

# Identification of Volatile Compounds Isolated from Round Kumquat (*Fortunella japonica* Swingle)

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Volatile constituents of round kumquat fruit were isolated by steam distillation and simultaneous purging/extraction (SPE) methods. The isolated volatiles were identified by gas chromatography and gas chromatography/mass spectrometry. Steam distillation led to the identification of 84 compounds, and 35 compounds were identified following SPE. Among a total of 91 constituents identified, 47 were identified for the first time in kumquat fruit. *d*-Limonene was the most abundant compound, comprising 87% of the sample from steam distillation and 97% of the sample from SPE. In addition to *d*-limonene, linalool, myrcene, and geranyl acetate were found in the sample from steam distillation as major constituents; myrcene,  $\alpha$ -pinene, and  $\beta$ -phellandrene were identified in the sample from SPE as major components.

**Keywords:** *Citrus oil; kumquat; volatile compounds*

## INTRODUCTION

A round kumquat (*Fortunella japonica* Swingle) tree, native to central China, grows to about 2 m in height. It has fine stems, few thorns, and dark green pointed leaves. The fruit varies in shape from round to slightly oval (~2-cm diameter), averaging around 10–12 g. Kumquat is a novelty that is not cultivated extensively. The fruit is quite sour, but its peel is edible. However, its size would make processing difficult. Kumquat oil has a typical citrus oil character; Koyasako and Bernhard (1983), who identified 71 volatile compounds in the oil of kumquat, reported that the most abundant compound was *d*-limonene, comprising 93% of the whole oil.

The history of essential oil use stretches back centuries. By the 17th century, over 170 different essential oils had been produced. By the middle of the 19th century, constituents of essential oils had begun to be determined. Citrus oils have been widely used in both perfumery and food industries. The constituents of such major citrus fruits as lemon (Clark and Bernhard, 1960), orange (Shaw and Coleman, 1974), and grapefruit (Umamo and Shibamoto, 1988) are well characterized. However, there are only a few reports on rare citrus fruits such as kumquat.

The method for isolation of volatile compounds from plants is always one of the most important and crucial processes in obtaining high-quality oils. Steam distillation has been most commonly used on both industrial and laboratory scales. However, steam temperature is not ideal because certain components are susceptible to heat degradation and produce unpleasant off-flavors; citrus oils in particular are very susceptible to heat degradation.

To obtain authentic fruit flavors, a headspace sampling method is ideal because headspace constituents are those that one actually smells in the natural fruits. However, only low amounts of volatile chemicals are present in headspace. It is, therefore, necessary to

concentrate the headspace sample. Recently, we developed a simple apparatus to recover headspace samples and successfully used it to isolate and concentrate intact grapefruit oil (Umamo and Shibamoto, 1988). This apparatus includes a tandem gas washing bottle and a liquid–liquid continuous extractor system. In the present study, volatile compounds from round kumquat were isolated with this apparatus and by conventional steam distillation under reduced pressure. The isolated oils were analyzed by gas chromatography/mass spectrometry.

## MATERIALS AND METHODS

**Materials.** Fresh round kumquat fruits were obtained from Wakayama prefecture, Japan. Authentic chemicals for gas chromatographic analysis were purchased from reliable commercial sources or were donated by Takata Koryo Co., Ltd., Osaka, Japan.

**Isolation of Volatile Compounds by Steam Distillation under Reduced Pressure.** After seeds were removed, fresh kumquat fruits (756 g) were mixed with dry ice and homogenized with an electric blender. The homogenized sample was mixed with 1.8 L of deionized water and 200 mL of saturated sodium chloride solution and then was steam-distilled at 32 °C and 35 mmHg. The distillate (900 mL) was extracted with 130 mL of dichloromethane using a liquid–liquid continuous extractor for 6 h. After the extract was dried over anhydrous sodium sulfate, the solvent was removed by distillation through a Vigreux column. The solvent was further removed with a purified nitrogen stream, and approximately 100 mg of fresh fruit essence was obtained. The essence was stored at 5 °C until analysis.

