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CHANGES IN VOLATILES OF MUSCAT GRAPES DURING RIPENING

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Abstract—The changes in linalool and other major volatile compounds in extracts from Muscat of Alexandria grapes were followed during the course of ripening and related to changes in reducing sugar and titratable acid. Linalool was first detected 2 weeks after the onset of sugar accumulation and increased steadily for as long as fruit was left on the vine. Other volatiles of possible significance in the aroma of the ripe grape appeared 2–4 weeks after linalool was first observed and then increased sharply. Hexanal and hexenal, which were probably formed during processing of the fruit, reached a maximum about 2 weeks after the beginning of sugar accumulation and then declined. Some of the volatile components of near-ripe and ripe fruit disappeared during storage of the extracted volatiles, with a concomitant formation of several new compounds including limonene and myrcene.

INTRODUCTION

Vitis vinifera L., cv. Muscat of Alexandria (Muscat Gordo Blanco) is one of a few grape varieties whose fruit have a distinctive varietal aroma. The composition of a volatile fraction from Muscat grapes has been studied by several workers. A predominant role in "muscat" aroma has been ascribed to linalool[1–3] although several other terpene alcohols and hydrocarbons have also been reported.[1,2,4]

Although it is accepted that the intensity of grape aroma increases during ripening, the changes in composition of the volatile fraction have not been studied. At the onset of ripening in grapes, there is a rapid increase in reducing sugar concentration and a decline in organic acids, which events have been correlated with changes in relevant enzyme activities.[5,6] This paper describes changes in linalool and other volatiles from the fruit of Muscat grapes and relates them to changes in reducing sugar and titratable acidity.

RESULTS AND DISCUSSION

The gas chromatograms of the volatiles isolated at different stages of ripening in the 1969 season are shown in Figs. 1–5 and the corresponding changes in reducing sugar and titratable acid in Fig. 6A. Similar changes were also observed in the 1968 season. Linalool was first detected on 19 February 1969, about 2–4 weeks after the onset of the rapid increase in sugar concentration and decline in titratable acid. The concentration of linalool then

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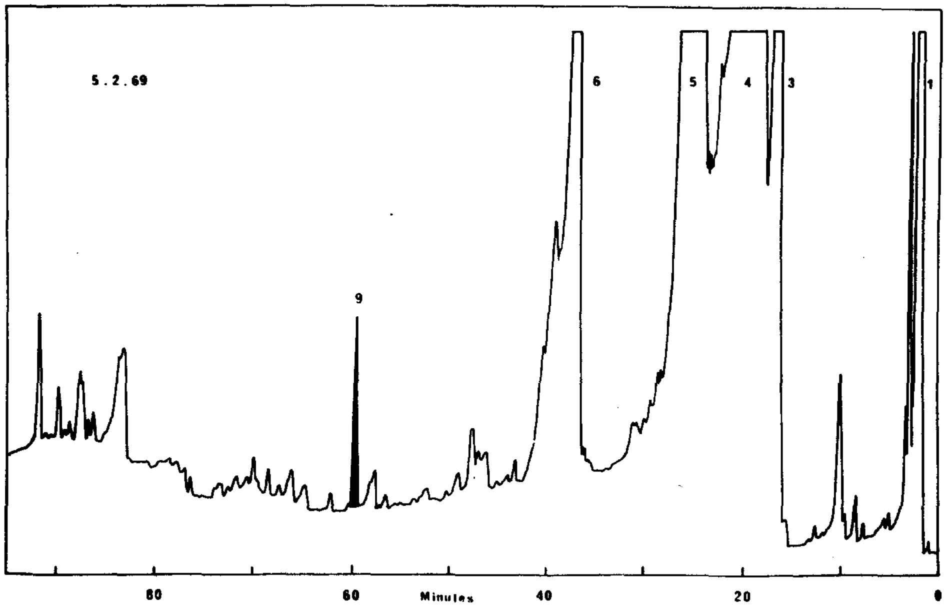
[3] K. W. O. WENZEL and M. J. DE VRIES, *S. African J. Agr. Sci.* **11**, 273 (1968).
[4] R. CORDONNIER, *Ann. Technol. Agr.* **5**, 75 (1956).
[5] J. S. HAWKER, *Phytochem.* **8**, 9 (1969).
[6] J. S. HAWKER, *Phytochem.* **8**, 19 (1969).


