Kasetsart J. (Nat. Sci.) 37 : 468 - 476 (2003)

Real Time Monitoring and Analysis of Flavour Volatile Release from Cucumber and Tomato using Dynamic Vapor Sorption Atmospheric Pressure Chemical Ionization Mass Spectrometry

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ABSTRACT

A real time monitoring of main volatile compounds released during cucumber and tomato fruit tissue disruption was measured by atmospheric pressure chemical ionization-mass spectrometry (APCI-MS). This technique showed promise for simple, rapid, and artifact-free method for monitoring the rapid volatile release. The key volatiles of fresh cucumber were mainly attributed to C-6 aldehydes (hexanal and *(E)*-2-hexenal) and C-9 aldehydes ((*(E)*-2,*(Z)*-6-nonadienal and *(E)*-2-nonenal) whereas only C-6 aldehydes were responsible for the predominant compounds of fresh tomato volatiles. Both C-6 and C-9 aldehydes were enzymatically produced through the fatty acid oxidation pathway during plant tissue disruption. The combination gas chromatograph (GC) with simultaneous electron impact (EI) and APCI-MS was also used to confirm the identification of these volatile compounds in fresh cucumber and tomato fruits.

Key words: cucumber, tomato, flavour volatile, APCI-MS

INTRODUCTION

The volatile composition in the ripening tomato fruit has been studied extensively (Kazeniac and Hall, 1970; Buttery *et al.*, 1971; Buttery *et al.*, 1987; Buttery *et al.*, 1988). Over 400 compounds have been identified as volatile components of fresh tomatoes and tomato products (Petro-Turza, 1987). Only a small number of these compounds have been studied and to be responsible for the main characteristic of fresh tomato aroma. Several reports have indicated that the following compounds play major volatiles in fresh tomato such as hexanal, *(Z)*-3-hexenal, *(E)*-2-hexenal, 1-penten-3-one, 6-methyl-5-hepten-2-one, β-ionone, ethanol, methanol, *(Z)*-3-hexenol, 2-and 3-methylbutanal, 2-isobuthylthiazole (Buttery *et al.*, 1987; Baldwin *et al.*, 1991). Some of these compounds are formed by deamination and decarboxylation of amino acids and carotenoid during fruit ripening (3-methylbutanal and 3-methylbutanol) (Yu *et al.*, 1968). Others are produced by lipid oxidation of unsaturated fatty acids when the tissue is disrupted such as hexanal and hexenal (Galliard *et al.*, 1977). Isomerization by isomerase activity can convert *(Z)*-3-enals to *(E)*-2-enals forms. All aldehydes can potentially be converted to the corresponding alcohols by alcohol dehydrogenase (ADH). All the C-6 aldehydes have been contributed in the "green' or "fresh" note to the flavor of tomato (Kazeniac and Hall, 1970).

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Received date : 13/10/03 Accepted date : 1/12/03

Cucumber contributes acyl-hydrolase and lipoxygenase, the latter has highly specific to the formation of the 9-hydroperoxide of linoleic acid as does the tomato lipoxygenase. The flavor of fresh cucumbers has been mainly attributed to *(E)*-2,*(Z)*-6-nonadienal and *(E)*-2-nonenal (Forss *et al.*, 1962; Kemp *et al.*, 1974; Scieberle *et al.*, 1990). Cucumber flavor volatiles are formed by enzymatic reactions. The involvement of fatty acid hydroperoxides that appears when tissues are disrupted (Fleming *et al.*, 1968; Hatanaka *et al.*, 1975). The major volatile products are *(Z)*-3-nonenal and hexanal which are derived from 9- and 13-hydroperoxides of linoleic acid respectively, whereas *(Z)*-3, *(Z)*-6-nonadienal and *(Z)*-3-hexenal are formed from 9- and 13-hydroperoxides of linolenic acid, respectively (Phillips and Galliard, 1978).

