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Causal Effects of Aroma Compounds on Royal Gala Apple Flavours

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Abstract: Volatile flavour compounds produced by Royal Gala apple have been identified by GC-MS. Major components were 2-methylbutyl acetate, butyl acetate, hexyl acetate, butanol, 2-methylbutanol and hexanol. Odour-port evaluation of the components separated by GC indicated that the first four compounds were important contributors to the aroma and flavour. Use of analytical sensory panels revealed that 2-methylbutyl acetate, butanol and hexyl acetate had the greatest causal effect on those aroma and flavour attributes considered important for Royal Gala apples.

Key words: apple, sensory panel, flavour components, causal effects.

INTRODUCTION

The volatile flavour constituents of apple have been the studied for over 50 years and have been reviewed by Dimick and Hoskin (1983). Although over 200 volatile compounds have been reported to be in various cultivars of apple there is little information on the contribution these chemicals have on the sensory perception consumers have of apple flavour. Flath *et al* (1967) recognised the importance of sensory evaluation and the influence of varietal differences. The importance of cultivar was reinforced by an investigation on cider apples (Williams *et al* 1980). The flavour volatiles of some of the older commercial cultivars such as Delicious, Golden Delicious, Cox's Orange Pippin and McIntosh has been the subject of several studies (Flath *et al* 1967; Sapers *et al* 1977; Williams and Knee 1977; Dirinck *et al* 1983). The early studies clearly show that the characteristic apple aroma/flavour results from a complex mixture of alcohols, esters, aldehydes and ketones.

The Royal Gala apple (*Malus domestica* Borkh) is a natural sport of Gala apple which in turn is a cross of Kidds Orange and Golden Delicious cultivars. It is one of the major commercially grown cultivars in New Zealand and has a strong aroma when ripe. However consumer feedback has indicated that there is a loss of flavour after storage. Little work has been reported on the volatile flavour constituents of Royal Gala apples. The objective of this study has been to identify the volatile flavour compounds associated with this apple variety and to use an analytical sensory panel to determine the contribution that selected components make to important sensory attributes.

METHODS

Isolation of flavour volatiles

Royal Gala apples in good condition were purchased from the local supermarket. The apples (2·1 kg) were peeled, cored and sliced (1–3 mm thick) and placed into a 3 litre reaction vessel (Quickfit) fitted for vacuum steam distillation (Young *et al* 1983). No water was added and no stirring was done. The distillation was carried out over a period of 6 h at 1·7–1·9 kPa, yielding 135 ml of aqueous distillate which was then divided into 10 ml portions and kept frozen (−20°C) until required. The organic components were recovered from the aqueous distillate (10 ml) by passing it through a C_{18} solid phase extraction column made by combining the packing material from two 6 ml Supelclean ENVI-18 (Supelco Inc, USA) tubes into a single unit, then recovering the adsorbed material by elution with diethyl

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ether (purified by distillation from $LiAlH_4$) until *c* 500 μl of diethyl ether was collected. A further 300 μl of ether was passed through the column and collected separately. The residue water which was collected along with the first ether layer was removed with a syringe and extracted with the second ether fraction. The two ether fractions were combined and concentrated by slow static distillation (Blomberg and Roeraade 1988) to 100 μl for GC, GC-MS and gas chromatography-olfactometry.

Gas chromatography (GC)

GC was carried out using DBWAX or DB1 fused silica capillary columns (J and W Scientific, CA, USA), flame ionisation detection (210°C) and split/splitless injection (150°C). For analytical work the columns were 30 m × 0·32 mm id and the carrier gas was H_2 at 30 cm s^{-1}. The film thickness of the DBWAX and DB1 columns was 0·5 μm and 1 μm, respectively. The column oven temperature programme was 30°C, hold 6 min, 3°C min^{-1} to 190°C.