5.2.69
6
5
4
3
1
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80
60
Minutes
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20
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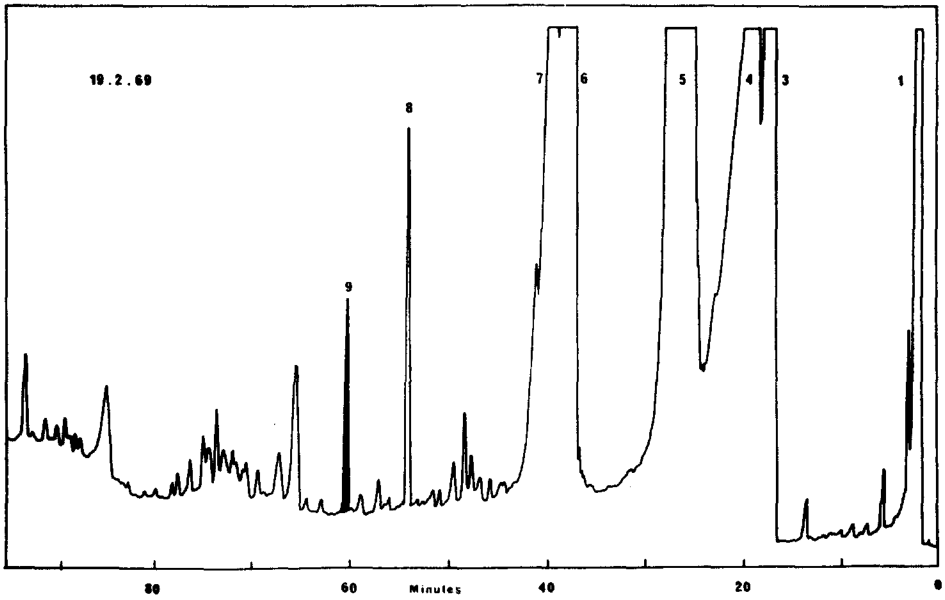
FIG. 1


19.2.69
7
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5
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3
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8
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80
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20
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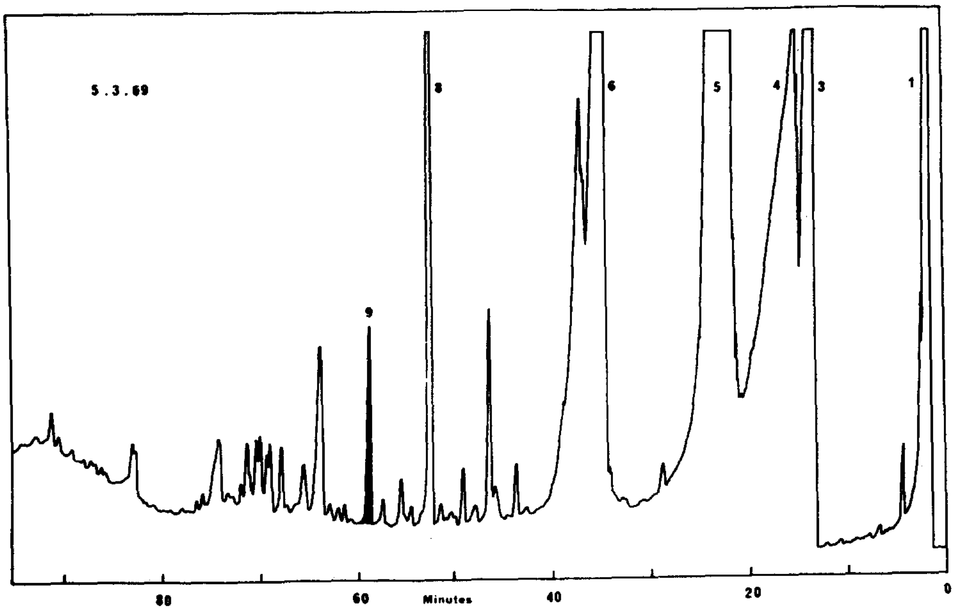
FIG. 2


