ponents were probably present as such in the leaves. The data may therefore be useful in taxonomic studies, as well as in identification of compounds in leaf oils obtained commercially by steam distillation.

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Importance of Thymol, Methyl *N*-Methylanthranilate, and Monoterpene Hydrocarbons to the Aroma and Flavor of Mandarin Cold-Pressed Oils

Charles W. Wilson, III,* and Philip E. Shaw

Various combinations of thymol, methyl *N*-methylanthranilate, (DMA), γ-terpinene, and β-pinene were added to tangerine cold-pressed oil at levels found in Argentine (Sicilian) mandarin oil. The aromas of the compounded oils and Argentine mandarin oil were compared, and then the flavors of tangerine juice containing the compounded oils and mandarin oil were compared. The presence of both β-pinene and γ-terpinene in addition to thymol and DMA was necessary to give the tangerine oil an acceptable mandarin aroma. However, additional citrus components were necessary to give the tangerine oil an acceptable mandarin flavor in tangerine juice. Flavor thresholds were determined for DMA in water and for thymol and DMA in single-strength tangerine juice.

Mandarin oranges are a large and distinctive group of loose-skinned citrus fruit that show more variation among cultivars than do oranges or grapefruit (Hodgson, 1967). Horticulturists usually restrict the name tangerine to a class of mandarins having a deep red color characteristic of the variety Dancy tangerine (Hodgson, 1967). Kugler and Kovats (1963) attributed the distinctive flavor and aroma of Sicilian mandarin peel oil to the presence of methyl *N*-methylanthranilate and thymol but gave no evidence to support this claim. In addition, the Sicilian mandarin oil they examined was designated *Citrus reticulata* Blanco, whereas Hodgson (1967) described the principal mandarin in Sicily (and in Argentina) to be the Mediterranean cultivar *Citrus deliciosa* Tenore.

Thymol has been identified in Dancy tangerine peel oil and both methyl *N*-methylanthranilate (DMA) and thymol have been identified in Sicilian mandarin peel oil (Shaw, 1977). The reported quantities of thymol vary from 0.04 to 0.2% of the oil (Shaw, 1979), while the quantity of DMA as quantitated only once in mandarin peel oil was 0.9%. The flavor threshold of thymol in water has been reported to be 1.7 ppm (Moshonas et al., 1972), but the flavor threshold of DMA in water has not been reported. Neither threshold has been determined in mandarin or tangerine juice. Thus, the relative contributions of these two constituents to the flavor and aroma of mandarin peel oil or juice are difficult to assess from the available information.

The current study reports quantities of DMA and thymol present in Argentine mandarin and tangerine peel oils. The relative importance of these two constituents to the flavor of mandarin and tangerine cold-pressed oils and to tangerine juice flavored with these oils was determined.

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MATERIALS AND METHODS

Mandarin oil from Argentina was obtained from J. Manheimer, Inc., Long Island City, NY; commercial cold-pressed tangerine oil (a blend of oil from mostly Dancy plus a small percentage of Robinson tangerines) and frozen concentrated tangerine juice wthout added oil or other flavor fractions (evaporator pump out) were obtained from Citrus Central, Inc., Orlando, FL. Thymol (NF, Fisher Scientific Co., Fair Lawn, NJ) was 99.5% pure by gas chromatography (GC). DMA (ICN Pharmaceuticals Inc., Plainview, NY) was purified by thin-layer chromatography (TLC) on Silica gel G in 90:10 hexane–acetone prior to use, and the purified sample was shown to be 99.8% pure by GC.

β-Pinene (Columbia Organic Chemicals, Columbia, SC) and γ-terpinene (SCM Corp., Jacksonville, FL) were purified by GC on a 4 mm i.d. × 5 m glass column containing

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15% Carbowax 20M coated on 80–100-mesh Gas-Chrom P. The purified samples were shown to be better than 99.0% pure by GC.

Analyses of Oils. Quantitative analyses of thymol and DMA in cold-pressed tangerine and mandarin oils were performed on a Hewlett-Packard Model 5840 GC equipped with a 5840A GC terminal, and the instrument and analytical parameters were the same as those previously reported for the analysis of cold-pressed grapefruit oil (Wilson and Shaw, 1980). Separations were performed on a 30-m fused silica capillary column coated with methyl silicone (Hewlett-Packard, Avondale, PA), and 1-phenyl-2-propanol was the internal standard.

