Flavour volatile compound analysis in strawberry (Fragaria x ananassa Duch.) fruits: comparison of two mass spectrometer techniques for identifying volatile compounds

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Introduction

Analysis of volatile compounds in strawberry (Fragaria x ananassa Duch.) poses problems due to the high metabolic rate of the fruits and subsequent loss or altering of the profile of volatile components (Perkins-Veazie 1995). The fruits are highly perishable because they contain a high percentage (up to 98%) of water as fresh weight (Tucker 1993, Richardson and Kosikattrun 1995, Pomper and Breen 1997, Hancock 1999). The high respiration rate of strawberries associated with the thin cuticle of the fruits results in rapid turgor loss and subsequent alteration of the volatile profile of fruits (Hancock 1999). Our previous preliminary work has shown that freezing of strawberry fruits for later analysis is not possible because the volatile profile is altered. There is therefore a need for a reliable technique to perform volatile compound analysis quickly so as to avoid degradation of compounds during analysis.

Atmospheric Pressure Chemical Ionisation–Mass Spectrometry (APCI–MS) is ideal for flavour studies as it allows all the volatiles present in the samples to be measured simultaneously within a very short time (Brauss et al. 1998). The system, however, resolves compounds on the basis of mass only. It cannot differentiate between stereoisomers and positional isomers or compounds producing ions of the same mass. On the other hand, a Gas Chromatograph–Mass Spectrometer (GC–MS) can separate the ions and compare them with a Masslynx library to enable their identification. There was good agreement on the compound identity using the two techniques for nine out of fourteen selected volatile compounds. However the GC–MS gave a different identity to five of the selected compounds. The APCI–MS offers a rapid method of compound analysis while the GC–MS is slow but can differentiate between stereo-isomers.
colour chart of KG Fruits Ltd, UK was used to determine ideal harvesting stage) were harvested from the experimental plant material grown in a glasshouse. The experiment was carried out at the School of Biosciences, University of Nottingham, UK.

Three whole fruits were macerated with a pestle so as to disrupt the fruit cells in order to release volatiles as described previously (Baldwin et al. 2000, Forney et al. 2000), and a tenax trap was inserted in an air-tight 100ml bottle. A pressure pump was activated to blow the volatiles onto the tenax trap in the bottle for 10min. The flow rate was set at 40–50ml sec⁻¹. The GC–MS uses tenax to adsorb volatiles and sample injection is via re-concentration in a cooling trap. Mass spectrometry was then used to separate and identify the compounds.

There were fourteen major compounds for Selected Ion Recording (SIR). These were obtained by performing a full scan for major peaks of the volatile compound profile by APCI–MS. The compounds were acetic acid, heptanone, ethyl methyl butyrate, furanone, ethyl hexanoate, acetone, methyl acetate, ethyl acetate, 3-(hexenal) (Z), hexanal, acetalddehyde, methyl butyrate, ethyl butyrate, and ethyl methyl hexanoate.

The two instruments, the GC–MS (GC-5890 Series II, Hewlett Packard and MS-Fisons Instruments, MD 800) and the APCI–MS (Platform LC2, Micromass, Manchester, UK) were connected and used simultaneously to compare corresponding peaks. The ion mass was entered on the APCI in order to obtain the peaks each time a measurement was made, and the highest peak was selected, while the GC was used to separate the ions. Ions were integrated and compared with the Masslynx library (Masslynx v 3.2, Micromass Ltd, Manchester, UK). The library is able to identify the particular compound and a quality fit shows the correlation of the compound with the fit.

Results and Discussion

Examples of peak scans and identities of some compounds are shown (Figures 1–4).

For the fourteen major compounds for SIR, there was good agreement on the identity of nine compounds using the APCI–MS and the GC–MS techniques. The GC–MS technique gave a different identity to five of the selected volatiles (Table 1). These were acetic acid (Figures 3 and 4), heptanone, furanone, ethyl methyl butyrate and ethyl hexanoate. Compounds that were similarly identified by both instruments were acetaldehyde (Figures 1 and 2), acetone, methyl acetate, ethyl acetate, 3-(hexenal) (Z), hexanal, methyl butyrate, ethyl butyrate and ethyl methyl hexanoate (Table 1). Acetone is, however, not a natural volatile in strawberry fruits. It could result from expired air that we breathe or it may be an organic pollutant (Dirinck et al. 1977).

Brauss et al. (1998) determined that aldehydes (acetalddehyde, hexenal and hexanal) are the first volatile compounds to be formed in ripening tomatoes. They further intimated that 3-(hexenal) (Z) and hexanal were derived from linoleic and linolenic acids respectively. Both aldehydes can be reduced to hexanol and cis-hexanol by alcohol dehydrogenase. High levels of acetaldehyde may reduce flavour quality by conversion to ethanol, which may be further metabolised to ethyl acetate, also an indication of loss of flavour (Larsen and Watkins 1995).

Esters (ethyl and methyl) provide between 25% and 90% of the total volatiles of fresh ripe fruit depending on the cultivar. They are therefore reported to be the most predominant group of volatile compounds in strawberries (Dirinck et al. 1981, Forney et al. 2000). Volatile organic acids are precursors to the formation of esters. Esters provide the fruity and floral aroma in flavour of fruits.