**Isolation of Volatile Compounds by a Simultaneous Purging/Extraction (SPE) Apparatus.** After seeds were removed, fresh kumquat fruits (982 g) were mixed with dry ice and homogenized with an electric blender. The homogenized sample was placed in a 2-L three-neck, round-bottom flask. The flask was connected to the SPE designed by Umamo and Shibamoto (1987). The headspace volatiles were purged into 250 mL of deionized water using a purified nitrogen stream at a flow rate of 1 mL/s. The flask containing the homogenized sample was heated at 40 °C in a water bath during purging. The volatiles trapped by the water were simultaneously and continuously extracted with 30 mL of dichloromethane for 2 h. The water temperature was kept at 10 °C by a Brinkman RM6 constant-temperature water circulator. The dichloromethane extract was dried over anhydrous sodium sulfate for 12 h, and then the extract was

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**Table 1. Volatile Compounds Identified in the Essential Oils of Kumquat Obtained by Steam Distillation (SD) and Simultaneous Purging/Solvent Extraction (SPE)**

| compound                           | Kovats index | GC peak area % <sup>a</sup> |       | compound                                       | Kovats index | GC peak area % <sup>a</sup> |      |
|------------------------------------|--------------|-----------------------------|-------|--|--------------|-----------------------------|------|
|                                    |              | SD                          | SPE   |  |              | SD                          | SPE  |
| acetaldehyde <sup>b</sup>          | 714          | c                           | d     | α-bourbonene (tentative)                       | 1514         | d                           | c    |
| octane                             | 800          | c                           | 0.01  | octyl propionate <sup>b</sup>                  | 1542         | d                           | c    |
| acetone <sup>b</sup>               | 813          | 0.01                        | c     | linalool                                       | 1549         | 1.44                        | 0.01 |
| tetrahydrofuran <sup>b</sup>       | 861          | d                           | c     | octanol  | 1561         | 0.01                        | d    |
| ethyl acetate                      | 885          | d                           | c     | nonyl acetate                                  | 1578         | 0.02                        | c    |
| nonane <sup>b</sup>                | 900          | c                           | 0.02  | β-elemene                                      | 1586         | 0.04                        | c    |
| decane <sup>b</sup>                | 1000         | d                           | c     | β-humulene <sup>b</sup>                        | 1589         | d                           | c    |
| α-pinene                           | 1020         | 0.83                        | 0.45  | 4-terpineol                                    | 1601         | 0.27                        | 0.01 |
| 2-methyl-3-buten-2-ol <sup>b</sup> | 1036         | 0.02                        | c     | dihydrocarvone <sup>b</sup>                    | 1621         | d                           | c    |
| toluene <sup>b</sup>               | 1037         | c                           | 0.01  | p-mentha-cis-2,8-dien-1-ol <sup>b</sup>        | 1629         | 0.14                        | c    |
| camphene <sup>b</sup>              | 1065         | c                           | d     | trans-β-terpineol                              | 1632         | 0.04                        | c    |
| hexanal                            | 1079         | 0.02                        | d     | (+)-aromadendrene <sup>b</sup>                 | 1635         | 0.05                        | c    |
| (Z)-2-pentenal <sup>b</sup>        | 1105         | d                           | c     | α-humulene                                     | 1663         | 0.06                        | d    |
| undecane <sup>b</sup>              | 1100         | 0.01                        | c     | (E)-β-farnesene <sup>b</sup>                   | 1665         | 0.02                        | c    |
| β-pinene                           | 1108         | c                           | d     | p-mentha-trans-2,8-dien-1-ol <sup>b</sup>      | 1672         | 0.09                        | c    |
| sabinene                           | 1119         | d                           | 0.03  | decyl acetate                                  | 1679         | 0.03                        | c    |
| (E)-2-pentenal <sup>b</sup>        | 1125         | d                           | c     | γ-murrolene (tentative)                        | 1681         | 0.06                        | c    |
| (Z)-3-hexenal <sup>b</sup>         | 1138         | 0.12                        | c     | α-terpieneol                                   | 1697         | 0.13                        | d    |
| δ-3-carene                         | 1146         | d                           | d     | germacrene D (tentative)                       | 1702         | 0.81                        | 0.03 |
| 1-penten-3-ol <sup>b</sup>         | 1157         | d                           | d     | α-murrolene (tentative)                        | 1720         | 0.03                        | c    |
| myrcene                            | 1160         | 1.32                        | 2.12  | neryl acetate                                  | 1726         | 0.08                        | c    |
| α-terpinene                        | 1176         | d                           | c     | 1-carvone                                      | 1728         | 0.23                        | c    |