The pathway of C-6 and C-9 aldehydes formation in cucumber was found to be similar to that in tomato. An important characteristic between volatile formation in cucumber and tomato is that in the former, both the 9- and 13-hydroperoxide isomers of both linoleic acid and linolenic acid were cleaved to yield the C-9 and C-6 aldehydes, respectively. In tomato, lipoxygenase favors the formation of the 9-hydroperoxides isomer with the 9- to 13-hydroperoxides ratio of 24:1 (Galliard and Matthew, 1977; Regdel *et al.*, 1994). The hydroperoxide lyase was found to act specifically on 13-hydroperoxides, giving rise to the C-6 aldehydes alone. The volatile cleavage product is hexanal when 13-hydroperoxides-linoleic acid is the substrate, whereas *(Z)*-3-hexenal is produced on cleavage of 13-hydroperoxide of linolenic acid (Galliard *et al.*, 1977). The fate of the 9-hydroperoxide is not clear.

The analyses of cucumber or tomato volatiles have been used the solvent extraction followed by GC-MS for identification and quantification. Aroma extraction dilution analysis (AEDA) was also used to evaluate the potent odorants of volatile components in cucumbers (Scieberle *et al.*, 1990). In order to monitor and investigate the volatile release from cucumber and tomato, a rapid method is required to measure the volatile formed. However, the times of extraction and analysis are relatively long and limit the number of samples analysis. Therefore, it is difficult to monitor the dynamic volatile release in samples in a short time analysis. APCI-MS has considerable potential for monitoring of rapid volatiles production through the lipid oxidation pathway and it is possible to achieve the study on the generation of volatile compounds both cucumber and tomato. The objective of this study was to monitor in real time and determine the flavour volatile release from cucumber and tomato homogenates to the headspace using APCI-MS.

MATERIALS AND METHODS

Source of plant materials

Cucumbers (*Cucumis sativus*) were purchased from local supermaket in Loughborough, UK. They were washed with tab water prior to use. Cross-sectional slices taken from at least 3 cm from the ends of cucumber were provided for the analysis.

Tomato fruits (*Lycopersicon esculentum* Mill. cv. Ailsa Craig) were grown in the glasshouse at Sutton Bonington campus, during the winter season (October 2001 – May 2002), following the university handbook procedure (BBSRC glasshouse user guide; the University of Nottingham, UK). Tomato fruits were picked at vine–ripe for volatile analysis.

APCI-MS

A Micromass Platform II quadrupole mass spectrometer *(Micromass, Manchester, UK)* operating in the gas phase using a positive ion, selective ion mode was fitted with a specifically designed air-sampling interface (Linforth and Taylor, 1998). Five key volatile compounds in cucumber homogenates and ten different volatile

compounds in tomato homogenates were monitored. For all volatile compounds, the corona pin voltage used was 4 kV and dwell time was 0.5 sec. The cone voltages (cv) for each ion mass (m/z) was adjusted to give a maximum sensitivity of $[M+H]^+$. All data were collected and acquired by MassLynx software.

GC-EI/APCI-MS

The analysis was conducted using GC with simultaneous EI-MS and APCI-MS detection (Figure 1). A sample of cucumber or tomato was blended in a commercial blender *(Phillips, HR-2914)* for 1 min. A hundred grams of homogenates was placed into a 250 ml glass bottle *(Schott)*. All volatile compounds released from the homogenates were collected onto a Tenax trap *(10.5 cm × 0.3 cm i.d.; SGE, Milton Keynes, UK)* by purging nitrogen gas for 20 min at flow rate of 30 ml/min. The Tenax trap was thermally desorbed at 240°C for 10 min in the GC injector *(Unijector SGE, Milton Keynes, UK)* by purging helium gas at column head pressure 18 psi as carrier gas. The compounds were cryofocused onto a 400 mm region of BP-1 column (*25 m × 0.22 mm i.d., 1*m*m film thickness; SGE*) with liquid nitrogen and were then chromatographed *(Hewlett Packard, HP5890 Series II gas chromatograph)* after holding for 1.50 min. After desorption, the column was held at 35°C for 2 min, then temperature programmed from 35°C to 106°C at 4°C/min, subsequently at 15°C/min to 145°C and held at 145°C for 8 min. The GC column was split using a Y-piece *(SGE)* and conducted to the APCI-MS and the EI-MS sources with a deactivated fused silica tube *(0.53 mm i.d., SGE)*. The APCI-MS was operated in a positive ion full scan mode for the mass range of 40-200 with a scan time 0.5 sec and inter-scan delay time 0.02 sec. The EI-MS was operated in the full scan mode at an ionization voltage of 70eV with a scan range of m/z 35-190 and a scan time 0.4 sec. Compounds were identified by linear retention indices (LRI) where authentic standards were available or mass spectral matching with NIST library.