A fraction rich in DMA was isolated from 200 μL of tangerine oil with a silica Sep-Pak cartridge (Waters Associates, Inc., Milford, MA). The Sep-Pak was rinsed with 2 mL of hexane, and then 2 mL of hexane–diethyl ether (50:50 v/v) was added to elute a carbonyl fraction that afforded DMA by GC on a 4 mm i.d. × 5 m Carbowax 20M column. Identification of DMA was made by comparison of the infrared (IR) spectrum with that of an authentic sample and by comparison of retention times on a nonpolar glass capillary column.

Aroma and Flavor Evaluations. The following oil samples were used in the aroma and flavor panel studies listed in Table I. Cold-pressed mandarin oil was compared with tangerine cold-pressed oil alone or fortified with various combinations of thymol, DMA, γ-terpinene, and β-pinene at levels present in the mandarin oil (Table I). Also, tangerine oil and limonene both containing thymol and DMA at levels found in mandarin oil were compared with tangerine oil. The aromas of the oil samples were evaluated by a paired comparison test by a panel of 12 experienced judges (Larmond, 1974). Each panelist was presented two pairs of samples for a total of twenty-four aroma judgments. Replicate samples were presented at least 20 min apart to each panelist to minimize fatigue. Sample presentations were randomized and included both like and unlike pairs. The judges were asked whether the samples were alike or different.

Juice samples were prepared by thoroughly mixing 120 μL of one of the above oils with 200 g of frozen concentrated tangerine juice (61.66 °Brix) and then adding 996 g of tap water to reconstitute the single-strength juice (11.0 °Brix; 0.012% oil). Single-strength juice samples were evaluated by a triangle comparison difference test with two presentations each to the twelve panelists (Larmond, 1974). Aroma panels were performed first and flavor panels were conducted as soon as possible afterward. An initial test comparing tangerine oil to mandarin oil was conducted to be certain the panel could distinguish between the two oils.

Flavor Threshold Determinations. Flavor thresholds for DMA in water and for DMA and thymol in single-strength tangerine juice were determined by paired comparison, and the results were interpolated from the linear regression of the log of concentration vs. probability at four different concentrations (Harrison and Elder, 1950). At least one concentration above and below the threshold value was included in each test. The individual compounds were dissolved in absolute ethanol (250 μL) and then added to 1200 mL of water or 200 g of frozen concentrated tangerine juice. An equivalent amount of ethanol was added to the control sample in each case. The concentrated tangerine juice was reconstituted to single-strength juice as described above.

RESULTS AND DISCUSSION

The importance of selected volatile components to the flavor and aroma of Argentine (Sicilian) mandarin oil was determined by comparing it to compounded oils containing mixtures of thymol, DMA, γ-terpinene, and β-pinene. The compounded oils were prepared by addition of the individual components at the levels found in mandarin oil to either cold-pressed tangerine oil or to limonene. Table I lists the oils tested and the results of aroma tests performed on neat oils. It also lists results of flavor tests on the oils added to single-strength tangerine juice prepared from "evaporator pumpout" (frozen concentrated tangerine juice without added oil or other flavor fractions).

Table I. Effects of Thymol, Methyl *N*-Methylanthranilate (DMA), β-Pinene, and γ-Terpinene on the Aroma and Flavor of Tangerine and Mandarin Cold-Pressed Oils

	confidence level, %	
oil samples compared	aroma panel[a] with oils	flavor panel[b] with juices
tangerine vs. mandarin	99.9	99.9
tangerine, DMA vs. mandarin	99.9	99.9
tangerine, thymol, DMA vs. mandarin	99.9	99.9
limonene, DMA vs. mandarin	99.9	99.9
limonene, thymol, DMA vs. mandarin	95.0	99.9
tangerine, thymol, DMA, γ-terpinene vs. mandarin	95.0	
tangerine, thymol, DMA, γ-terpinene, β-pinene vs. mandarin	NS[c]	99.0
limonene, thymol, DMA vs. tangerine	95.0	99.9
tangerine, thymol, DMA vs. tangerine	95.0	99.0

[a] Paired comparison difference test. [b] Triangle comparison difference test. [c] NS, not significant at the 95% confidence level.

Table II. Levels of Important Flavor Components in Mandarin and Tangerine Oils

	wt %	
component	tangerine oil	mandarin oil
thymol	0.022	0.182
methyl *N*-methylanthranilate	0.072	0.652
γ-terpinene	1.74	14.0
β-pinene	0.17	1.8

Quantities of individual compounds that had to be added to limonene or tangerine oil so that they equaled the quantities in mandarin oil were determined on the basis of the glass capillary gas chromatographic analysis of the two cold-pressed oils, as shown in Table II. The current values were similar to those reported earlier for these compounds in mandarin and tangerine oil (Shaw, 1979). One component, DMA, had not been identified previously in cold-pressed tangerine oil. Its presence in this sample of tangerine oil was confirmed by its infrared spectrum.

