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Short Communication

Glass capillary gas chromatographic identification of volatile components recovered from orange essence by continuous liquid extraction

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ABSTRACT

Volatile components extracted from two different commercial orange essences by continuous liquid–liquid extraction were analyzed by glass capillary gas chromatography. From the evaluation of identified volatile components, one of the essences seemed to be a true orange essence, whereas the other seemed to be a refined citrus peel oil. We also showed that the continuous liquid–liquid extraction provided an easy, useful and very convenient procedure for the preparation of samples.

INTRODUCTION

The recovery of flavour compounds from fruits and their products prior to gas chromatographic (GC) separation is one of most important aspects of flavour chemistry, even though the development of glass capillary GC and GC–mass spectrometry (MS) has also contributed significantly to recent advances in flavour analytical chemistry. The concentration and nature of the flavour compounds detected often depend on the sample preparation methods, and three general procedures have been used: analysis of the headspace above the sample, steam distillation of the sample, and extraction (either cold or at room temperature) of the sample with a low-boiling organic solvent followed by evaporation of the solvent. Of these three methods, solvent extraction procedures have been widely used in citrus flavour analysis [1–5]. Solvent extraction procedures, however, are often tedious and time consuming using a separating funnel.

Orange essence recovered during industrial concentration processes is often added to a number of citrus fruit products to impart fresh flavour. Recently, flavour loss of citrus juices and drinks packed in flexible pouches has become a serious problem [6–8], and in this study we applied a continuous liquid–liquid solvent extractor for analysing flavour compounds in the orange essence.

This paper deals with the identification of volatile components extracted from two different commercial orange essences by a continuous liquid–liquid extraction, using wall-coated open-tubular (WCOT) glass capillary GC and GC–MS.

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EXPERIMENTAL

Materials

Two commercial orange essences, (A) W-9735 and (B) P-0522, were purchased from Takasago Perfume (Tokyo, Japan). W-9735 was produced from volatiles recovered during the concentration of orange juice and P-0522 was prepared from peel oil recovered from the extraction of orange fruits [9].

Flavour recovery from orange essence solution

Distilled water (250 g) was added to 250 g of orange essence. The essence solution obtained was continuously extracted for 8 h with 360 ml of *n*-pentane–diethyl ether (2:1) at room temperature using a liquid–liquid extractor as shown in Fig. 1. The flask which contained the solvent was heated to and maintained at 45°C. The combined solvent extract was dehydrated with sodium sulphate and then the extract was concentrated *in vacuo* (28°C, 10 mmHg). The yields of extract concentrates were 1.535 and 8.483 g for essences A and B, respectively.

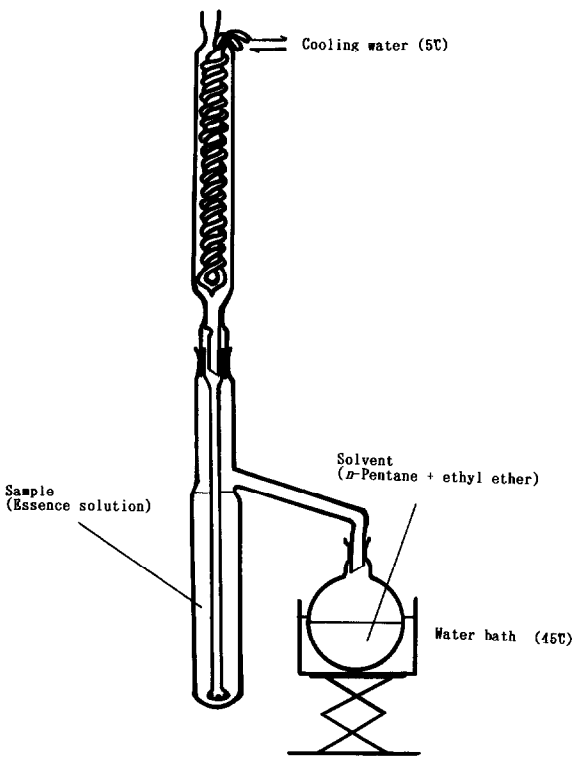

Fig. 1. Schematic diagram of the continuous liquid–liquid extractor.