RESULTS AND DISCUSSION

The volatile release profiles by APCI-MS

The release profile of main volatiles from cucumber and tomato homogenates are shown in Figure 2 and Figure 3, respectively. Cucumber was blended at *t=0 min* and the release of its volatiles were monitored for 3 min after maceration. Hexanal was released first with the highest intensity in the headspace during blending followed by

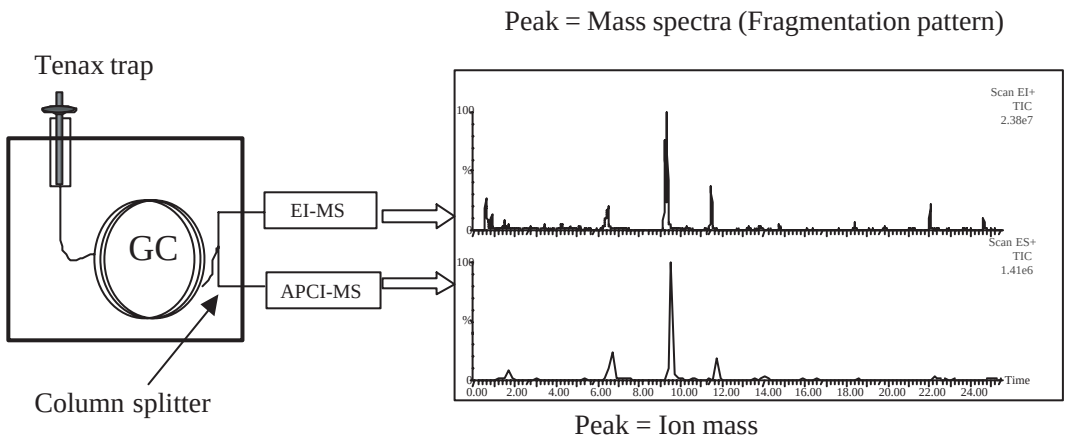

Figure 1 Schematic of the GC with simultaneous EI-MS and APCI-MS detection.

nonadienal and nonenal, respectively. The shape of each volatile release was similar, raising up to the maximum intensity then rapidly dropped to the end of the time after maceration. Figure 3 shows a typical release profile of ten volatiles of tomato homogenates above the headspace during a 3 min maceration. Some compounds were released rapidly such as methylbutanal, methybutanol and 6-methyl-5-hepten-2-one, suggesting these compounds were preformed in the intact tomato fruits during ripening (Yu *et al.*, 1968; Buttery and Ling, 1993). It has been suggested that these compounds were synthesized by deamination and decarboxylation of amino acids during fruit ripening. While, some compounds (hexanal, hexenal and hexanol) showed a slower release due to the fact that they were generated only after tissue disruption by the lipid oxidation pathway (Buttery and Ling, 1993). No C-9 aldehydes (nonenal and nonadienal) were detected in tomato due to the activity of some specific enzymes on the cleavage of hydroperoxide fatty acid were not favored. This result confirmed the work of Galliard and co-workers (1977) who found that only C-6 aldehydes were formed by the enzymatic degradation of acyl lipid in disrupted tomato fruits.

Identification and confirmation of ions monitored by GC-EI/APCI/MS

Volatile compounds monitored in cucumber and tomato homogenates sample were identified and confirmed by the combination of GC-EI/APCI-MS. The chromatograms from both GC-EI and APCI-MS were very similar qualitatively, indicating same sensitivity to compounds between the two systems. Mass spectra from the EI-MS were represented to identify of each peak so that the ion mass of corresponding peak by the APCI-MS could be match to that compound.