5.3.69
1
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5
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80
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Minutes
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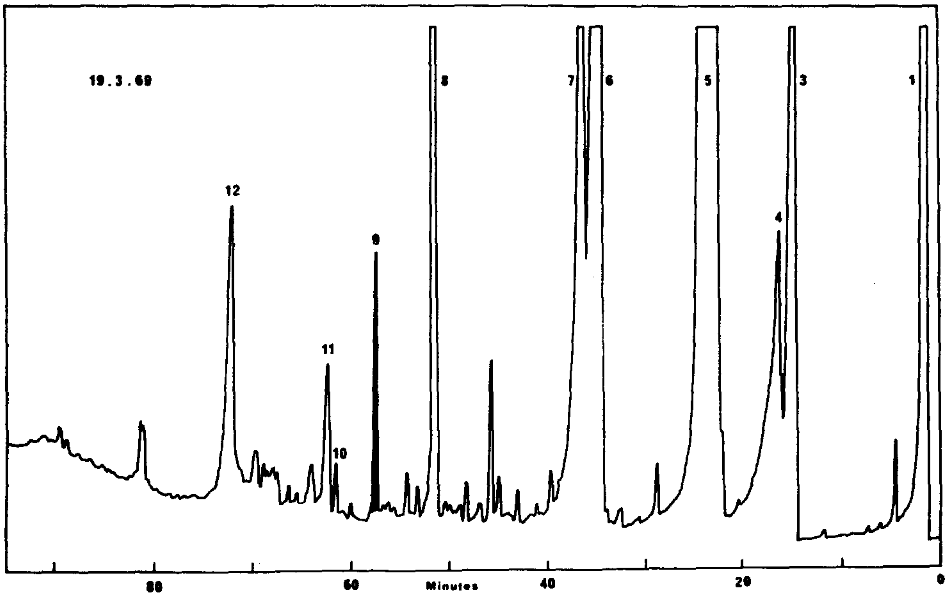
FIG. 3


19.3.69
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11
12
80
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Minutes
40
20
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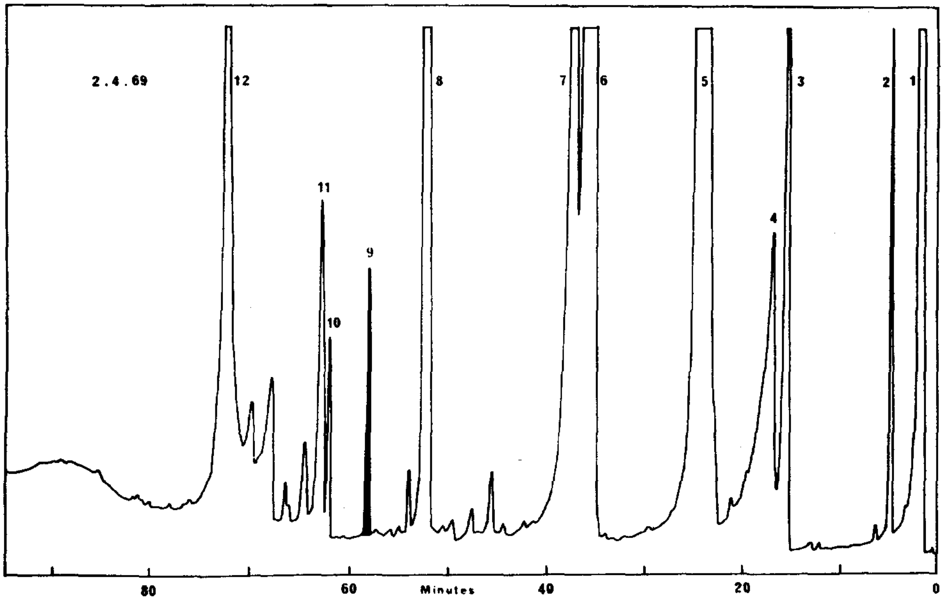
FIG. 4



FIGS. 1–5. GAS CHROMATOGRAMS OF VOLATILES IN EXTRACTS FROM MUSCAT GRAPES HARVESTED AT THE DATES INDICATED.