| d-limonene                         | 1196         | 87.07                       | 97.04 | p-mentha-2,8-dien-1-yl acetate <sup>b</sup>    | 1731         | 0.12                        | d    |
| β-phellandrene <sup>b</sup>        | 1212         | 0.19                        | 0.16  | 1-carvenyl acetate <sup>b</sup>                | 1734         | 0.02                        | c    |
| (E)-2-hexenal <sup>b</sup>         | 1216         | 0.08                        | c     | (E,E)-α-farnesene <sup>b</sup>                 | 1748         | 0.08                        | c    |
| cis-β-ocimene <sup>b</sup>         | 1234         | d                           | 0.01  | isopiperitenol (tentative)                     | 1748         | 0.10                        | c    |
| γ-terpinene                        | 1243         | 0.03                        | 0.03  | δ-cadinene (tentative)                         | 1753         | 0.19                        | d    |
| trans-β-ocimene                    | 1250         | 0.08                        | 0.06  | isopiperitenol (tentative)                     | 1753         | 0.24                        | c    |
| p-cymene                           | 1266         | d                           | d     | geranyl acetate                                | 1756         | 1.12                        | d    |
| terpinolene                        | 1280         | 0.04                        | 0.02  | 1-perillaldehyde <sup>b</sup>                  | 1776         | 0.46                        | c    |
| acetoin <sup>b</sup>               | 1280         | d                           | c     | p-menth-1-en-9-yl acetate <sup>b</sup>         | 1818         | 0.17                        | c    |
| octanal                            | 1287         | d                           | c     | isopiperitenone (tentative)                    | 1833         | 0.02                        | c    |
| tridecane <sup>b</sup>             | 1300         | d                           | c     | trans-carveol                                  | 1836         | 0.04                        | c    |
| (Z)-2-pentanol <sup>b</sup>        | 1322         | 0.02                        | c     | p-cymen-8-ol <sup>b</sup>                      | 1848         | 0.01                        | c    |
| 3-methyl-2-buten-1-ol <sup>b</sup> | 1323         | 0.02                        | c     | geraniol                                       | 1851         | 0.02                        | c    |
| hexanol                            | 1355         | 0.01                        | d     | p-mentha-1,8-dien-9-yl acetate                 | 1855         | 0.76                        | d    |
| heptyl acetate <sup>b</sup>        | 1374         | 0.01                        | d     | cis-carveol                                    | 1866         | 0.01                        | c    |
| (Z)-3-hexenol                      | 1386         | 0.07                        | d     | p-menth-1-en-9-yl propanoate <sup>b</sup>      | 1883         | d                           | c    |
| nonanal                            | 1390         | d                           | c     | perillyl acetate <sup>b</sup>                  | 1900         | 0.40                        | c    |
| tetradecane                        | 1400         | d                           | c     | p-mentha-1,8-dien-9-yl propanoate <sup>b</sup> | 1920         | d                           | c    |
| (E)-2-hexenol <sup>b</sup>         | 1419         | 0.01                        | d     | p-menth-1-en-9-ol <sup>b</sup>                 | 1933         | 0.01                        | c    |
| dehydro-p-cymene <sup>b</sup>      | 1433         | 0.02                        | c     | p-mentha-1,8-dien-9-ol <sup>b</sup>            | 1996         | 0.02                        | c    |
| linalool oxide cis-furanoid        | 1443         | d                           | c     | 1-perillalcohol <sup>b</sup>                   | 2005         | 0.04                        | c    |
| α-cubebene <sup>b</sup>            | 1455         | d                           | c     | trans-nerolidol                                | 2042         | 0.02                        | c    |
| δ-elemene (tentative)              | 1467         | 0.19                        | d     | methyl N-methylanthranilate <sup>b</sup>       | 2068         | d                           | c    |
| linalool oxide trans-furanoid      | 1471         | d                           | c     | elemol <sup>b</sup>                            | 2079         | d                           | c    |
| octyl acetate                      | 1476         | 0.21                        | 0.01  | guaiacol <sup>b</sup>                          | 2089         | 0.02                        | c    |
| α-copaene                          | 1488         | 0.02                        | d     | hinesol <sup>b</sup>                           | 2186         | 0.02                        | c    |
| pentadecane                        | 1500         | d                           | c     | β-eudesmol <sup>b</sup>                        | 2226         | 0.03                        | c    |
| decanal                            | 1496         | 0.02                        | c     | γ-dodecalactone <sup>b</sup>                   | 2365         | d                           | c    |

<sup>a</sup> Solvent excluded. <sup>b</sup> Newly identified. <sup>c</sup> Not detected. <sup>d</sup> Less than 0.01.

concentrated to 1 mL as before. The solvent was further removed with a purified nitrogen stream, and approximately 200 mg of fresh fruit essence was obtained. The essence was stored at 5 °C until analysis.