Gas chromatography-olfactometry (GCO)

GCO analysis (odour-port assessment) was carried out using the methodology based on the CHARM procedure described by Cunningham *et al* (1986). The GC column used was a 0·53 mm id DBWAX column (30 m) and carrier gas was H_2 at 30 cm s^{-1}. Detector (flame ionisation) and injector temperatures were 210 and 150°C, respectively. Column oven temperature programme was 30°C, hold 4 min, 5°C min^{-1} to 195°C. The outlet of the GC column was connected to an outlet splitter with 95% to the sniffing port and 5% to the detector. Make up gas (N_2) at 9 ml min^{-1} was added immediately before the splitter. The eluate was assessed by three assessors familiar with Royal Gala aroma. Each assessor assessed the sample for 'Royal Gala apple', 'red apple' and 'unknown' at 1 ×, 3 ×, 15 × and 75 × dilution in separate runs. Assessors indicated start and end of detection of a given peak without regard to intensity.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS was carried out on a VG-70SE spectrometer, operated in the electron impact mode at 70 eV, fitted with a directly coupled GC. Gas chromatographic conditions were similar to that for GC. The carrier gas was replaced with He.

Preparation and sensory evaluation of samples

A total of 256 samples were prepared for sensory testing by adding flavour components to 30 ml aliquots of a base solution. The base solution was prepared from an apple juice concentrate (obtained from ENZA Processors, Hastings, New Zealand) diluted to 12°Brix and passed down an Amberlite XAD-2 column to remove residual aroma material. Each of the four compounds (Table 1) was added to the base at one of the four predetermined levels, according to a full factorial design (based on four compounds and four levels of each chemical). Levels were chosen in a pilot study such that level 2 was the lowest level at which the compound could be reliably detected while level 4 was the limit at which it was considered to be slightly more intense than of a freshly cut Royal Gala apple.

Stock solutions of the volatile flavour compounds were prepared so that 50 μl of the solution when added to 30 ml of the base gave the final level required (Table 1). Each solution was prepared by dissolving the compound in ethanol (7·5 ml) and made up to 15 ml with reverse osmosis (RO) purified water.

Sixteen trained sensory panellists were asked to assess aroma (by sniffing) and flavour (by tasting) of four samples over four sessions for intensity of the following attributes: 'overall aroma', 'red apple aroma', 'sweet aroma', 'acid aroma', 'overall flavour', 'red apple flavour', 'characteristic apple flavour', 'sweet flavour' and 'acid flavour'. Intensities were recorded on 150 mm line scales with descriptive anchors at each end.

Statistical analysis

For each attribute an accumulated analysis of variance table was generated from a regression model. This

TABLE 1
Concentrations (mg per 30 ml) of volatile flavour compounds added to the base solution

	Level 1	*Level 2*	*Level 3*	*Level 4*
Butyl acetate	0	0·045	0·096	0·180
Hexyl acetate	0	0·021	0·048	0·066
S-2-Methylbutyl acetate	0	0·035	0·096	0·186
Butanol	0	0·333	0·651	0·987

included panellist effects and up to third order interactions. This was followed by a REML analysis with random panellist effects. F statistics from the accumulated analysis of variance table were used as a guide to which interactions to consider.

RESULTS

The compounds found in the volatile flavour distillate were identified by comparison of mass spectra with library spectra and are shown in Table 2. Not listed in Table 2 is hexanal which was only present in trace amount. Hexanal was masked on the tail of the intense butyl acetate peak and was only detected by MS using the single ion chromatogram technique. The identification of 1-methoxy-4-(2-propenyl)-benzene (estragole) with its spice-like or aniseed aroma (Williams *et al* 1977) is interesting since these descriptors have not been used in the sensory characterisation of Royal Gala. The configuration of the 2-methylbutyl acetate has been tentatively assigned the S form by analogy with results reported in the literature for the related 2-methylbutanoate esters found in Granny Smith apple (Mosandl *et al* 1991).

GCO analysis suggested four important contributors to the flavour of Royal Gala apples. These were 2-methylbutyl acetate, butyl acetate, hexyl acetate and butanol.

The main effects of the four selected components on the sensory attributes studied are shown in Fig 1. Graphs with solid lines indicate that at the levels used the compound has a statistically significant effect (Tukey's LSD, $P < 0{\cdot}05$) on that sensory attribute. Several first order interactions were also apparent (Figs 2–5).