Volatile compounds are usually analysed by Gas Liquid Chromatography, GC–MS and High Pressure Liquid Chromatography (Dirinck et al. 1977, Buttery et al. 1988, Baldwin et al. 2000, Forney et al. 2000). Each technique or combination of techniques yields a slightly different volatile profile, and analysis of volatile compounds is not rapid. This may result in degradation of compounds during analysis and subsequent alteration of the volatile profile of the fruits.

The APCI–MS is characterised by being a soft ionisation technique that has real time monitoring. It is highly sensitive, therefore can measure very low headspace concentrations of volatile compounds, and many samples can be measured in a relatively short time. The APCI–MS method has been

Table 1: Comparison of identities of compounds by APCI–MS and by GC–MS

| Mass  | APCI–MS identity       | GC–MS identity         |
|-------|------------------------|------------------------|
| 61.2  | Acetic acid            | Ethyl acetate          |
| 115.2 | Heptanone              | Methyl propyl acetate  |
| 131.2 | Ethyl methyl butyrate  | Methyl hexanoate       |
| 143.2 | Furanone               | Hexyl acetate          |
| 145   | Ethyl hexanoate        | Methyl propyl butyrate |
| 45.3  | Acetaldehyde           | Acetaldehyde           |
| 59.2  | Acetone                | Acetone                |
| 75.2  | Methyl acetate         | Methyl acetate         |
| 89.3  | Ethyl acetate          | Ethyl acetate          |
| 99.3  | 3-(Hexenal) (Z)        | 3-(Hexenal) (Z)        |
| 101.2 | Hexanal                | Hexanal                |
| 103.2 | Methyl butyrate        | Methyl butyrate        |
| 117.2 | Ethyl butyrate         | Ethyl butyrate         |
| 159.2 | Ethyl methyl hexanoate | Ethyl methyl hexanoate |

Figure 1: Largest peak recovered for compound with ion mass 45.1m/z. APCI–MS identified the compound as acetaldehyde.
used previously to determine the volatile profile of tomatoes (Brauss et al. 1998). However, its use in measuring the flavour volatile compounds in strawberries does not appear to have been evaluated in detail.

Although the APCI–MS technique does not differentiate isomers of aroma compounds, its ability to be used for rapid analyses of a large number of samples makes it ideal for the analysis of flavour compounds.

The results have shown that the APCI–MS technique requires validation of the volatile compounds by GC–MS so that a true identity of the compounds is established. The GC–MS technique thus gives a correct identity of the compounds since it is able to differentiate between isomers.

We conclude that once the identity of the volatile compounds of interest has been confirmed through the GC–MS technique, the APCI–MS method can then be used for rapid analysis of aroma flavour compounds.

References

*Baldwin AE, Scott JW, Shewmaker CK, Schuch W (2000) Flavor trivia and tomato aroma: Biochemistry and possible mechanisms for control of important aroma components. HortScience 35: 1013–1031*

*Brauss MS, Linforth RST, Taylor AJ (1998) Effect of variety, time of eating and fruit-to-fruit variation on volatile release during eating of tomato fruits (*Lycopersicon esculentum*). Journal of Agricultural Food Chemistry 46: 2287–2292*

*Buttery RG, Teranishi G, Ling LC (1988) Fresh tomato aroma volatiles: A quantitative study. Journal of Agricultural Food Chemistry 36: 1022–1025*

*Dirinck PJ, De Pooter HL, Willaert GA, Schamp NM (1981) Flavor quality of cultured strawberries: the role of sulphur compounds. Journal of Agricultural Food Chemistry 29: 316–321*

*Dirinck P, Schreyen L, Schamp N (1977) Aroma evaluation of tomatoes, apples and strawberries. Journal of Agricultural Food Chemistry 25: 759–762*

*Forney CF, Kalt W, Jordan MA (2000) The composition of strawberry aroma is influenced by cultivar, maturity and storage. HortScience 35: 1022–1025*
Hancock JF (1999) Strawberries. CABI Publishing, London, UK. ISBN 0–85199–339–7
Larsen M, Poll L (1992) Odour thresholds of some important aroma compounds in strawberries. Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung 195: 120–123
Larsen M, Watkins CB (1995) Firmness and concentration of acetaldehyde, ethyl acetate and ethanol in strawberries stored in controlled and modified atmospheres. Postharvest Biology and Technology 5: 39–50
Perkins-Veazie P (1995) Growth and ripening of strawberry fruit. Horticultural Review 17: 267–297
Pomper KW, Breen PJ (1997) Expansion and osmotic adjustment of strawberry fruit during water stress. Journal of American Society for Horticultural Science 122: 183–189
Richardson DG, Kosikattrun M (1995) Off-flavour development of apples, pears, berries and plums under anaerobiosis and partial reversal in air. In: Rouseff RL, Leahy MM (eds) Fruit Flavors. American Chemistry Society, Washington DC, pp 211–222. ISBN 0–8412–3227–X
Tucker GA (1993) Fruit ripening. In: Seymour G, Taylor J, Tucker G (eds) Biochemistry of Fruit Ripening. Chapman and Hall, London, pp 1–20. ISBN 0–412–40830–9