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Application of Simultaneous Purging and Solvent Extraction Technique for Flavour Monitoring of Natural Products

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ABSTRACT

A newly developed simultaneous purging and solvent-extraction apparatus was used to investigate variations of volatiles formed from natural bananas during ripening periods. Five alkylacetates and five alkylbutyrates, which give characteristic flavours to banana, were chosen for flavour monitoring during banana ripening. Their formation increased during the ripening period. After 10 days, ester formation reached a maximum at the onset of the fruit's senescence period. Three alkyl alcohols increased steadily in concentration for 5 days and plateaued until the late senescence stages. Fresh celery volatiles were also collected using the same apparatus in order to investigate its possible application for collecting headspace volatiles of fresh vegetables. The compounds isolated and identified in the headspace sample from celery were 14 terpenes and two aromatic compounds.

INTRODUCTION

Analysis of volatile components is the major task in order to understand the nature of flavour in natural or processed foods. Consequently, numerous analytical studies have been done on various foods in the last three decades. Among many techniques developed for flavour analysis, headspace analysis is an ideal technique for the study of natural flavours because its

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constituents are those which one actually smells from the foods. Among the disadvantages of the direct headspace sampling method is that the amount of sample obtained is relatively small, and often some concentration procedure is required (Clark & Cronin, 1975; Murray, 1977). The most commonly used concentration method for headspace samples is the application of adsorbents such as Tenax (Buckholz *et al.*, 1980) and Porapak Q (Jennings *et al.*, 1972). However, these methods require some heat treatment (100–300°C) to recover trapped volatiles, which may cause certain alteration of samples.

A simultaneous purging and solvent extraction method (SPE) developed in our laboratory offers some advantages over conventional headspace concentration methods (Umano & Shibamoto, 1987). This method could maintain mild experimental conditions to avoid alteration of constituents. Also, a relatively large quantity of sample (1–10 mg) is obtainable (Umano & Shibamoto, 1988). In the present study, flavour chemicals formed in banana during ripening were collected periodically using the SPE and were analyzed by gas chromatography (GC).

A headspace sample of ground celery was also collected with the same apparatus in order to examine its possible application for studies on vegetable flavours. Celery was chosen because analysis of celery constituents has been previously conducted by many researchers (Gold & Wilson, 1961; Macleod *et al.*, 1988; Macleod & Ames, 1989; Van Wassenhove *et al.*, 1990), but most of these works were done using steam distillation and solvent extraction.

MATERIALS AND METHODS

Materials

Fresh green bananas (*Musa accuminata*) and celery (*Apium graveolens* var. *dulce*) were purchased from a local market. Authentic reference compounds were obtained from reliable commercial sources and used without further purification.

Sample preparations

Collection of banana headspace sample

Green bananas (500 g) were placed in a 10-litre desiccator interfaced to an SPE apparatus. A schematic diagram of this apparatus was shown in a previous report (Umano & Shibamoto, 1988). The headspace of banana samples was purged with a purified air stream (60 ml/min) into a water trap

(250 ml) of an SPE. Volatile chemicals trapped in the water were simultaneously and continuously extracted with 50 ml of dichloromethane. The water temperature was maintained at 10°C by a Brinkman RM6 constant-temperature water circulator. Fresh dichloromethane (50 ml) was replaced periodically every 12 h for 11 days. Each extract was concentrated to 2 ml using a Vigreux column and was then analyzed by GC with methyl valerate as an internal standard.

Collection of celery headspace sample

Fresh celery stalks (300 g) were chopped and homogenized with 600 ml 1 M NaCl solution using an Osterizer blender. The celery slurry was placed in a 10-litre desiccator interfaced to an SPE apparatus. A purified air stream (40 ml/min) was bubbled into the sample and the headspace gas was purged into the SPE for 4 h. The extraction was performed with 75 ml of dichloromethane four times and the extracts were combined. The sample was concentrated to 2 ml and analyzed by GC.