TABLE 2
Compounds identified in the volatile flavour extract from Royal Gala apple[a]

Components	*RI*	*μg g*[b]	*ID*
Ethanol	946	1·51	MS, RI
Propyl acetate	979	0·32	MS, RI
2-Methylpropyl acetate	1017	0·07	MS
Propanol	1049	2·72	MS, RI
n-Butyl acetate	1077	13·06	MS, RI
2-Methylpropanol	1100	0·59	MS, RI
2-Methylbutyl acetate	1125	2·59	MS, RI
n-Butyl propanoate	1145	0·04	MS, RI
n-Butanol	1156	38·79	MS, RI
n-Pentyl acetate	1177	0·25	MS, RI
2-Methylbut-3-enyl acetate	1199	0·01	MS
2-Methylbutanol	1214	2·96	MS, RI
3-Methylbut-3-enyl acetate	1234	0·01	MS
3-Methylbut-3-enol	1251	0·01	MS, RI
3-Methylbut-2-enyl acetate	1254	0·01	MS, RI
n-Pentanol	1257	0·23	MS, RI
n-Hexyl acetate	1277	4·98	MS, RI
E-Hex-3-enyl acetate	1312	0·01	MS, RI
Z-Hex-3-enyl acetate	1320	0·01	MS, RI
Hex-4-enyl acetate	1329	0·29	MS
E-Hex-2-enyl acetate	1337	1·06	MS, RI
n-Hexanol	1360	5·82	MS, RI
Z-Hex-3-enol	1390	0·71	MS, RI
E-Hex-2-enol	1413	0·01	MS, RI
n-Hexyl-2-methylbutanoate	1430	0·01	MS, RI
n-Heptanol	1462	0·03	MS, RI
Camphor	1529	0·02	MS, RI
n-Octanol	1565	0·11	MS, RI
n-Oct-2-enol	1625	0·02	MS, RI
1-Methoxy-4-(2-propenyl)-benzene	1682	0·44	MS, RI

[a] Identification by Retention Index (RI) and/or Library Mass Spectra (MS).
[b] Semi-quantitatively data assuming 100% recovery.