Apparatus

Analytical GC was carried out on a Shimadzu Model 8A gas chromatograph with flame ionization detection (FID). A WCOT glass capillary column (50 m × 0.25 mm I.D.) coated with Carbowax 20M was used. The temperature of both the injection port and detector were maintained at 250°C. The column oven temperature was maintained at 65°C for 4 min and then programmed from 65 to 195°C at 2°C/min. Helium was used as the carrier gas at a flow-rate of 0.93 ml/min with a splitting ratio of 108:1. The sample size was 0.2 μl. Peak areas were integrated by means of a Shimadzu Chromato-pack C-R3A integrator.

GC–MS was performed on a Hitachi Model M-80A mass spectrometer combined with a Hitachi Model 063 gas chromatograph, under similar conditions to the GC analysis. Other operating parameters were as follows: carrier gas, helium; ionizing voltage, 20 eV; ion source temperature, 200°C.

RESULTS AND DISCUSSION

The yields (w/w) of extract concentrates were 0.61% for essence A and 3.39% essence B.

Gas chromatograms of the extract concentrates A and B from two orange essences using FID are given in Figs. 2 and 3, respectively. The presence of peaks of *m/z* 127 for A and *m/z* 121 for B was demonstrated; 50 components for A and 44 for B were identified as known compounds by comparing and matching the mass spectra and GC retention times or from the mass spectra only (Table I).

The peak-area percentages, excluding peaks for the solvents and ethanol, were calculated; ethanol is used in preparing commercial fruit essence [9].

Concerning the identified terpene content, there was a great difference between essences A and B. As expected, the largest fraction of the extract concentrate B consisted of terpene carbohydrates; principally *d*-limonene and 1,8-cineole (89.71%), myrcene (1.81%) and α-pinene (0.64%) were present in the greatest quantities. Methyl butyrate, ethyl 2-methylbutyrate, dihydrolinalool, isopulegol and nonanol were lacking with essence B compared with essence

