Journal of Chromatography, 268 (1983) 336–340
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,003

Note

Glass capillary gas chromatographic analysis of oil components extracted from Yuzu (*Citrus junos*) juice

HIDEAKI OHTA*

Agricultural Technical Center, National Federation of Agricultural Co-operative Associations, Hiratsuka-shi, Kanagawa 254 (Japan)

and

YUTAKA OSAJIMA

Department of Food Science and Technology, Faculty of Agriculture, Kyushu University, Fukuoka-shi, Fukuoka 812 (Japan)

(Received May 24th, 1983)

Recent advances in flavour chemistry have been greatly dependent on the development of glass capillary gas chromatography (GC) and GC–mass spectrometry (MS). The minor components of citrus essential oil have also been isolated and identified by glass capillary GC[1–4].

Yuzu, *Citrus junos* Sieb. ex Tanaka, is a sort of sour orange. The fruit and its juice are used as a vinegar and seasoning in some parts of western Japan because of the pleasant flavour. The constituents of cold-pressed Yuzu oil have already been investigated using stainless-steel capillary GC[5] and packed column GC[6]. However, the flavour components in Yuzu juice have not yet been reported.

This paper describes the identification of oil components recovered with *n*-pentane from commercial Yuzu juice produced by a belt-press juice extractor, using wall-coated open tubular (WCOT) glass capillary GC and GC–MS.

EXPERIMENTAL

Materials

Yuzu fruits, harvested at Kouchi prefecture in the autumn of 1981, were used. The Yuzu juice was extracted by a belt-press juice extractor (Yamaoka Tetsukoh) from 40 kg of Yuzu.

Recovery of oil from the juice

Yuzu oil in the juice (4 kg) was extracted three times with *n*-pentane (total 2400 ml). The combined *n*-pentane extract was concentrated *in vacuo* to remove the solvent.

Silica gel column chromatography

The *n*-pentane extract was fractionated in terpene hydrocarbons and oxygen-containing compounds by silica gel (Merck) column (25 × 2.5 cm I.D.) chromatography using *n*-hexane and diethyl ether as the eluents, respectively.

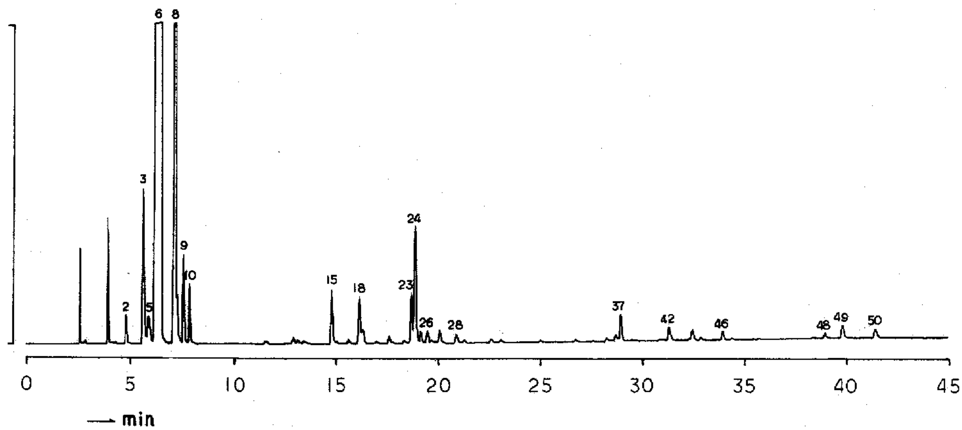

Fig. 1. Gas chromatogram of an *n*-pentane extract of Yuzu juice. A WCOT glass capillary column (25 m × 0.25 mm I.D.) and flame ionization detector were used. The column oven temperature was programmed from 50°C to 190°C (4°C/min). Other operating conditions are in the Experimental section and peak identities are given in Table I.

GC and GC–MS

Analytical GC was performed on a Hitachi Model 163 chromatograph equipped with a flame ionization detector. A WCOT glass capillary column (25 m × 0.25 mm I.D.) coated with Carbowax 20M was used. The temperatures of both detector and injection port were maintained at 250°C. The column oven temperature was programmed from 50°C to 190°C at a rate of 4°C/min, then held at 190°C for 20 min. Nitrogen carrier gas was used at a flow-rate of 1.0 ml/min with a splitting ratio of 1:80. Peak areas were integrated by a Chromato-pack C-R1B (Shimadzu).