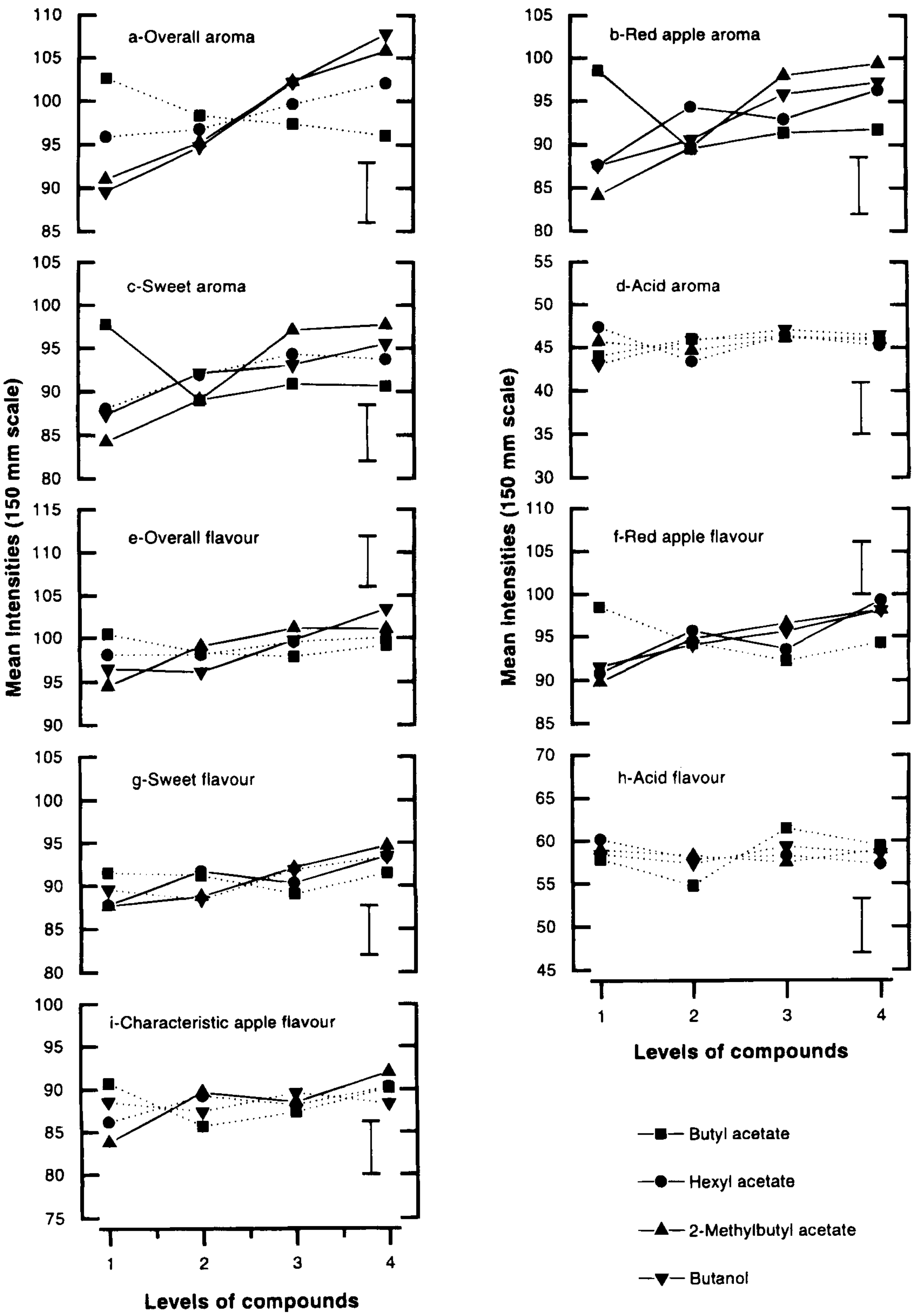

Fig 1. Mean intensities (measured on 150 mm line scales) for the sensory attributes at various levels of added compounds. Y-axis = mean intensities measured on a 150 mm scale, X-axis = levels of added compounds. For concentrations of the added chemicals refer to Table 1. Solid lines represent significant effects. All intensities are plotted on the same relative scale.

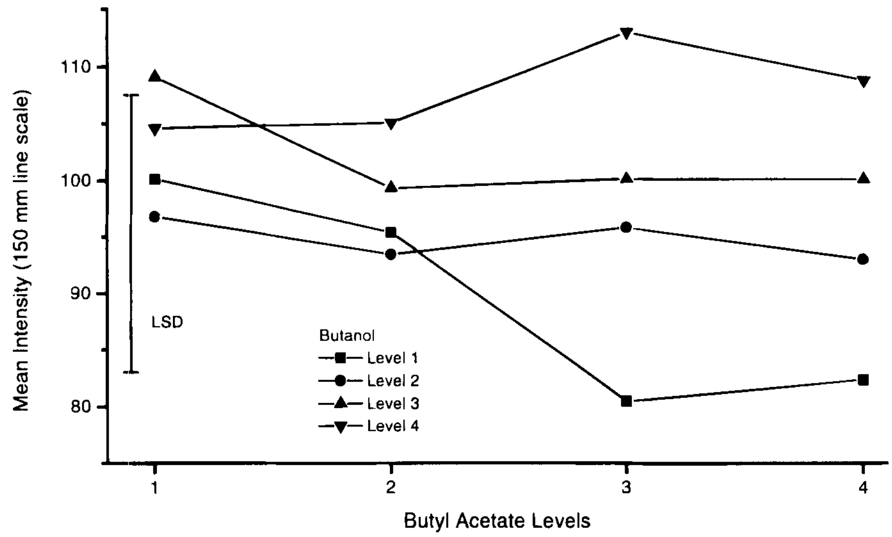

Fig 2. The effects of a butyl acetate/butanol interaction on 'overall aroma' LSD (Tukey, $P = 0{\cdot}05$) = 24·5.