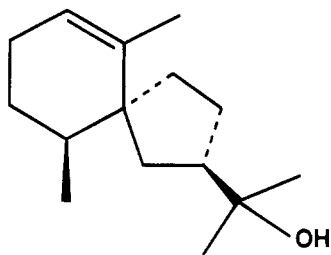
**Analysis of Volatiles.** Identification of volatile constituents of the kumquat samples was made by comparison of their Kovats gas chromatographic retention indices and mass spectra to those of authentic compounds.

**Instruments.** A Hewlett-Packard (HP) Model 5890 gas chromatograph (GC) equipped with a 60 m × 0.25 mm i.d. DB-Wax fused silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID) were used for routine analysis. The oven temperature was held at 40 °C for 2 min and then programmed to 200 °C at 2 °C/min. The detector and injector temperatures were 250 °C. The linear velocity of the helium carrier gas was 30 cm/s, with a split ratio of 1:30.

A Varian 3500 gas chromatograph interfaced to a Finnigan MAT Model 800 ion trap detector was used for mass spectral (MS) identification of the GC components. Column and oven conditions were as stated above.

## RESULTS AND DISCUSSION

The yields of total volatiles (relative to the kumquat fruit used) were 0.011% (w/w) from steam distillation and 0.019% (w/w) from SPE. Table 1 shows the compounds identified in the samples along with their GC peak area percent and Kovats index on a DB-Wax column. For some compounds, formulas were deduced from MS fragmentation, but authentic compounds were not available for comparison. Those compounds are listed as "tentatively" identified. Peak area percent of overlapped peaks, such as (E,E)-α-farnesene and isopiperitenol, was calculated using a GC/MS total ion chromatogram (TIC). Among a total of 91 volatile compounds positively identified in the samples, 84 were found in the sample from steam distillation and 35 were found in the sample from SPE. Forty-seven compounds were identified for the first time in kumquat oil.



**Figure 1.** Structure of hinesol.

As in other citrus fruits, *d*-limonene was the most abundant compound, comprising 87.07% of the sample from steam distillation and 97.04% of the sample from SPE. Terpene alcohols and terpene esters were major abundant chemicals in the sample from steam distillation, but they were found only in trace amounts in the sample from SPE. Monoterpenes were the most abundant chemicals found in the sample from SPE, but other chemical groups were found in rather low levels. Many esters were found in the sample from steam distillation but only a few in the sample from SPE. The major differences between the samples from steam distillation and SPE were in the amounts and the numbers of sesquiterpenes recovered. The presence of at least 31 sesquiterpenes and related compounds was recognized in the sample from steam distillation by a MS fragmentation. On the other hand, only 9 sesquiterpenes were detected in the sample from SPE.

The major compounds (except *d*-limonene) found in the sample from steam distillation were linalool (1.44%), myrcene (1.32%), geranyl acetate (1.12%),  $\alpha$ -pinene (0.83%), and germacrene D (0.81%). The major compounds (in addition to *d*-limonene) found in the sample from SPE were myrcene (2.12%),  $\alpha$ -pinene (0.45%), and  $\beta$ -phellandrene (0.16%). It is interesting that  $\beta$ -pinene, one of the most abundant monoterpenes in essential oils, was found in trace amounts in the sample from SPE but was not detected in the sample from steam distillation.

Some compounds that are known to be responsible for off-flavors were found in the samples, in particular from steam distillation. They are *p*-cymene, dehydro-*p*-cymene, and *p*-cymen-8-ol. It has been hypothesized

that they form from monoterpenes and related compounds as a result of heat treatment (Kimura et al., 1984).

Hinesol has never been reported in citrus essential oils prior to this study. It is a sesquiterpene alcohol (Figure 1) and postulated to form from cyclization of two cyclodeca-1,6-diene conformers (McSweeney et al., 1970).

Kumquat oil contains more sesquiterpenes and sesquiterpenoids than other citrus oils such as orange and lemon (Shaw, 1979). Kumquat oil gives a somewhat bitter aroma rather than the fruity aroma of orange or lemon. This may be due to the low levels of aldehydes and esters in kumquat oil. Kumquat oil has not been widely used compared to other citrus oils. However, it contains interesting volatile chemicals, and further study of possible applications for this oil is in order.

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