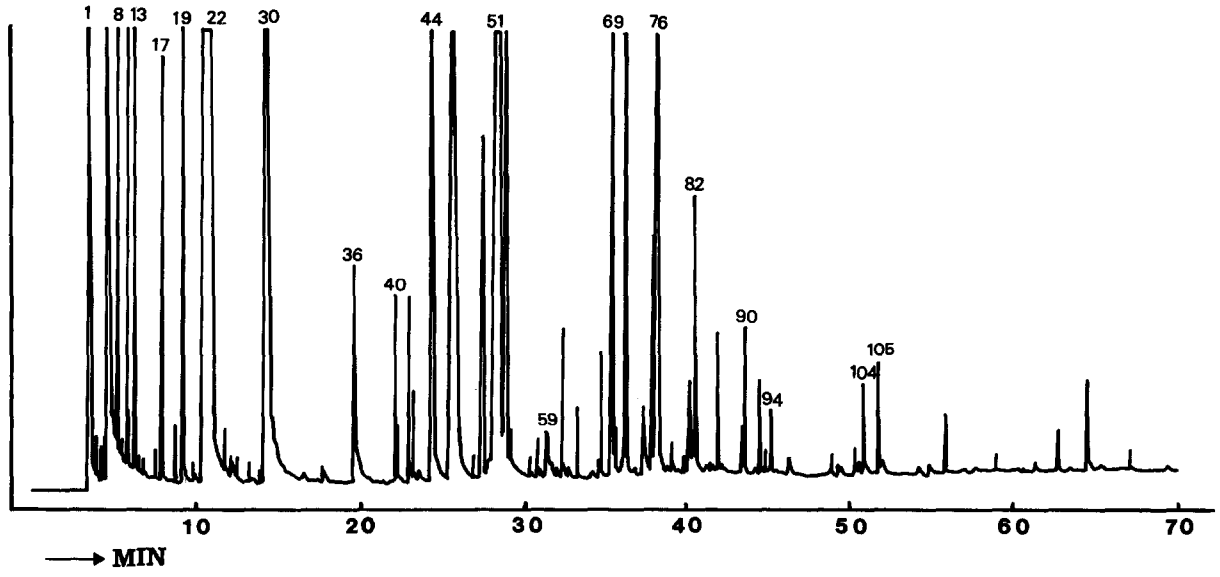

Fig. 2. Gas chromatogram recorded with FID of an extract concentrate of orange essence W-9735 (A). Sample size, 0.2 μl. A WCOT glass capillary column (50 m × 0.25 mm I.D.) coated with Carbowax 20M was used. The column oven temperature was kept at 65°C for 4 min and then programmed from 65 to 195°C at 2°C/min. Other operating conditions were as given under Experimental and peak identities are given in Table I.

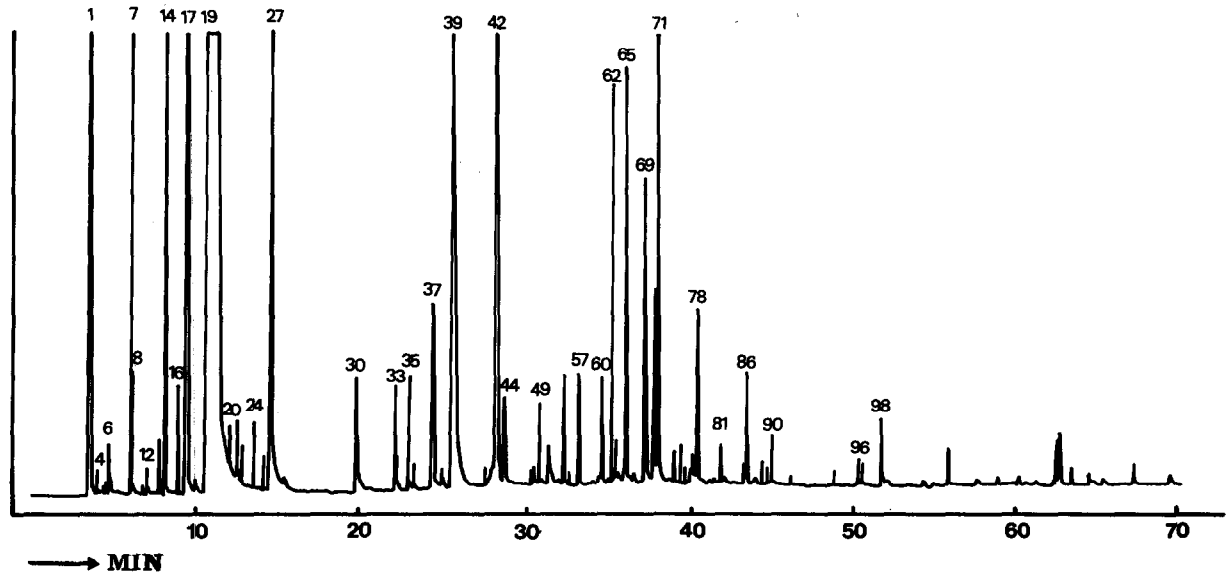

Fig. 3. Gas chromatogram recorded with FID of an extract concentrate of orange essence P-0522. (B) GC conditions and peaks as in Fig. 2.