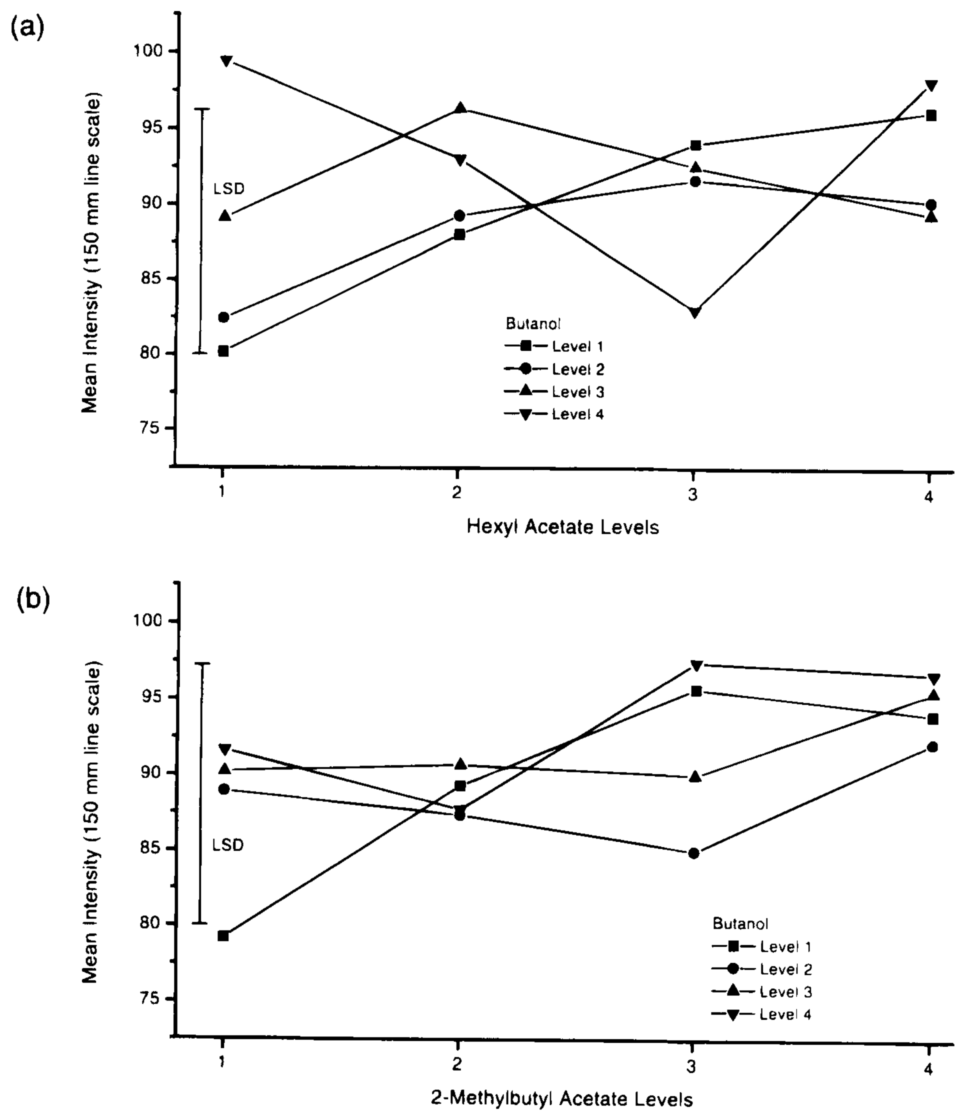

Fig 3. The effects of (a) a hexyl acetate/butanol interaction on 'sweet flavour', LSD (Tukey, $P = 0{\cdot}05$) = 16·2; and (b) a 2-methylbutyl acetate/butanol interaction on 'sweet flavour', LSD (Tukey, $P = 0{\cdot}05$) = 17·2.