Instruments

A Hewlett–Packard 5790 gas chromatograph equipped with a 30 m × 0·25 mm i.d. bonded-phase DB-1 fused-silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector was used for qualitative and quantitative analyses of the samples. The oven temperature was held at 30°C for 5 min and then programmed to 120°C at 3°C/min for banana samples, or held at 50°C for 10 min and then programmed to 150°C at 5°C/min for celery samples. The injector and detector temperatures were 250°C.

A Hewlett–Packard Model 5890 GC interfaced to a VG Trio II mass spectrometer with VG 11-250 computer data system was used for MS identification of the GC components at MS ionization voltage 70 eV. The column and oven conditions for GC/MS were as described for the HP 5790 GC.

RESULTS AND DISCUSSION

Figure 1 shows a gas chromatogram of the headspace sample obtained from bananas after 10 days of ripening. Table 1 shows the compounds used to monitor formation variations throughout the entire ripening process. Figure 2 shows the amount of volatiles formed at different stages of fruit ripening. The ester formation increased steadily until 10 days after the ripening process began, reached a maximum just before senescence started and then

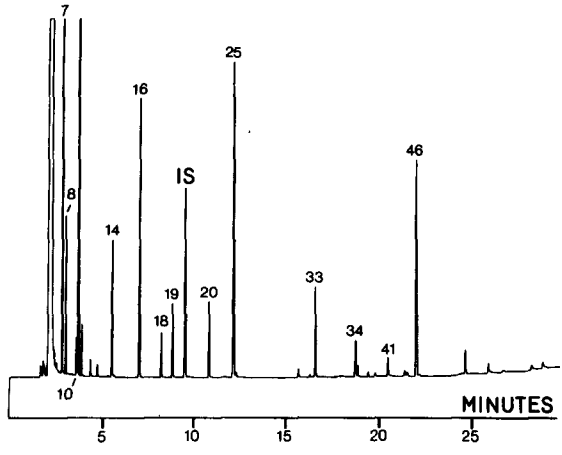

Fig. 1. A gas chromatogram of the headspace sample obtained from bananas after 10 days of ripening (see Table 1 for peak indentification).

declined slightly. Alcohols increased until 5 days and then plateaued for the rest of the ripening process. The results were consistent with previous reports on bananas (Macku & Jennings, 1987), on cantaloupe (Horvat & Senter, 1987), and on golden delicious apples (Schamp & Dirinck, 1982). Maximum concentrations of volatiles were not reported in the above studies, suggesting that the methods used had certain limitations for long-term use.

TABLE 1
Volatile Aroma Chemicals Found in the Headspace from Fresh Bananas and Used to Monitor their Formations over Different Time Periods[a]

Peak number in Fig. 1	*Compound*	*I*[b]	*GC peak area (%)*
7	Ethylacetate	600	41·9
8	Iso-butanol	616	2·90
10	Butanol	651	0·80
14	Iso-pentanol	723	4·30
16	Iso-butylacetate	761	9·80
18	Ethylbutyrate	788	1·60
19	Butylacetate	800	2·80
20	2-Pentylacetate	844	3·00
25	Iso-amylacetate	868	13·9
33	Iso-butylbutyrate	946	4·00
34	Butylbutyrate	983	1·60
41	2-Pentylbutyrate	1 014	0·80
46	Iso-amylbutyrate	1 044	10·4

[a] See Fig. 2.
[b] Kovats Index on DB-1.