TABLE I

VOLATILE COMPOUNDS IDENTIFIED IN THE ORANGE ESSENCES W-9735 (A) and P-0522 (B)

Peak No.[a] (Fig. 2)	Compound	Peak area (%)		Evidence	
		A	B	GC	GC–MS
1	*n*-Pentane (solvent)			+	+
2	Diethyl ether (solvent)			+	+
5	Ethyl acetate	0.02	Tr[b]	+	+
7	Ethanol			+	+
8	Methyl butyrate	0.26	–[b]	+	+
10	α-Pinene	0.32	0.64	+	+
11	Ethyl butyrate	0.03	0.04	+	+
13	Ethyl 2-methylbutyrate	0.57	–	+	+
16	β-Pinene	0.02	–	+	+
17	Sabinene	0.27	0.38	+	+
18	δ-3-Carene	0.05	0.09	+	+
19	Myrcene	1.23	1.81	+	+
20	Heptanal	0.02	0.01	+	+
21'[c]	*d*-Limonene + 1,8 cineole	58.05	89.71	+	+
22'	Ocimene + ethyl caproate	0.03	0.02	+	+
24	α-Terpinene	0.02	0.03	+	+
26	4-Cymene	0.01	0.02	+	+
27	Terpinolene	0.01	0.03	+	+
30	Octanal	3.56	0.67	+	+
36	Nonanal	0.37	0.13	+	+
41	Limonene-1,2-oxide	0.18	0.07		+
42	Linalool-3,6-oxide	0.09	0.02		+
44	Citronellal	0.75	0.18	+	+
47	Decanal	2.10	0.91	+	+
49	Dihydrolinalool	0.43	–	+	+
51	Linalool	21.80	2.08	+	+
52	Octanol	0.67	0.08	+	+
53	Isopulegol	0.07	–	+	+
57	Terpinen-4-ol	0.05	0.06	+	+
59	Undecanal	0.07	0.05	+	+
67	Nonanol	0.03	–	+	+
68	3-Pinen-2-ol	0.12	0.07	+	+
69	Neral	1.38	0.25	+	+
72	α-Terpineol	1.77	0.28	+	+
74	Dodecanal	0.16	0.29	+	+
75	α-Terpinyl acetate	0.22	0.13	+	+
76	Geranial	2.29	0.47	+	+
81	β-Citronellol	0.12	0.03	+	+
82	Perillaldehyde	0.30	0.12	+	+
86	Nerol	0.15	0.03	+	+
90	*cis*-Carveol	0.15	0.08		+
92	Geraniol	0.10	0.02	+	+
94	*trans*-Carveol	0.07	0.04		+
102	Laurinal	0.04	0.03	+	+
104	Dodecanol	0.11	0.02	+	+
105	*p*-Menta-1,8-dien-9-ol	0.13	0.06		+

[a] Peak numbers on the left-hand side give the elution order on the 50-m Carbowax 20M column in Fig. 2.

[b] Tr, <0.01%; –, not detectable.

[c] In the case of two overlapped peaks in Fig. 2, the peak numbers are marked with primes. The overlapped peaks were separated by GC (column temperature 60°C).

A. In contrast, some terpene alcohols (including linalool, nerol and citronellol) and aldehydes (including octanal, decanal, neral and geranial) were relatively abundant in essence A. Especially the linalool ratio relative to *d*-limonene and 1,8-cineole of essence A (37.6%) is sixteen times greater than that of essence B (2.3%).

Of the compounds in Table I, the oxygen-containing components, including carbonyls (octanal, decanal and neral, etc.) and alcohols (linalool, citronellol and nerol, etc.), are recognized as the major contributors to the fresh and pleasant flavour of orange essences, because of their low threshold and their olfactory characteristics [5,10,11].

The yield of extract concentrate B was approximately five times higher than that of extract A, owing to its higher content of terpene hydrocarbons (Table I). On the other hand, essence A was evaluated as a higher quality flavouring ingredient for orange product processors, as it contained a relatively larger proportion of the oxygen-containing compounds. These results suggested that essence A was a true orange essence whereas the GC profile of concentrate B was very similar to that of a refined citrus peel oil [9,12].

Although the convenient headspace analysis procedure for sample preparation has been widely applied recently by flavour chemists using Tenax resins, etc., solvent extraction is also necessary for the identification of high-boiling flavour compounds, although tedious and time consuming using a separating funnel. In this study to evaluate chemically the flavour compounds from commercial citrus essence, we have demonstrated that a continuous liquid–liquid extractor provides an easy, useful and very convenient procedure for preparing samples.

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