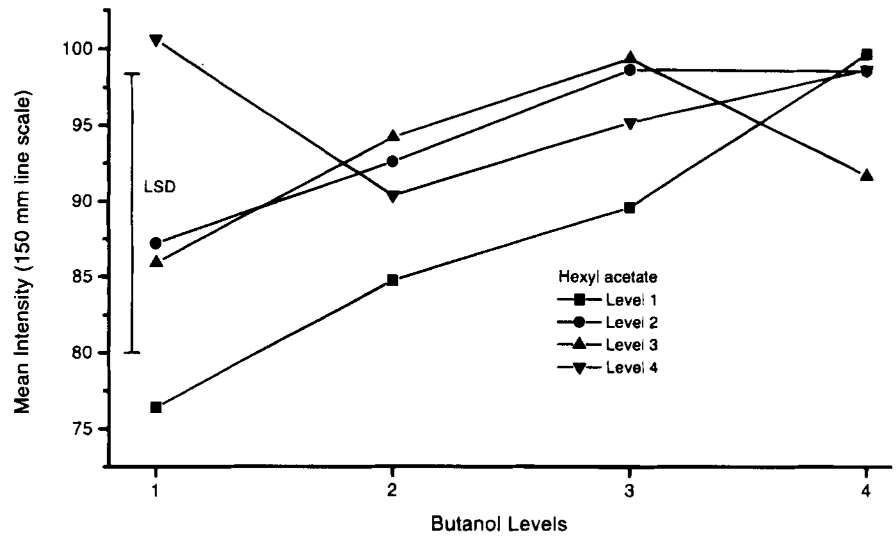

Fig 4. The effects of a hexyl acetate/butanol interaction on 'red apple aroma', LSD (Tukey, $P = 0{\cdot}05$) = 18·4.

'Overall aroma' and 'overall flavour' increased with increasing levels of 2-methylbutyl acetate and butanol (Fig 1a and 1e). In the absence of butanol and increasing levels of butyl acetate, 'overall aroma' showed a downward, although non-significant, trend (Fig 2). In contrast, when butanol was added butyl acetate had little effect on 'overall aroma'. A hexyl acetate/butanol interaction was detected but no obvious pattern was discernible (data not shown).

'Sweet aroma' increased with increasing levels of 2-methylbutyl acetate and butanol (Fig 1c) while 'sweet flavour' increased with hexyl acetate and 2-methylbutyl acetate (Fig 1g). The presence of butyl acetate decreased the intensity of 'sweet aroma'. A hexyl acetate/butanol and a 2-methylbutyl acetate/butanol interaction was found for 'sweet flavour'. With both interactions the effects of increasing acetate were greatest in the absence of butanol (Fig 3a and 3b).

'Acid aroma' and 'acid flavour' were not significantly affected by any of the added volatile chemicals. No interactions that affected 'sweet aroma' or 'acid flavour' were detected.

'Red apple aroma' and 'red apple flavour' increased with increasing levels of 2-methylbutyl acetate, butanol and hexyl acetate (Fig 1b and 1f). The addition of butyl acetate to level 2 decreased the intensity of 'red apple aroma', although further addition did not continue the trend. A hexyl acetate/butanol interaction was found for 'red apple aroma'. In the absence of butanol this attribute increased with increasing levels of hexyl acetate, whereas in the absence of hexyl acetate the attribute increased with increasing butanol level (Fig 4).

'Characteristic apple flavour' increased with 2-methylbutyl acetate levels while butyl acetate caused an initial, although not significant decrease (Fig 1i). There is evidence for a 2-methylbutyl acetate/butanol inter-