Peak 1: solvent; 2: ethyl acetate; 3: hexanal; 4: hexenal; 5: 2-hexenal; 6: hexenol + hexanol; 7: hexenol; 8: linalool; 9: diethyl succinate (internal standard); 10: α-terpineol; 11: unknown; 12: geraniol (tentative).

increased throughout all subsequent harvests (Fig. 6B). The changes in sugar and acid were most rapid at the beginning of ripening whereas the increases in linalool and other volatiles of possible significance in the aroma of the fruit were greatest near the end of the season. The peak height of linalool at the final harvest, assuming quantitative recovery, corresponds to a concentration of 0·3 mg/l. of grape juice, as compared with values of 0·12 to 0·23 mg/l. in wines of different vintages made from grapes of the same variety.[3]

Several other components appeared in the chromatograms obtained from the later harvests (Figs. 4 and 5), which were not observed at early harvests. Peak 12 (Figs. 4 and 5) has the same retention time as geraniol, which has previously been reported as a major volatile in Muscat grapes.[1,4] The samples of extract analysed by mass spectrometry were first stored at −10° for 8 weeks and then at room temperature for 3–4 days. The chromatogram obtained from the extract made at the final harvest, after storage under these conditions, is shown in Fig. 7. The peak corresponding by retention to geraniol and several other peaks shown in Fig. 5 had disappeared and several new components were observed. Table 1 gives the identities of some of these compounds, which included several monoterpene hydrocarbons, limonene and myrcene being predominant. Limonene and myrcene have been reported before in oils prepared from Muscat grapes.[1,4] The present results suggest that they are not present in the intact fruit but are formed during storage of the extracts, perhaps from the decomposition of geraniol. Linalool appeared to be relatively stable in storage. Several unidentified terpene alcohols or carbonyls (Fig. 7, peaks 15, 16, 24) and several components tentatively identified as cyclohexanone derivatives (peaks 26–29) which may derive from the C-6 aldehydes or alcohols present in the extracts, also appeared.

In all of the extracts, 2-hexenal and hexanal were major components and were probably formed during processing of the grapes.[7] They may, nevertheless, make important contributions to the aroma of grape juice as they have strong grassy odours. 2-Hexenal and hexanal reached a maximum 2–4 weeks after the onset of sugar accumulation and then declined (Fig. 6B), in contrast with linalool and other components which were absent from the unripe grapes and increased during ripening. The significance of these changes in 2-hexenal and hexanal is obscure, though they may reflect the changing levels of precursors such as linolenic and linoleic acids.[7] Another component (peak 4, Figs. 1–5) having a mass spectrum similar to that of 2-hexenal was also present in extracts from immature grapes and declined during ripening. Mass spectra obtained from peak 6 (Figs. 1–5) indicated the presence of both hexanol and hexenol. In early harvests, this component appeared from its mass spectrum to

