reactions only if an active phytochrome or similar system is present.

L. W. Mapson
T. Swain

Low Temperature Research Station,
Downing Street, Cambridge.

[1] Mapson, L. W., *Biochem. J.*, **85**, 360 (1962).
[2] Mapson, L. W., *Phytochem.*, **3**, 429 (1964).

Determination of 2-Phenylethanol in Cider

Methods used for the determination of 2-phenylethanol in fermented beverages have utilized solvent extraction of the beverage or of a distillate, followed by measurements of absorption spectra[1–3] or by gas chromatography[4–8]. The compound is not included in the determination of fusel oils by the colorimetric procedures usually adopted[8,9]. The methods described have certain disadvantages: preliminary extraction may be incomplete unless prolonged, absorption measurements may involve previous purification of the fraction containing 2-phenylethanol, and in the gas chromatographic methods described the retention time of the compound may exceed 1 h with column temperatures above 100°. The method to be described is simple in operation and has been found satisfactory for cider: by the use of a column of low capacity of the type described by Hishta *et al.*[10] the retention time of 2-phenylethanol has been reduced to 8 min.

Cider (50 ml.) was distilled without fractionation and 25 ml. of distillate collected; 25 ml. of water was added to the flask and a further 25 ml. distillate collected. The process was repeated and the third distillate containing only traces of 2-phenylethanol collected separately. To the third distillate was added sodium chloride (3 M) and it was then extracted at about 5° C with two volumes each of 15 ml. ethyl chloride. These extracts were used to extract the main distillate after addition of sodium chloride; it was then extracted twice further with 10 ml. ethyl chloride. The combined ethyl chloride solutions were washed with minimal volumes of water, freed from water droplets by passage through a layer of sodium sulphate (anhydrous) and concentrated to one-third volume by distillation of the solvent. The solution was transferred to a weighed tube, 2 ml. of 50 per cent aqueous ethanol added and the ethyl chloride removed by a slow stream of dry air. The solution, of known weight, was used directly for gas chromatography, using 5 μl. injections.

The apparatus used for gas chromatography was built in this laboratory and consisted of a 122 cm × 4·5 mm. internal diam. glass column packed with 0·2 per cent 'Apiezon *L*' on 60 mesh (A.S.T.M.) glass micro-beads, run isothermally at 80° with flame ionization detector. The flow-rate was approximately 15 ml./min with nitrogen–hydrogen in 1 : 1 mixture as carrier gas. Under these conditions 2-phenylethanol gave a reasonably narrow peak so that the amount present could be estimated from peak height taking as standards solutions of 2-phenylethanol in 50 per cent aqueous alcohol that gave peaks of comparable size.

The retention times relative to benzene of the main fusel oil components found in cider[11,12] were, as shown below, well separated from that of 2-phenylethanol; they all emerged within 2 min, whereas the latter gave a peak maximum at 7 min 42 sec.

Table 1. Fusel Oil Components of Cider and their Retention Times (relative to benzene)

Methanol	1·32
Ethanol	1·37
1-Butanol	1·59
1-Hexanol	5·05
2-Methyl-1-propanol	1·39
3-Methyl-1-butanol	2·90
β-Methyl-propyl ethanoate	1·75
γ-Methyl-butyl ethanoate	8·45
2-phenylethanol	44·85

The chromatograms showed two main peaks, the more volatile components forming one unresolved peak, the second being that corresponding to 2-phenylethanol. There is as yet no evidence for the presence of other cider components contributing to the second peak. The chromatograms were readily reproducible and estimates of the amount of 2-phenylethanol present did not vary by more than ± 5 per cent.

Extraction with ethyl chloride gave recoveries of 2-phenylethanol exceeding 90 per cent from synthetic fusel oil solutions corresponding to the composition of cider, or from distillates of the solution. Continuous extraction with *n*-pentane required more than 14 h for completion; direct extraction with ether or ether–pentane mixtures gave recoveries below 80 per cent. Persistent emulsions were formed when cider itself was shaken with solvents. The extraction of cider distillates with ethyl chloride was therefore adopted, this being the most efficient and selective solvent.

As the method of determination is based solely on a comparison of retention time with that of a standard, a semi-quantitative isolation of 2-phenylethanol from cider was made using a sample estimated to contain 100 p.p.m. The distillate from cider (1,560 ml.) was extracted with ether (5 vol. of 200 ml.) and the ether extract washed with N caustic soda and with water. The ether was removed, the liquid residue (10 ml.) extracted with ethyl chloride and the extract washed with water and dried. After removal of the solvent the liquid residue (3·1 g) was distilled in a slow air stream at 24 mm and 50° C until the residue had no odour of amyl alcohols. On raising the temperature to 70° a fraction with an odour of 2-phenylethanol condensed in the distillation head, from which it was removed with ether; 90 mg of this material was collected. 50 mg converted to the 3 : 5 dinitrobenzoate gave an 88 per cent yield of crude product, giving 62 mg of m.p. 105° C, raised by re-crystallization to 107°; mixed with an authentic specimen of m.p. 107° C the m.p. was unchanged. The infra-red spectra of the two specimens were identical.

Table 2. 2-Phenylethanol Content of Ciders

No.	Fermentation	p.p.m.
1	Yeasted	9
2	,,	7
3	,,	9
4	,,	15
5	Not yeasted	100
6	,, ,,	102

The amounts of 2-phenylethanol found in ciders were found to vary widely (as shown in Table 2); the amounts in yeasted fermentations were below 20 p.p.m., whereas sulphited juices fermented by members of the natural yeast flora (Nos. 5, 6) gave products containing about 100 p.p.m. A figure of 121 p.p.m. for a cider has been found by Morgan[13].

Margaret E. Kieser
A. Pollard
Pauline M. Stevens
O. G. Tucknott

Research Station
(University of Bristol),
Long Ashton, Bristol.

[1] Äyräpää, T., *J. Inst. Brew.*, **68**, 504 (1962).
[2] Äyräpää, T., *Nature*, **194**, 472 (1962).
[3] Shigematsu, N., *Bull. Brew. Sci.*, Tokio, **8**, 44 (1962); *J. Inst. Brew.*, **69**, 277 (1963).
[4] Stevens, R., *J. Inst. Brew.*, **67**, 329 (1961).
[5] Stevens, R., *Nature*, **191**, 913 (1961).
[6] Strating, J., and Venema, A., *J. Inst. Brew.*, **67**, 525 (1961).
[7] Webb, A. D., Riberceau-Gayon, P., and Boidron, J. N., *Acad. Agric. de France, C.R.*, **49**, (2), 115 (1963).
[8] Sihto, E., and Arkima, V., *J. Inst. Brew.*, **69**, 20 (1963).
[9] Hough, J. S., and Stevens, R., *J. Inst. Brew.*, **67**, 488 (1961).
[10] Hishta, C., Messerly, J. P., and Reschke, R. F., *Anal. Chem.*, **32**, 1730 (1960).
[11] Matthews, J. S., Sugisawa, H., and MacGregor, D. R., *J. Food Sci.*, **27**, 355 (1962).
[12] Anon., *Ann. Rep. Long Ashton Res. Sta. for 1963*, 40 (1964).
[13] Morgan, K. (private communication).