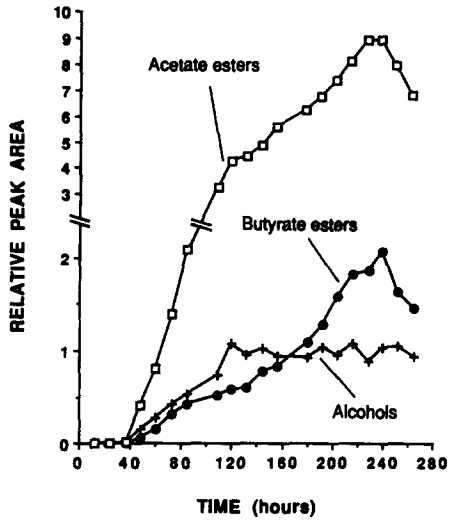

Fig. 2. The amount of volatiles formed at different stages of fruit ripening.

Figure 3 shows a gas chromatogram of the headspace sample obtained from celery. Table 2 shows compounds identified in this sample. The major constituents of celery headspace were terpene hydrocarbons, including *d*-limonene (GC peak area = 61·2%), β-pinene (12·7%), and γ-terpinene (11·0%). Among the terpenes found, α-thujene, camphene, sabinene, α-terpinene, cis-β-ocimene, trans-β-ocimene, and terpinolene were found in a headspace sample from celery for the first time.

Phthalides, such as sedanonic anhydride, have been found as one of the major constituents of celery volatiles (Macleod & Ames, 1989) and are reportedly important contributors to celery flavour (Gold & Wilson, 1963;

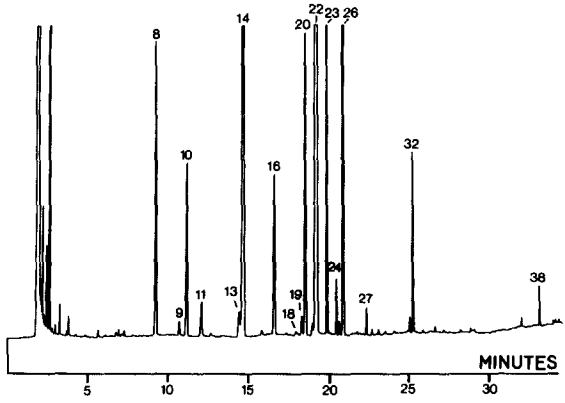

Fig. 3. A gas chromatogram of the headspace sample obtained from celery (see Table 2 for peak identification).

TABLE 2
Compounds Isolated and Identified in the Headspace from Celery[a]

Peak number in Fig. 3	*Compounds*	*I*[b]	*GC peak area (%)*
8	Branched hydrocarbon	900	2·40
9	α-Thujene	929	0·10
10	α-Pinene	936	1·70
11	Camphene	948	0·30
13	Sabinene	972	0·20
14	β-Pinene	974	12·7
16	Myrcene	981	1·60
18	α-Phellandrene	1 000	0·03
19	α-Terpinene	1 005	0·20
20	*p*-Cymene	1 009	2·60
22	*d*-Limonene	1 023	61·2
23	Cis-β-ocimene	1 033	3·30
24	Trans-β-ocimene	1 044	0·60
26	γ-Terpinene	1 053	11·0
27	Terpinolene	1 079	0·20
32	Amylbenzene	1 149	1·00
38	β-Caryophyllene	1 418	0·30

[a] See Fig. 3.
[b] Kovats Index on DB-1.

Uhlig *et al.*, 1987). No phthalides were found in the headspace sample from celery in the present study, even though the sample possessed a characteristic celery aroma. The absence of phthalides in a headspace sample may be due to their lesser volatility. This result is consistent with a previous report (De Pooter *et al.*, 1985). The terpenes found in the present study may play as important a role in the aroma of celery as phthalides do. Our results indicate that SPE gives consistent results with the method used previously, even though its procedure is simpler and more sensitive. One of the major advantages of the SPE method is that a sample can be collected over long periods of time. Changes in volatile formations of fruits or vegetables under various atmospheric conditions can be monitored. For example, if oxygen is required to maintain a biological system, air or oxygen can be used as a purging gas. It can be also applied for volatile formations by microorganisms over a certain period of time (Berger *et al.*, 1990).

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