The chromatogram of cucumber homogenates volatiles is shown in Figure 4. Only five key volatile compounds were detected by GC-EI and APCI-MS. C-6 and C-9 aldehydes generated by the lipid oxidation pathway were the predominant volatile compounds in cucumber (Phillips and Galliard, 1978). Generally, the APCI ion mass is equal to the molecular weight (M) plus one, due to this technique produces the protonated molecular ion $[M+H]^+$ for almost volatile compounds. However, some alcohols can be

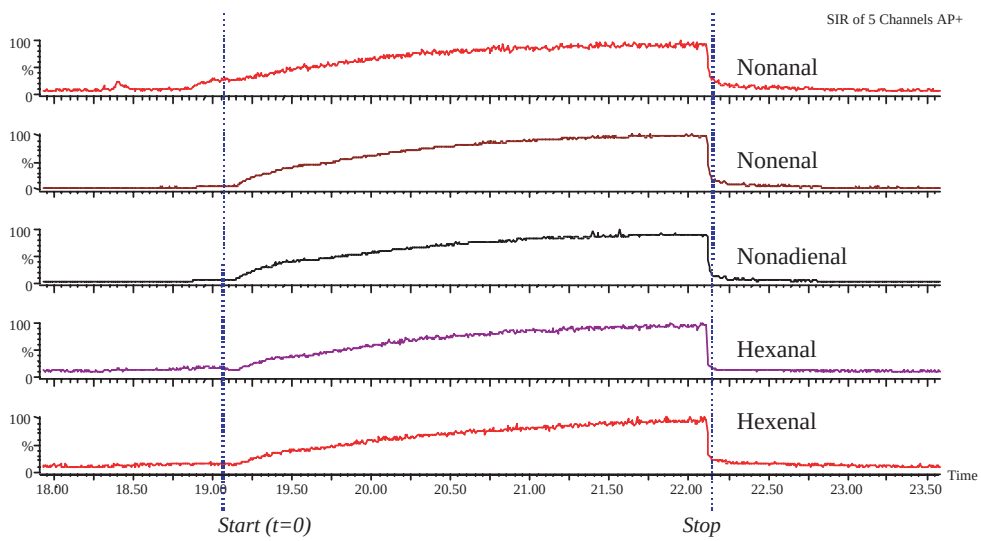

Figure 2 The volatile release profile of cucumber homogenates after maceration (*t = 0 min*); intensities expressed on a relative scale.

dehydrated, resulting a $[M\text{-}H_2O+H]^+$ (Taylor *et al.*, 2000). The major ion mass obtained from the full scan data of APCI-MS was a single compound. The ion mass of 139 and 141 was represented of *(E)*-2, *(Z)*-6-nonadienal and *(E)*-2-nonenal, respectively, corresponding the identification by matching with library mass spectra by EI-MS (Table 2).

Thirteen volatile compounds from tomato were obtained by comparing their mass spectra from the EI-detector with mass spectra and linear retention indices of authentic compounds and mass spectra held in library database (Figure 5 and Table 2).

