Composition of Peel Oil from Citrus Unshu†

By Tei YAMANISHI, Akio KOBAYASHI, Yasuko MIKUMO, Yoko NAKASONE,
Masae KITA and Satohiko HATTORI*

Laboratory of Food Chemistry, Ochanomizu University

**Takasago Perfumery Co., Ltd.*

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Constituents of aroma of peel oil (both oleoresin and cold-pressed oil) from Citrus Unshu were isolated by column chromatography and gas chromatography (GLC), and identified mostly by infrared (IR), partially by mass (MS) and nuclear magnetic resonance (NMR) spectroscopy.

One hundred and seven compounds were found in the oil and among them, four sesquiterpene hydrocarbons, five acetates, five carbonyls and five alcohols were identified as a main constituents which contributed to difference in flavor from other citrus peel oils. Characteristics of aroma of C. Unshu was discussed on the basis of quantitative analysis.

Constituents of various citrus peel oils have been investigated by many researches.¹⁻⁵⁾

Citrus Unshu, produced in the limited area (only in Japan and China), has some characteristic aroma.

The present paper describes a procedure for isolation of the aroma components of peel oil from C. Unshu followed by identification with the use of IR, MS, and NMR and or thin layer chromatography (TLC) and elementary analysis.

Combination of silica-gel column chromatography and GLC was particularly effective to get a complete separation and also to presume

a class of the compound from a position on the diagram obtained by plotting the fraction number of silica-gel column chromatography against the peak number of GLC.

EXPERIMENTAL AND RESULT

Material

1) **Peel oleoresin.** Peel of C. Unshu, picked at Kanagawa Prefecture during 1964~66 season, was extracted with acetone after drying and powdering. From the extract, excellent aroma-containing oleoresin was obtained by distilling off acetone under reduced pressure, below 40°C. Oleoresin was obtained in about 1.5% yield of dried peel.

2) **Cold-pressed peel oil.** Peel from the same C. Unshu was used for preparation of cold-pressed oil.

Apparatus

Gas chromatographic separations were carried out in a Shimadzu Model 1C containing a 3 mm id. by 185 cm stainless U tube packed with 23% Ucon 50 HB 270 X on Shimalite W (60/80 mesh). The oven temperature was 150°C or 160°C, isothermal except the case of carbonyl fraction in which programming from

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1) R. W. Wolford, J. A. Attaway, G. E. Alberding and C. D. Atkins, *J. Food Sci.*, **28**, 320 (1963).

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70°C to 150°C (rate 2°C per min) was employed. Helium flow rate was 20 ml to 30 ml per min. The materials represented by the peaks were collected into cold capillary tube immersed in a dry ice-acetone mixture for identification.

Column chromatography was accomplished in a 3 cm od. by 50 cm column containing silica-gel. Elution was carried out with *n*-

hexane, mixtures of ether and *n*-hexane of several kinds of proportion and methanol as shown in Fig. 4.

Infrared spectra were obtained using a Japan Spectroscopic Co. Model IR-S. MS and NMR were obtained with a Hitachi Ltd. RMU-6E and Model JNM-C-60 of a Japan Electron Optics Laboratory Co. Ltd. respectively.

FIG. 1. Gas Chromatograms of the Essential Oil from Peel Oleoresin of *C. Unshu* and the Carbonyl and Non-carbonyl Fractions.

- (A) Whole essential oil
(B) Non-carbonyl fraction
(C) Carbonyl fraction

Column Temp.:

- (A) } 150°C
(B) } Isothermal
(C) 70°C~150°C programming rate 2°C/min

TABLE I. FRACTIONATION OF COLD-PRESSED PEEL OIL BY VACUUM DISTILLATION

Fraction No.	Distilled temperature	Yield	Color	Aroma
1	~67°C/7 mm	91.0%	Pale yellow	Limonene like, somewhat acidic and bitter odor
2	35~52°C/3 mm	2.1	Pale yellow	Limonene like
3	67~79°C/3 mm	0.5	Yellow	Sweet orange
4	77~87°C/3 mm	0.7	Greenish yellow	Sweet and heavy aroma of Citrus Unshu
5	Residue	5.7	Dark brown	Woody and resine like

Separation and analysis

The essential oil was prepared from the oleoresin by steam distillation under the reduced pressure by the same method as used in the previous work on tea flavor,⁶⁾ and analyzed as follows: A portion of the essential oil was separated into acidic, basic and neutral fractions by routine procedure and examined by GLC. The aroma and the chromatogram of neutral fraction were very similar to those of original essential oil.

Therefore, without removing acidic and basic fractions, major portion was directly separated into carbonyl and non-carbonyl fractions by Girard-T reagent and subjected to GLC. Chromatograms of the essential oil and these two fractions are shown in Fig. 1. IR spectra showed several single peaks due to more than one compound.

As for cold-pressed oil, other procedure was employed. First, cold-pressed oil was subjected to a fractional vacuum distillation under

FIG. 2. Gas Chromatograms of the Four Fractions of Cold-pressed Peel Oil from C. Unshu.

6) T. Yamanishi, T. Kiribuchi, M. Sakai, N. Fujita, Y. Ikeda and K. Sasa, *Agr. Biol. Chem.*, **27**, 193 (1963).

the stream of nitrogen and separated into five fractions as shown in Table I. GLC of the four fractions are in Fig. 2. Fraction No. 1 consisted of about 96% of *d*-limonene, 2.5% of *p*-cymene, 1% of β -pinene and myrcene. Fraction No. 2 contained 62% of *d*-limonene and 20% of *p*-cymene.