From aroma and flavor panel studies on tangerine and mandarin oils (Table I), we can make some conclusions about the importance of thymol and DMA to the flavor and aroma of mandarin oil. In two tests conducted with DMA and DMA plus thymol added to tangerine oil, panelists were able to distinguish a difference in both aroma and flavor between the mandarin oil and tangerine oil containing either DMA or DMA plus thymol at the 99.9% confidence level. When limonene containing DMA or DMA plus thymol was compared to mandarin oil, the panel detected a difference in the aroma and flavor of the oils.

Table III. Flavor Threshold Values for Thymol and Methyl *N*-Methylanthranilate in Water and in Tangerine Juice

compd	threshold, ppb	r^2
in water		
thymol	1700[a]	ND
methyl *N*-methylanthranilate	20.3	0.98
in tangerine juice		
thymol	147 000	0.93
methyl *N*-methylanthranilate	157	0.96

[a] Reported earlier by Moshonas et al. (1972); r^2 was not determined (ND).

Results of these limited flavor panel tests and comments by the panelists suggested that mandarin oil contains other constituents besides thymol and DMA that differentiate its aroma and flavor from tangerine oil. A GC profile of Argentine mandarin oil showed several terpene hydrocarbons to be the major components of the oil. In addition to limonene, the major hydrocarbons were β-pinene and γ-terpinene (Table II). Tests were conducted to determine the influence of these two hydrocarbons on the aroma and flavor of mandarin oil. The levels of thymol, DMA, and γ-terpinene in tangerine oil were adjusted to the levels found in mandarin oil, and the compounded oil was compared to mandarin oil (Table I). Panelists were able to detect a difference in the aromas of the two oils at the 95% confidence level. Since the aroma was still deficient in mandarin character, β-pinene was added to the above mixture, and the compounded oil was compared to mandarin oil. No significant difference in aroma of the two oils was detected by the panel, but a difference in flavor was detected at the 99.0% confidence level. Thus, it appears that thymol and DMA contribute to the aroma and flavor of mandarin but not as much as previously stated. Terpene hydrocarbons, especially β-pinene and γ-terpinene, in addition to thymol and DMA appear to be necessary for a full mandarin aroma.

Tangerine oil is a commercially valuable oil and has an aroma distinctively different from that of Sicilian mandarin oil (Manheimer, 1979). When the flavor panelists compared neat tangerine oil with either tangerine oil or limonene containing thymol plus DMA at the levels found in mandarin oil, they were able to detect a difference in aroma at the 95% confidence level and a flavor difference at the 99.0% confidence level or better. Thus, the relatively low levels of thymol and DMA found in tangerine oil seem important to the distinctive aroma and flavor differences between tangerine and mandarin oils.

To better assess the influence of thymol and DMA on the flavor of tangerine and mandarin juices, we determined the flavor thresholds for these two compounds in tangerine juice (Table III). The flavor threshold of DMA in water was also determined since it had not been reported earlier. DMA was by far the more potent flavor component. Its flavor threshold in water was ~100 times lower than that of thymol, and its flavor threshold in bland tangerine juice was almost 1000 times lower than that of thymol. We estimated the thymol and DMA levels in tangerine juice flavored with mandarin oil to be 200 and 782 ppb, respectively, based on an oil level of 0.012% in the juice. The latter level is ~5 times higher than the threshold level of DMA in tangerine juice, whereas the former level is almost 700 times *lower* than the flavor threshold level for thymol in tangerine juice. Thus, DMA is much more likely to influence the flavor of juices containing Argentine mandarin oil than is thymol.

We attempted to estimate the amount of DMA present in tangerine evaporator pumpout by TLC according to Shaw and Wilson (1981). Lower limits of detection by TLC were ~500–1000 ppb in the pumpout reconstituted to single-strength juice. Attempts to determine DMA by direct injection of 1-μL samples of single-strength juice onto a glass capillary GC column showed that virtually none of this compound was present, but the lower limit of detection was not determined. However, the blandness of the pumpout suggests that the amounts of DMA in the pumpout were below threshold levels.

In conclusion, adding a mixture of thymol and DMA to tangerine cold-pressed oil did not afford an oil similar to Argentine mandarin oil in aroma and flavor even though the DMA was added at above the threshold level. Addition of two other components, β-pinene and γ-terpinene, to the above mixture produced a synthetic oil with an aroma that could not be distinguished from mandarin oil aroma by an aroma panel. Just as with orange flavor (Shaw, 1977), a mixture of several components in the proper proportions seems essential for simulating a mandarin flavor.

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