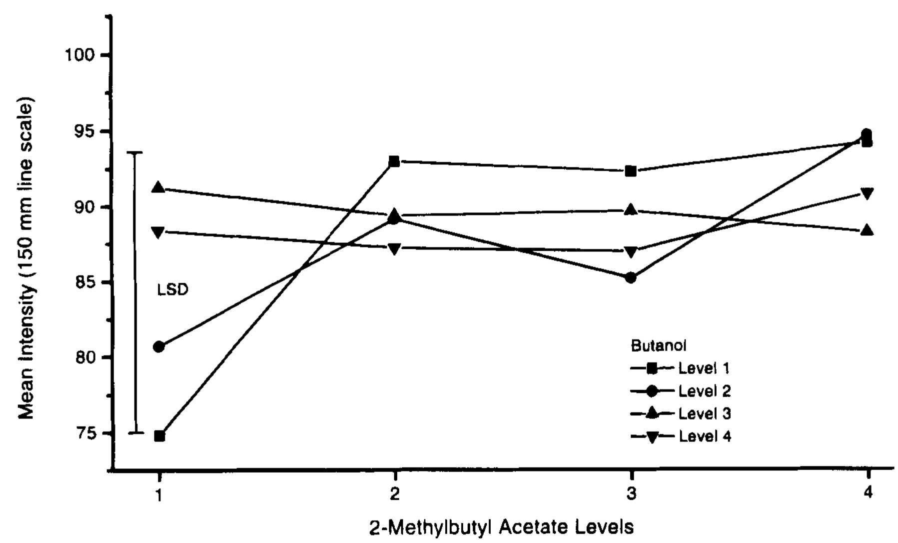

Fig 5. The effects of a 2-methylbutyl acetate/butanol) interaction on 'characteristic apple flavour', LSD (Tukey, $P = 0{\cdot}05$) = 18·6.

action (Fig 5). 2-Methylbutyl acetate had the greatest effect in the absence of butanol. With butanol present at the highest level, 2-methylbutyl acetate did not affect this attribute.

DISCUSSIONS

Chemical and GCO analyses

The major volatile flavour components found in Royal Gala apple have all been previously found in apple (Dimick and Hoskin 1983). Although butanol was the most abundant compound in the extract prepared by vacuum distillation, the relative amounts of the components can vary with different isolation methods (Parliament 1986). For example, we have noticed that butyl acetate was the most abundant compound found when headspace sampling was used (results not shown).

Quantitatively the important volatile components in Royal Gala flavour are butanol, hexanol and the acetates. GCO was used to carry out a preliminary determination of the more important contributors to Royal Gala aroma. During sensory profiling, terms that emerged for describing the positive sensory attributes of Royal Gala apple were 'red apple aroma', 'overall aroma', 'sweet aroma', 'acidic aroma, 'red apple flavour', 'acidic flavour', 'sweet flavour' and 'characteristic apple flavour'. 'Red apple' as a descriptive term describes a perception of apple aroma and flavour that differentiates from other apple flavour perceptions such as 'green apple' associated with Granny Smith and 'woody apple' associated with Red Delicious. Through discussions with the panel it was found that the 'red apple aroma' and 'red apple flavour' terms were strongly associated with Golden Delicious apple by panellists, and not with the more traditional red apple varieties such as Red Delicious. Indeed ethyl 2-methylbutanoate, which was shown by Flath *et al* (1967) to be an important contributor to the flavour of Red Delicious apple juice, was not detected in Royal Gala flavour volatiles. Although one of the descriptors included in the GCO analysis was 'red apple', the data did not reveal any obvious contributors to 'red apple aroma'.

Analytical sensory panel data

The levels of the added compounds approximate those found in fresh fruit. It is difficult to precisely match levels in the test solutions with those present during the actual consumption of fresh apple. The flavour volatiles appear to be produced over a period of time when tissues have been disrupted eg during the chewing process and as noted above the extracted amounts are sensitive to the method and duration of extraction.

As expected the added compounds caused the greatest changes with the aroma attributes. Of the four compounds selected for study, butanol and 2-methylbutyl acetate had the greatest effects. However similar effects were echoed in the corresponding flavour attributes, although to a smaller extent. Thus the increasing trend in 'overall aroma' caused by 2-methylbutyl acetate and butanol (*c* 15 units) (Fig 1a) is also found in 'overall flavour' (*c* 5 units) (Fig 1e). A similar trend was observed between 'red apple aroma' and 'red apple flavour' (Fig 1b and 1f).