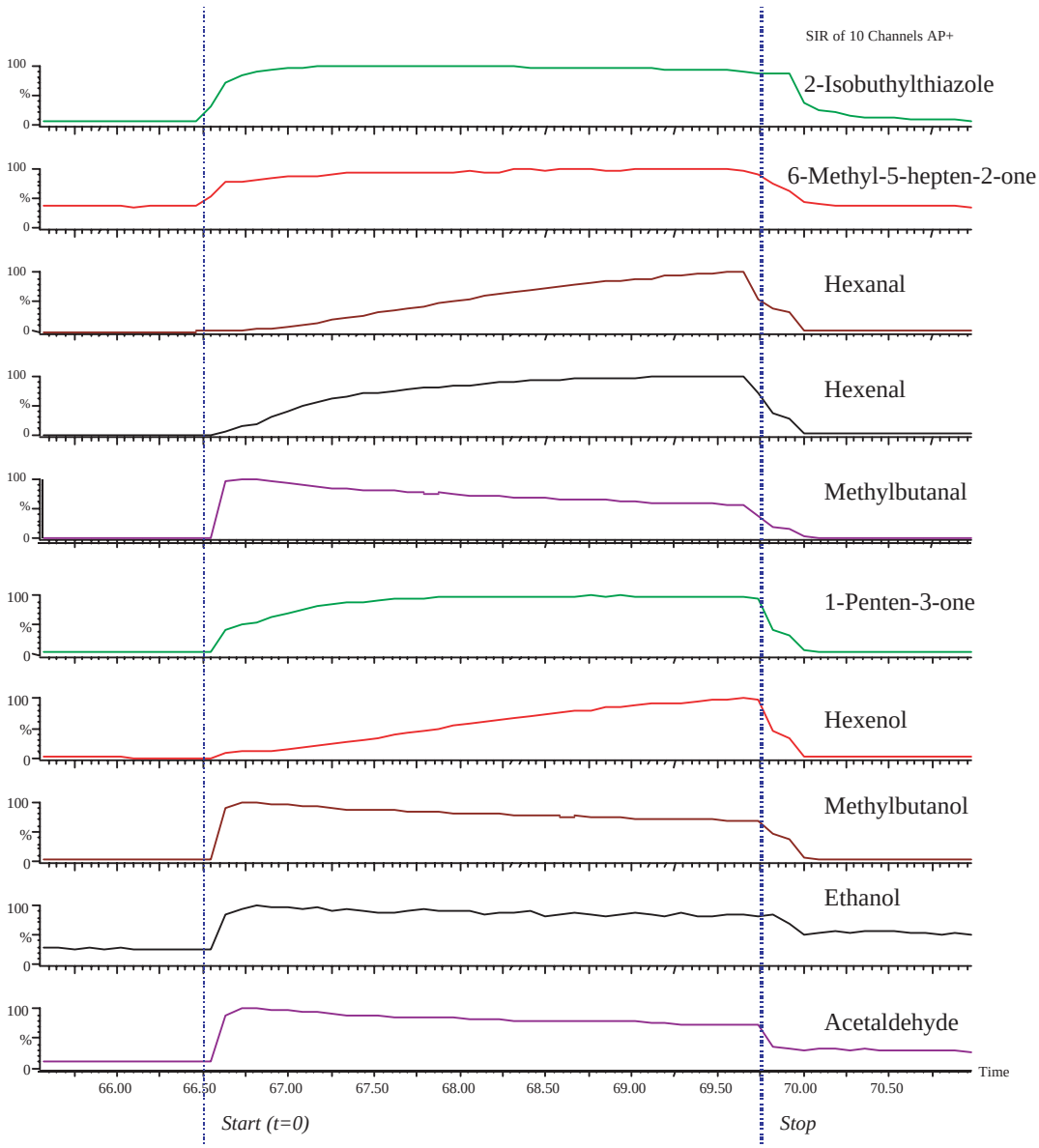

Figure 3 The volatile release profile of tomato homogenates after maceration (*t = 0 min*); intensities expressed on a relative scale.

