of Taylor and Kirsop¹⁵, was easily detected using the XAD-2 technique. Table II shows the extents to which several beer flavour components were removed, by XAD-2 resin, from a vacuum-steam distillate. It can be seen that whilst the two fatty acids were efficiently adsorbed by the resin, β -phenylethanol was only very poorly adsorbed. Therefore, in accord with the literature, the method appears to be most suited for

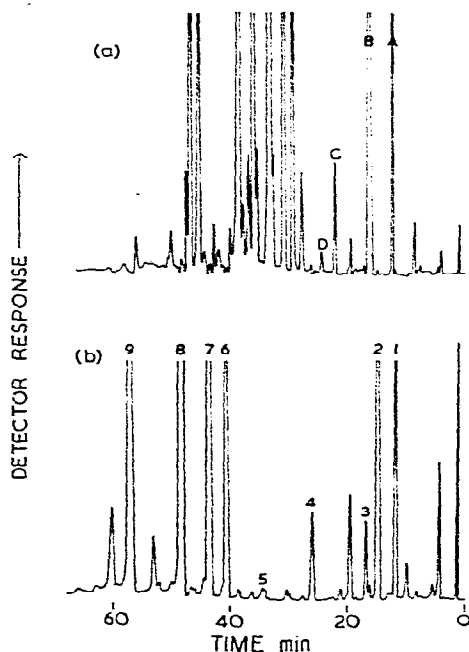


Fig. 1. Gas chromatography of beer extracts. Detection: (a) flame photometric; (b) flame ionisation. Peaks: A = S-methyl 2-methylpropanethioate; B = S-methyl 2-methylbutanethioate; C = S-methyl 4-methylpentanethioate; D = S-methyl hexanethioate; 1 = isoamyl acetate; 2 = isoamyl alcohol; 3 = ethyl hexanoate; 4 = ethyl octanoate; 5 = ethyl decanoate; 6 = β -phenylethyl acetate + hexanoic acid; 7 = β -phenylethanol; 8 = octanoic acid; 9 = decanoic acid.

detecting compounds of a relatively hydrophobic nature. Further work is in progress to establish whether this method provides the basis of a quantitative procedure for estimating beer flavour components which do not contain sulphur.

TABLE II

REMOVAL OF BEER FLAVOUR COMPONENTS FROM A VACUUM STEAM DISTILLATE BY XAD-2 RESIN

<i>Compound</i>	<i>Concentration in beer vacuum steam distillate (ppm)</i>	<i>Removed by XAD-2 resin (%)</i>
Decanoic acid	1.2	97
Octanoic acid	3.6	89
β -Phenylethanol	19.4	9

CONCLUSION

The technique provides a simple and sensitive method for detecting and quantifying traces of flavour compounds, particularly those containing sulphur, in beer.

Further work is being carried out to improve the sensitivity of the technique. It is believed that this method could be used to detect and quantify sulphur compounds in a wide range of foodstuffs by emulsifying samples in water prior to the initial vacuum steam distillation step.

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