Butyl acetate, which is quantitatively one of the major components found in the flavour volatiles of Royal Gala apple, did not increase the aroma or flavour attribute intensities with increasing levels. In fact its presence decreased 'red apple aroma' and 'sweet aroma' intensities. This compound is a reasonably powerful odorant (Flath *et al* 1967; Larson and Poll 1992) and was one of the compound targeted by GCO analysis as a possible contributor to the aroma. Butyl acetate was not considered to have apple-like quality when assessing Delicious apple essence (Flath *et al* 1967) and was not one of the components of a synthetic apple odour developed by Durr and Rothlin (1981). It is however considered as one of the important contributors to the flavour of Cox's Orange Pippin apple (Williams and Knee 1977). In a study by Knee and Hatfield (1976) the ratio of butanol to butyl acetate was *c* 0·08 and the ratio of 2- or 3-methylbutyl acetate to butyl acetate was *c* 0·02, in the equilibrium headspace over Cox's Orange Pippin apple discs. Although not directly comparable due to different sampling methods, the butanol : butyl acetate and butanol : 2-methylbutyl acetate ratios for Royal Gala apple were 3·0 and 0·20, respectively. Using the 10 ml total headspace and the average weight of an apple disc as 0·15 g (Knee and Hatfield 1976) the amount of butyl acetate in Cox's Orange Pippin would be 12·5 $\mu g\ g^{-1}$, compared with 13·1 $\mu g\ g^{-1}$ found for Royal Gala, indicating that the two cultivars may be specifically different in butanol and 2-methylbutyl acetate production. This information would suggest that the perceived aroma and flavour of Cox's Orange Pippin apple would be quite distinct from that of Royal Gala apple. Because of the butanol/butyl acetate interaction and butanol is one of the more abundant components of the flavour volatiles in Royal Gala apple, butyl acetate is not expected to contribute significantly to the sensory attributes.

Overall, increasing levels of hexyl acetate, 2-methylbutyl acetate and butanol resulted in increasing intensities of 'red apple aroma' and 'red apple flavour'. These two attributes have been considered by panellists as important sensory properties of Royal Gala apple. Hexyl acetate showed a downward trend between level 2 and level 3. Since it is observed in the aroma and flavour attributes it is unlikely that this is a spurious result. In the absence of butanol, 'red apple aroma' increased with increasing levels of hexyl acetate (75–101 units) while in the absence of hexyl acetate 'red apple aroma' increased with increasing levels of butanol. Because of this hexyl acetate/butanol interaction and as butanol and hexyl acetate are both found in the flavour volatiles of Royal Gala apple, 2-methylbutyl acetate is probably the most effective contributor to 'red apple aroma'. It is noteworthy that this compound is the only one which made a significant contribution to 'characteristic apple flavour'. The alkyl moiety of 2-methylbutyl acetate is branched while the other two acetates used in this study are *n*-alkyl esters. 2-Methylbutyl acetate is the major component of ripe Bisbee Delicious apple aroma and has been suggested as an indicator of physiological maturity (Mattheis *et al* 1991).

The increase in intensity of 'sweet aroma' and 'sweet flavour' with increasing levels of 2-methylbutyl acetate is consistent with the fact that aroma compounds can affect sweet flavour perception in fruit. Narain and Thomas (1990) report that ethyl butanoate affected the sweet flavour in peaches, ethyl acetate has been weakly correlated with sweetness in apple (Watada *et al* 1981), Binder and Flath (1989) found that methyl benzoate contributed to the sweet aroma of guava. Frank *et al* (1989) found that strawberry odour enhanced the sweetness of sucrose solutions. The enhancing of 'sweet aroma' and 'sweet flavour' by butanol is interesting as alcohols do not appear to have been previously reported as having causal effects on sweetness perception. Because of the hexyl acetate/butanol and 2-methylbutyl acetate/butanol interactions found for 'sweet flavour' the contribution of butanol to ' sweet flavour' intensity of Royal Gala apples is probably very small.

The lack of effects by the four compounds on 'acid aroma' and 'acid flavour' attributes is not totally unexpected since esters and alcohols have not been previously associated with acid flavour notes. It is also possible that 'acid flavour' is strongly influenced by the sugars and non-volatile acids.

CONCLUSIONS

The compound that had the most effect on the sensory attributes which are particularly important for Royal Gala apple is 2-methylbutyl acetate. This compound affected eight of the nine attributes. Butanol, the most abundant component found in the volatile flavour extract, also has a strong effect on the aroma and flavour attributes. The association of 2-methylbutyl acetate, hexyl acetate and butanol with the 'red apple' attributes confirm that 'red apple' as defined for Royal Gala is quite different from that of Delicious apple. The suppressive effect of butyl acetate on perceived intensities of the sensory attributes highlight the importance of extending odour-port results by use of thorough sensory testing.

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