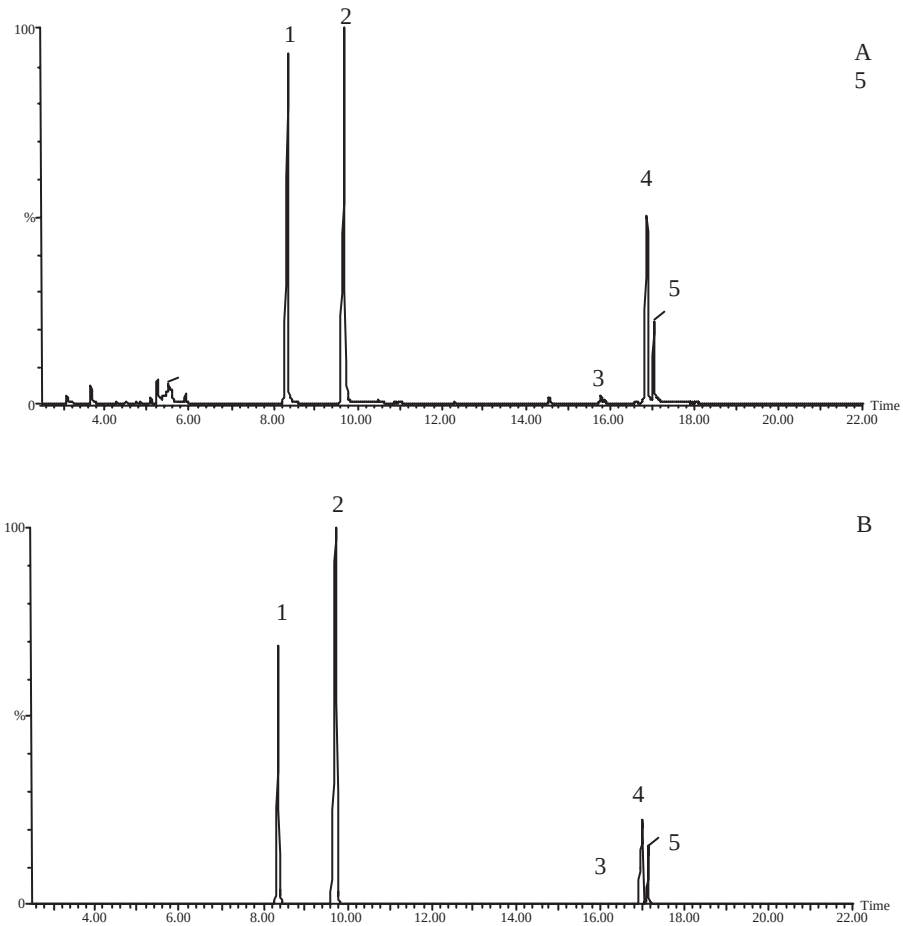

Figure 4 Chromatogram of volatile compounds from cucumber homogenates by GC-EI (A) and APCI-MS (B). Peak number 1-5 were identified in Table 1.

Table 1 Identification GC/APCI-EI/MS peaks of cucumber volatile compounds.

Peak no.	RT	Identification compound	MW	APCI-MS mass	Confirmation [a]
1	8.36	Hexanal	100	101	*
2	9.72	*(E)*-2-Hexenal	98	99	*
3	15.78	Nonanal	142	143	*
4	17.02	*(E)*-2,*(Z)*-6-Nonadienal	138	139	*
5	17.16	*(E)*-2-Nonenal	140	141	*

[a] Symbols are as follows: *-confirmation by library mass spectra and mass spectra of authentic standard.