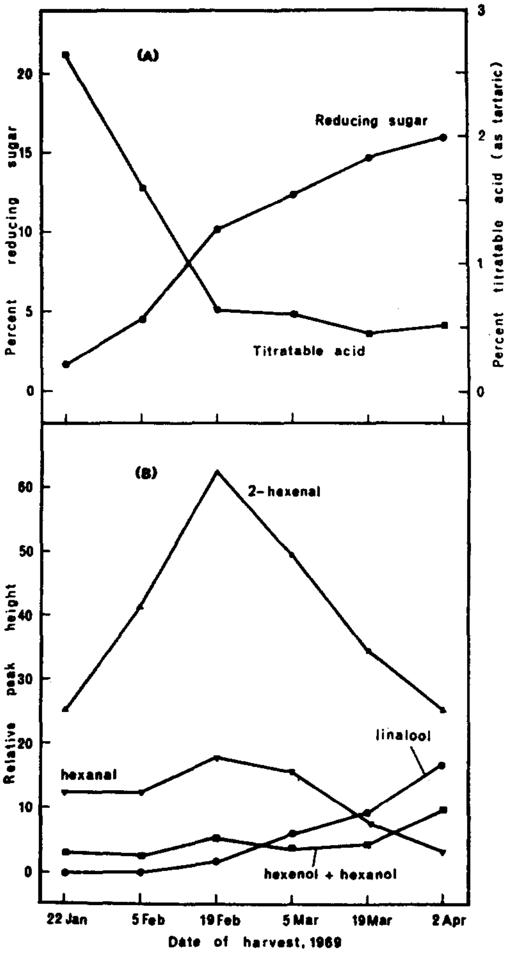


FIG. 6. CHANGES IN REDUCING SUGAR AND TITRATABLE ACID (A) AND IN THE MAJOR VOLATILE COMPONENTS (B) DURING RIPENING.

Relative peak height: peak height of compound/peak height of diethyl succinate.

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[7] F. DRAWERT, W. HEIMANN, R. EMBERGER and R. TRESSL, *Liebigs Ann. Chem.* 694, 200 (1966).

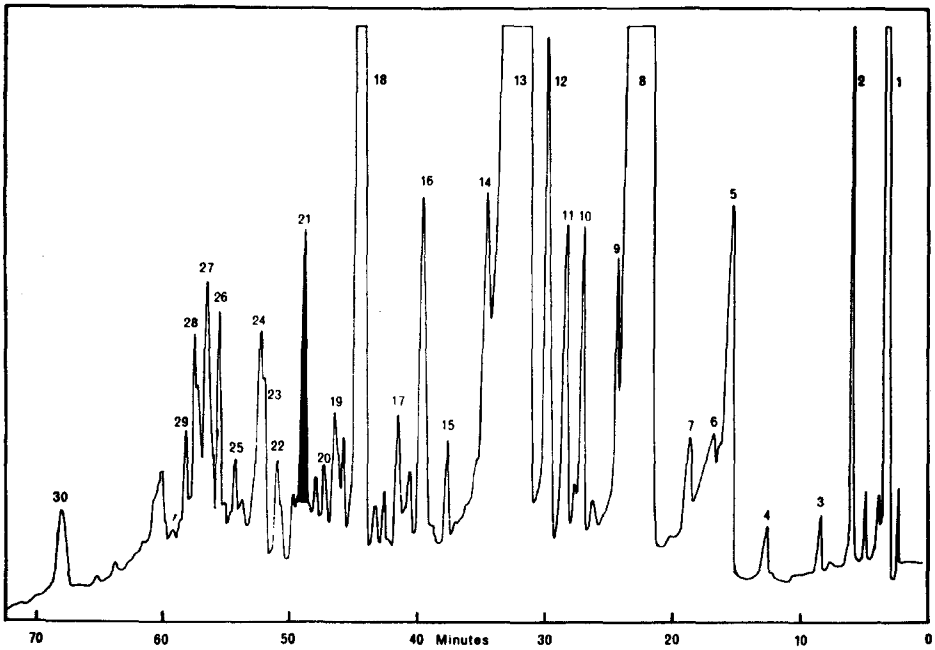

FIG. 7. CHROMATOGRAM OF AN EXTRACT FROM RIPE GRAPES (HARVESTED 2 APRIL) WHICH HAD BEEN STORED AT −10° FOR 8 weeks AND AT ROOM TEMPERATURE FOR 4 days.

For identities of the peaks see Table 1.

consists mainly of hexenol. The proportion of hexanol increased during ripening. Peak 7 (Figs. 1–5) also was tentatively identified as a hexenol. *Cis*- and *trans*-3-hexen-1-ol, *trans*-2-hexen-1-ol and hexanol are present in Muscat grapes.[1] The gas chromatogram of volatiles from immature grapes (Fig. 1) was similar to those of volatiles from leaves of Muscat grapevines.