GC–MS analysis was carried out on a Hitachi Model M-80A mass spectrometer combined with a Hitachi Model 063 gas chromatograph, under similar condi-

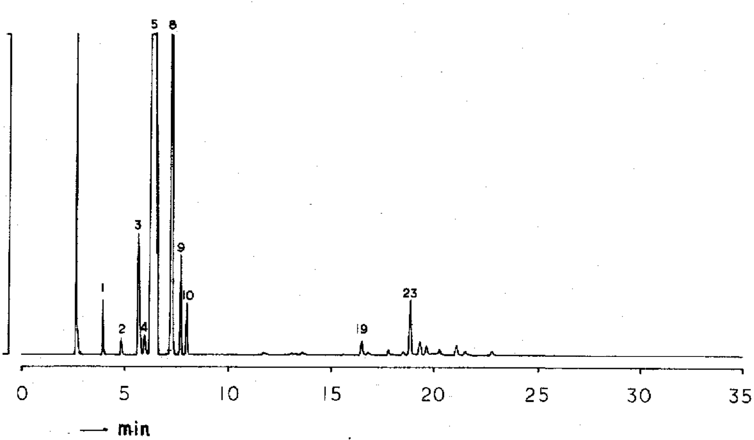

Fig. 2. Gas chromatogram of the hydrocarbon fraction of an *n*-pentane extract of Yuzu juice. See Fig. 1 for GC conditions.

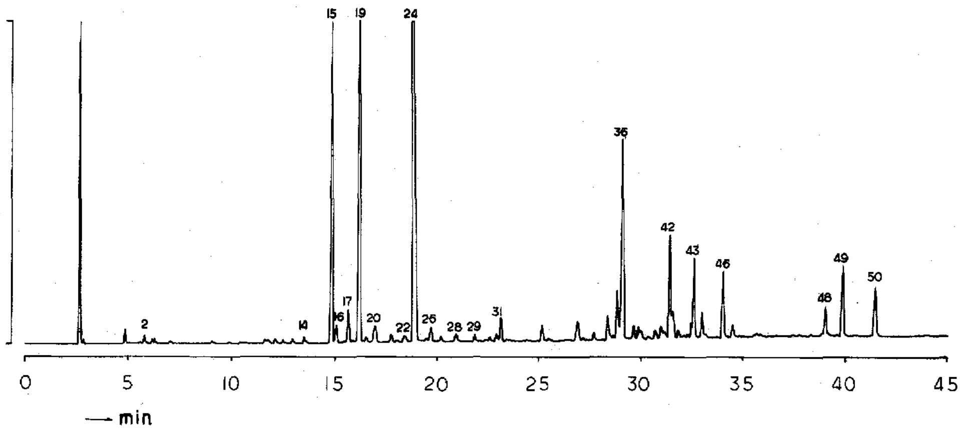

Fig. 3. Gas chromatogram of the oxygen-containing compound fraction in an *n*-pentane extract of Yuzu juice. See Fig. 1 for GC conditions.

tions 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 yield of *n*-pentane extract (recovered oil) was 0.26% of the weight of Yuzu juice used. The proportions of the terpene hydrocarbon fraction and oxygenated compound fraction were 94.7% and 6.3%, respectively.

A gas chromatogram of an *n*-pentane extract of Yuzu juice is shown in Fig. 1. Gas chromatograms of the hydrocarbon fraction and the oxygen-containing compound fraction are also given in Fig. 2 and Fig. 3 respectively.

The presence of 50 peaks was demonstrated in the *n*-pentane extract of Yuzu juice. Thirty-three of these peaks were identified by comparing and matching the mass spectra and GC retention times, and three peaks were identified by the mass spectra only. Of these, 11 compounds including one terpene hydrocarbon, one oxide, 4 alcohols and 5 esters were newly identified from Yuzu fruit in the present study.

Table I lists the compounds identified in the *n*-pentane extract of Yuzu juice. The peak area percentages were estimated by GC analysis of the *n*-pentane extract and the two chromatographic fractions. Peak numbers on the left-hand side show the elution order on the 25-m Carbowax 20M column (Fig. 1).

The largest fraction of the *n*-pentane extract consisted of terpene hydrocarbons of which limonene (77.10%), γ-terpinene (10.80%) and myrcene (2.35%) were present in the greatest quantities. The percentages of these terpene hydrocarbons were similar to those already reported[5,6]. The percentage of linalool was also similar to that obtained by Shinoda *et al.*[5], although less than that reported by Kusunose and Sawamura[6].