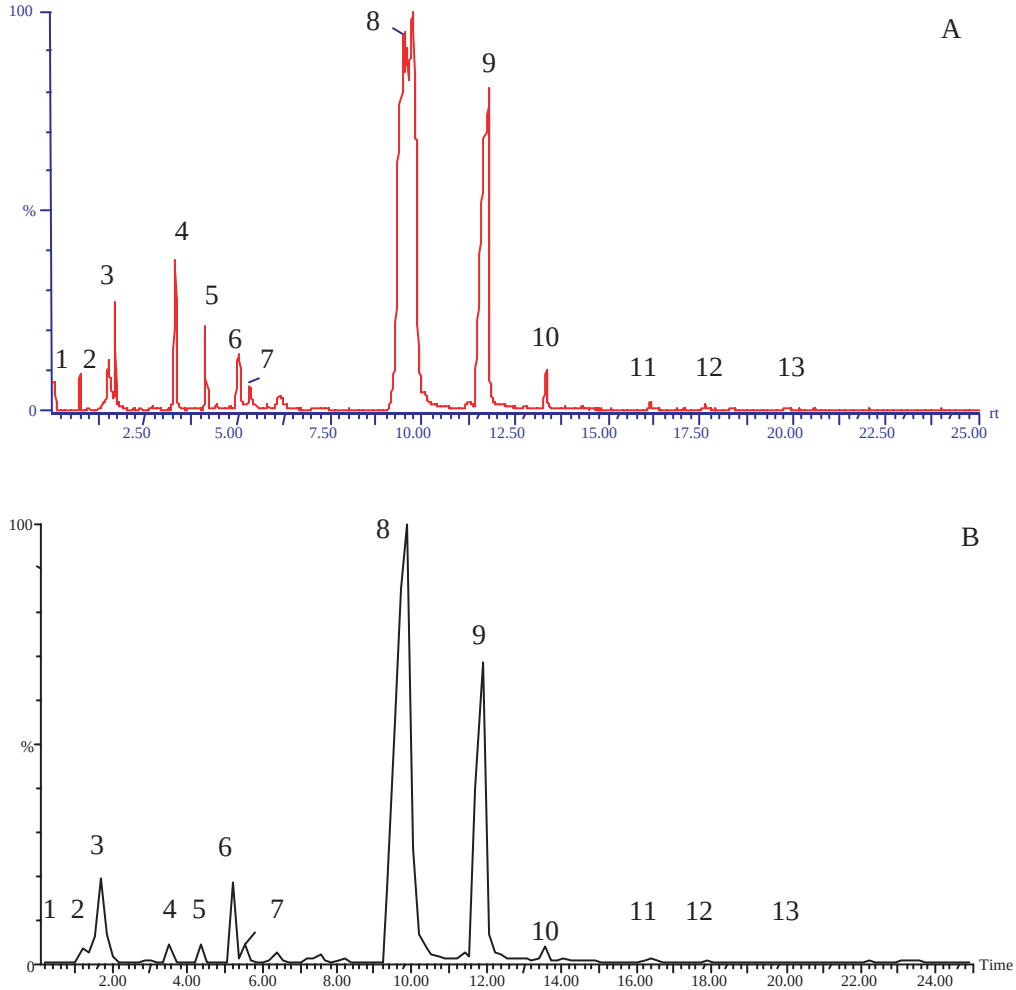

Figure 5 Chromatogram of volatile compounds from tomato homogenates by GC-EI (A) and APCI-MS (B). Peak number 1-13 were identified in Table 2.

CONCLUSION

The rapidly flavour volatiles released from cumcumber and tomato homogenates upon disruption were monitored simultaneously by APCI-MS. This technique was a real time and rapid method. The combination GC-EI and APCI-MS were accomplished to confirm the identified compounds in cucumber and tomato homogenates volatiles between the two methods. These techniques have considerable potential for monitoring in real time analysis of rapid volatile change during plant tissue disruption. The possibility to apply such techniques for the volatile analysis in other fruits and vegetables should be considered.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Royal Thai Government and the graduate school, Chiang Mai university for the financial support. We thank to Dr. Fabienne Boukobza for her valuable suggestions.

Table 2 Identification GC/APCI-EI/MS peaks of tomato volatile compounds.

Peak no.	RT	Identification compound	LRI	MW	APCI-MS mass	Confirmation[a]
1	0.96	Acetaldehyde	-	44	45	*
2	1.52	Acetone	-	58	59	*
3	1.70	Pentane	-	72	73	*
4	3.33	Ethyl acetate	602	88	89	**
5	4.15	3-Methylbutanal	633	86	87	**
6	5.04	1-Penten-3-one	667	84	85	**
7	6.73	3-Methylbutanol	722	88	71	**
8	9.88	Hexanal	803	100	101	**
9	11.23	*(E)*-2-Hexenal	834	98	99	**
10	12.14	Hexenol	855	100	83	**
11	16.12	*(Z)*-2-Heptenal	945	112	113	**
12	17.61	6-Methyl-5-hepten-2-one	978	126	127	**
13	19.80	2-Isobutylthiazole	1036	141	142	**

[a] Symbols are as follows: *-confirmation by library mass spectra, ** - confirmation by mass spectra and LRI of authentic standard.

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