This work shows that the development of Muscat aroma compounds is most rapid during the final stages of ripening and that measurements of sugar and acid content do not give an accurate indication of ripeness from an aroma point of view. It also illustrates the need to analyse grape volatiles immediately after isolation in order to avoid the changes in composition which occur during storage of the extracts.

EXPERIMENTAL

Extraction and Concentration of Volatile Components

Muscat of Alexandria grapes were harvested from a vineyard near Adelaide, South Australia, starting on 22 January 1969, when the berries were still hard, green, and sour. Fruit was collected at 2-weekly intervals until 2 April. On the day of harvest, the fruit was passed through a household juice extractor. The juice was distilled *in vacuo* using a rotary evaporator with a bath temperature of 35° and the condenser and receiver at 0–5°, until distillation ceased. Aqueous distillate (5·8 l.) was obtained from 7·2–7·8 l. of juice, depending on the sugar concentration of the latter. Volatile components were extracted from the distillates, starting immediately after distillation, using once-redistilled $CFCl_3$ in continuous liquid/liquid extractors for 24hr. This procedure gives near-quantitative recoveries of higher alkanols, alkanones and alkanoates from aqueous solutions.[8] An internal standard (diethyl succinate) was added to the $CFCl_3$ extracts (1.0 ml of a 0·01% solution of diethyl succinate in $CFCl_3$ equivalent to 5·8 l. of aqueous distillate). The extracts were concentrated to about 8 ml[8] and then stored at −10° in sealed glass ampoules.

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[8] P. J. HARDY, *J. Agr. Food Chem.* **17**, 659 (1969).

TABLE 1. IDENTITIES OF PEAKS RECORDED IN FIG. 7

Peak number	Compound identity or mass spectral data
1	Solvent
2	Ethyl acetate
3	Unknown, base peak 81, M = 96
4	Unknown, major peaks 41, 39, 67, 55, 27, 90, M = 118
5	Hexanal
6	Hexenal
7	Unknown, base peak 67, M = 118
8	2-Hexenal
9	Unknown, major peaks 68, 71, 139, 81, 121
10	Myrcene
11	Unknown, base peak 67, plus a monoterpene hydrocarbon
12	Limonene
13	Hexanol + hexenol
14	Monoterpene hydrocarbon, base peak 121
15	Terpene, M = 152
16	Monoterpene alcohol or carbonyl
17	Unknown, base peak 68
18	Linalool
19	Unknown
20	Monoterpene hydrocarbon, base peak 93
21	Diethyl succinate (internal standard)
22	Unknown
23	α-Terpineol
24	Monoterpene alcohol or carbonyl
25	Terpene
26 27	Major peaks 43, 98, 55, 41, 83, 69, M = 182 probably hexyl cyclohexanones
28 29	Major peaks 55, 41, 98, 91, 83, 69, M = 180 probably hexenyl cyclohexanones
30	Unknown, base peak 71

Analysis

At least two samples of each extract were analysed by gas chromatography immediately after the final harvest. They were concentrated in small pointed test-tubes until no further $CFCl_3$ could be removed and 1·5 μl. aliquots of the residue injected into a Perkin Elmer F-11 gas chromatograph equipped with a flame ionization detector. A support-coated open tubular column (50 ft × 0.02 in. i.d.) coated with Ucon Oil (Perkin Elmer: LB 550 X) was used with a helium flow rate of 5 ml/min. Temperature was increased from 32° at injection, to 165° at 1·5°/min.

Samples were also analysed using the same column in a combined gas chromatography–mass spectrometry apparatus,[9] with effluent split equally between the flame ionization detector and the mass spectrometer. In this case the temperature was programmed at 2°/min from 32° to 150°. Components were identified by comparison of the mass spectra with published data[10–12] and by retention time. A component was considered identified if both the mass spectrum and retention time were consistent. Reducing sugar and titratable acid were measured as described by Hawker.[5]

Acknowledgements—Help from Dr. E. H. Ramshaw, Mr. B. J. Michael and Mr. A. G. Thornton is gratefully acknowledged.

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