It is interesting that the percentages of α-terpineol and terpinen-4-ol were much greater than those mentioned in previous papers[5,6]. However, these alcohols were derived from larger quantities of terpene hydrocarbons present, especially α-pinene, β-pinene and limonene. Shimoda and Osajima[7] revealed that α-terpineol was formed

TABLE I

COMPOUNDS IDENTIFIED IN THE OIL EXTRACTED FROM YUZU JUICE

Peak No.	*Component*	*Peak area (%)*	*Evidence*		*Refs.*
			GC	*GC–MS*	
1	α-Pinene	0.30	+	+	5, 6
2	β-Pinene	0.28	+	+	5, 6
3	Myrcene	2.35	+	+	5, 6
4	1,4-Cineole*	0.02	+	+	
5	α-Terpinene*	0.59	+	+	
6	Limonene	77.10	+	+	5, 6
7	1,8-Cineole	0.01	+	+	6
8	γ-Terpinene	10.80	+	+	5, 6
9	*p*-Cymene	0.47	+	+	5, 6
10	Terpinolene	0.62	+	+	5
11	Nonanal	0.01	+	+	5, 6
12	Citronellal	0.01	+	+	5, 6
13	Decanal	0.01	+	+	5, 6
14	Octyl acetate*	0.02	+	+	
15	Linalool	0.74	+	+	5, 6
16	*p*-Menth-2-en-1-ol*	0.04	+	+	5, 6
17	Fenchol*	0.06	+	+	
18	Terpinen-4-ol	0.69	+	+	5, 6
19	Caryophyllene	0.25	+	+	6
20	β-Terpineol	0.03	+	+	6
22	Ocimenol*	0.01	+	+	
23	β-Farnescence	0.74	+	+	5
24	β-Terpineol	1.80	+	+	5, 6
26	Carvone	0.20	+	+	5, 6
27	Citronellol	0.25	+	+	5, 6
28	Geranyl acetate	0.18	+	+	5
29	Nerol	0.02	+	+	6
31	Geraniol	0.05	+	+	5, 6
36	T-Cardinol	0.13		+	5
38	α-Eudesmol*	0.03		+	
39	β-Eudesmol	0.02		+	5
42	Thymol	0.29	+	+	6
46	Ethyl palmitate*	0.16	+	+	
48	Ethyl oleate*	0.08	+	+	
49	Ethyl linolate*	0.23	+	+	
50	Ethyl linolenate*	0.17	+	+	

* Newly identified.

mainly from β-pinene and terpinen-4-ol from limonene by a nucleophilic substitution reaction (hydration) in stored Satsuma mandarin juice. This α-terpineol had already been proposed as an indicator of the storage history for orange juice[8].

In addition, it was reported that limonene is converted into 1,4- and 1,8-cineoles[9]. The aromatic compound *p*-cymene is a well-known product from γ-terpinene during deterioration of citrus oil[10].

Esters such as ethyl palmitate, ethyl oleate, ethyl linolate and ethyl linolenate were identified for the first time in Yuzu juice, suggesting that these were probably formed by condensation of fatty acids and alcohols in the juice.

Many lower-boiling oxygen-containing compounds in Table I were believed to contribute to the flavour of Yuzu juice because of their low threshold and their olfactory characteristics, especially the aldehydes (nonanal, citronellal and decanal), oxides (1,4- and 1,8-cineoles) and alcohols (citronellol, nerol and thymol, etc.).

REFERENCES

1 I. Yajima, T. Yanai, M. Nakamura, H. Sakakibara and K. Hayashi, *Agr. Biol. Chem.*, 43 (1979) 259.
2 C. W. Wilson, III and P. E. Shaw, *J. Agr. Food Chem.*, 28 (1980) 919.
3 C. W. Wilson, III and P. E. Shaw, *J. Agr. Food Chem.*, 29 (1981) 494.
4 M. G. Moshonas and P. E. Shaw, *J Agr. Food Chem.*, 31 (1983) 334.
5 N. Shinoda, M. Shiga and K. Nishimura, *Agr. Biol. Chem.*, 34 (1970) 234.
6 H. Kusunose and M. Sawamura, *Nippon Shokuhin Kogyo Gakkai-Shi*, 27 (1980) 517.
7 M. Shimoda and Y. Osajima, *Nippon Nôgei Kagaku Kaishi*, 56 (1982) 27.
8 V. A. Askar, H. J. Bielig and H. Treptow, *Deut. Lebensm.-Rundsch.*, 69 (1973) 162.
9 C. A. Slater and W. T. Watkins, *J. Sci. Food Agr.*, 15 (1964) 657.
10 R. M. Ikeda, W. L. Stanley, S. H. Vanmer and L. A. Rolle, *Food Technol.*, 15 (1961) 379.