Since the main substances which contributed to the characteristic aroma of C. Unshu seemed to be contained in Fraction No. 3 and No. 4, the fractions were subjected to silica-gel column chromatography and separated into fifty fractions. Each fraction was evaporated under reduced pressure at temperature below 50°C to remove the solvent and analysed by GLC. After analysis, each fraction was further

separated by GLC. IR spectra of main components as shown in Fig. 3 were quite identical with that of authentic compound (gas chromatographically pure). Besides IR, some peaks were identified by TLC, MS and NMR. Approximate quantitative data were also obtained by calculating of the yield of each fraction and peak area of GLC. The results are summarized in Table II.

To look over the aroma pattern, the authors obtained the diagram by plotting the fraction number of column chromatography against the peak number of GLC (numbered in the order of retention time) as shown in Fig. 4. Thus, one hundred and seven components in the peel oil were recognized on the diagram.

FIG. 3. Infrared Spectra of Main Components in Peel Oil from C. Unshu and of the Authentic Compounds.

(1) Component No. 3
α -Ylangene	(5) Component No. 45
<i>n</i> -Octyl Acetate	(9) Component No. 69
Perillyl Acetate	(13) Component No. 103
α -Terpineol
(2) Component No. 9
Caryophyllene	(6) Component No. 49
Citronellyl Acetate	(10) Component No. 91
Carvone	(14) Component No. 106
<i>cis</i> -Carveol
(3) Component No. 13
δ -Cadinene	(7) Component No. 51
Neryl Acetate	(11) Component No. 92
Perillaldehyde	(15) Component No. 109
<i>trans</i> -Carveol
(4) Component No. 15
β -Elemene	(8) Component No. 52
Geranyl Acetate	(12) Component No. 97
Isopulegol	

DISCUSSION

The characteristic aroma of C. Unshu seemed to be chiefly due to the larger contents of β -elemene and acetates of four terpene alcohols after comparing the composition with those of other citrus peel oils reported by other workers.¹⁻⁵⁾

There were two important components, i.e. an aldehyde and an ester, which seemed to contribute to the characteristic aroma of C.

Unshu, still remained unknown because of the difficulty to obtain an enough amount of samples for the elucidation.

Aroma of peel oleoresin was superior to that of cold-pressed oil. One reason for this fact seemed to depend on the higher concentration of oxygenated compounds in peel oleoresin than in cold-pressed oil.

Further investigation is now in progress on both identification of the unknowns and eluci-

TABLE II. IDENTIFICATION AND QUANTITATIVE VALUE OF THE AROMA CONSTITUENTS OF PEEL OIL FROM CITRUS UNSHU

Silica column chromat. Fraction No.	GLC Peak No.	No. on diagram	Constituent	Method of identification besides GLC	Cold-pressed oil wt % excluding monoterpene hydrocarbon	Oleoresin peak area % excluded limonene
2	15	3	<i>d</i> -Ylangene	TLC, IR, MS	6.0	6.3
3	1	6	<i>d</i> -Limonene	IR	(ca, 94)	—
"	2	7	<i>p</i> -Cymene	IR	(ca, 2.8)	—
"	18	9	Caryophyllene	IR	2.8	5.6/3*
"	29	13	δ -Cadinene	EA, ^{a)} IR, MS	5.0	8.9
4	17	15	β -Elemene	EA, IR, MS, NMR	24.6	11.9
29	8	45	<i>n</i> -Octyl acetate	IR	1.1	5.0/2*
"	9	46	<i>n</i> -Decanal	TLC(2,4-DNP), IR	5.0	4.0
"	17	49	Citronellyl acetate	IR	4.1	6.3
"	19	50	Terpinyl acetate	IR	1.6	5.6/3*
"	21	51	Neryl acetate	IR	3.0	9.6
"	23	52	Geranyl acetate	IR	4.0	7.3
33	17	56	Undecanal	TLC(2,4-DNP)	0.4	—
36	25	59	Dodecanal	TLC(2,4-DNP)	0.5	—
40	35	69	Perillyl acetate	IR	0.3	—
46	19	91	Carvone	IR	0.2	5.3/3*
"	33	92	Perillaldehyde	IR	0.5	—
49	8	96	Linalool	IR	2.2	5.0/2*
"	11	97	Isopulegol	IR	1.6	—
50	16	103	α -Terpineol	IR	1.6	—
"	24	106	<i>cis</i> -Carveol	IR	1.4	—
"	26	107	<i>trans</i> -Carveol	IR	0.1	—
5	28	17	α Sesquiterpene	IR	7.2	9.9
29	17	48	an Aldehyde (characteristic aroma of sweet orange)	IR, NMR	3.0	—
31	32	53	an Ester	IR	0.8	0.8
33	14	54	an Ester (characteristic aroma of sweet peach)	IR	1.3	0.8

Numbers in () present Wt% in whole peel oil.

Numbers of * mark is that of mixed components in single peak. a) EA means elementary analysis.

FIG. 4. Diagram of the Aroma Composition of Peel Oil from *C. Unshu*.

* Solvent

No.

1. *n*-Hexane
2. 1% Ether added
n-Hexane
3. 2% Ether
4. 3%
5. 4%
6. 6%
7. 8%
8. 12%
9. 20%
10. Methanol

Size of the square (◆)
presents the relative quantity.Mark (×) presents the small
contents less than 0.05%.Mark (●) shows outside of
calculation.(A) Area of Monoterpene
hydrocarbhone(B) Area of sesquiterpene
hydrocarbhone(C) Area of carbonyls
and Esters

(D) Area of Alcohols

dation of the reason of aroma difference between oleoesin and cold-pressed oil.

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