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Analysis of Volatile Constituents from Meyer Lemon Oil

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Thirty-one compounds were isolated and identified as Meyer lemon oil volatile constituents. Vacuum distillation, column chromatography, and gas chromatography were used for separation and purification and compound identifications were made by infrared spectroscopy and mass spectrometry.

Found were seven terpene hydrocarbons, seven sesquiterpene hydrocarbons, nine alcohols, seven carbonyl compounds, and the fungicide, *o*-phenylphenol. The taste threshold was determined for thymol, which was the major oxygenated constituent.

Expansion of Florida's lemon crop from 610,000 boxes in 1967 to 1,510,000 boxes 2 years later indicates a rapidly developing lemon industry in Florida (Florida Agricultural Statistics Citrus Summary, 1970). The Meyer lemon, which grows well in Florida, is considered by Swingle (1967) as probably a hybrid of lemon and some other species of citrus and is commonly designated as [*Citrus limon* × *C. sinensis*]. The juice is sufficiently similar to true lemon so that it is used for making both lemon concentrate and lemonade (Wenzel *et al.*, 1958). The oil has an unusual flavor and aroma and information on oil composition would be of value to the citrus industry.

The only previous studies on Meyer lemons compared juice yield and certain physical constants of 42 lemon varieties, but no individual components of the oils were identified (Kesterson and Hendrickson, 1958; Wenzel *et al.*, 1958). Wenzel *et al.* (1958) prepared and examined frozen concentrates from the 42 varieties and showed Meyer lemons to have the highest juice yield per box. However, they also noted that Meyer lemon peel oil causes an off-flavor in both the juice and concentrate. Kesterson and Hendrickson (1958) showed Meyer lemon oil to have the lowest aldehyde content of the 42 varieties studied.

The unique physical characteristics of Meyer lemon oil and the thymol-like aroma noted when handling whole fruit of this variety prompted an analytical study of its composition. The present paper describes the isolation and identification of volatile constituents from cold-pressed Meyer lemon oil and determination of the taste threshold of thymol, the major oxygenated component.

EXPERIMENTAL

Cold-pressed Meyer lemon oil (43 g) was obtained using an FMC in-line extractor from five boxes of fresh Meyer lemons picked on March 29, 1971.

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Gross Separation Procedures. DISTILLATION. This oil was distilled at a bath temperature of 34°C and 0.5 mm in a rotary evaporator until most of the terpene hydrocarbons (99% limonene) were removed (39.5 g). The liquid nitrogen trap located between the receiver and the vacuum pump contained 0.9 g of liquid and the nondistilled residue weighed 2.1 g.

Liquid Adsorption Chromatography. The 2.1 g of residue was further separated into three fractions on a 1-in. × 17-in. ice water-jacketed (9°C) column containing 60–100 mesh Florisil (Fisher Scientific Co.). The fractions were eluted successively with 400 ml of hexane to remove the hydrocarbons, 400 ml of a 50/50 hexane–ethyl ether solution to concentrate the carbonyl-containing compounds, and 300 ml of absolute ethanol to strip the column of any remaining compounds. The weight of material in each fraction upon removal of the solvent was as follows: hexane, 0.5 g; hexane–ether solution, 1.3 g; and ethanol, 0.2 g.

Glc Procedures. Analyses of these fractions as well as material collected in the liquid nitrogen trap and the distillate from the rotary evaporator were carried out on an F&M Model 810 gas chromatograph equipped with dual thermal conductivity detectors using either a 1/4-in. × 20-ft column packed with 20% Carbowax 20M on 60–80 mesh Gas Chrom P or a 1/4-in. × 20-ft column packed with 10% of the nonpolar liquid phase UCW-98 (Applied Science Laboratories, Inc., State College, Pa.) on 60–80 mesh Gas Chrom P (Applied Science Laboratories, Inc.). For all runs the injection temperature was 250°C, the detector temperature was 280°C, the detector milliamperage was 150, and the carrier gas was helium.

Mass and Infrared Spectral Methods. Fractions were either collected in short capillary tubes for infrared and mass spectral analysis or were run into the mass spectrometer directly from gas chromatography. Mass spectra were obtained with either the Bendix Model 3012 (TOF) or the CEC Model 21–490 mass spectrometer, and infrared spectra were obtained with a Perkin-Elmer Model 137 spectrophotometer.

Spectra for each compound identified were compared to

Table I. Compounds Identified in Meyer Lemon Peel Oil

Alcohols	Hydrocarbons
Thymol	Limonene
Linalool	γ -Terpinene
α -Terpineol	<i>p</i> -Cymene
Citronellol	Terpinolene
Nerol	α -Pinene
1- <i>p</i> -Menthen-9-ol	β -Pinene
1,8- <i>p</i> -Menthadien-9-ol	Myrcene
Terpinen-4-ol	α -Cubebene
<i>p</i> -Cymene-8-ol	α -Copaene
Aldehydes	<i>trans</i> - α -Bergamotene
Citronellal	α -Selinene
Neral	Farnesene
Geranial	β -Elemene
Esters	β -Bisabolene
Citronellyl acetate	Miscellaneous
Neryl acetate	<i>o</i> -Phenylphenol
Geranyl acetate	
1,8- <i>p</i> -Menthadien-9-yl acetate	

Table II. Flavor Thresholds of Thymol in Water Using Paired Comparison Tests

Thymol level, ppm	Correct judgments (30 presentations)	Confidence level, %
1.5	17	Not significant
1.7	21	95
2.0	26	99.9

those from authentic samples. Commercially available compounds were used to obtain spectra of authentic samples, with all but the following exceptions. Published spectra were used for identification of *p*-cymen-8-ol (Andersson *et al.*, 1964) and α -selinene (Wenninger *et al.*, 1967; Moshonas and Lund, 1970). The remaining compounds that could not be purchased were identified by spectral comparison with authentic samples obtained previously at our laboratory by the authors indicated: limonene, γ -terpinene, *p*-cymene, terpinolene, α - and β -pinene, myrcene, α -cubebene, α -copaene, α -bergamotene, farnesene, β -elemene, and β -bisabolene by Hunter and Brogden (1965); 1-*p*-menthen-9-ol, 1,8-*p*-menthen-9-ol, *o*-phenylphenol by Hunter and Moshonas (1966); and 1,8-*p*-menthadien-9-yl acetate by Moshonas (1967).

Taste Panel Methods. Flavor samples were made up in 1-l. batches using aliquots of a stock solution prepared by dissolving 10 mg of thymol (N.F., Fisher Scientific Co.) in 30 μ l of absolute ethanol, adding this solution to a little distilled water, and diluting to 100 ml with distilled water. An equivalent amount of absolute ethanol was added to the distilled water control sample each time (Berry *et al.*, 1967).

All flavor evaluations were made using the paired comparison tests described by Boggs and Hanson (1949) with 15 panelists each being given two presentations. Panelists were asked to select the sample that was not the control water sample.

RESULTS AND DISCUSSION

The compounds isolated and identified from Meyer lemon oil in this study are shown in Table I. Each compound was identified by comparison of its infrared or mass spectrum as well as glc retention time with that of an authentic sample. All compounds identified are being reported as Meyer lemon oil constituents for the first time. Nine of these constituents have not been previously found in any lemon oil study. These are: thymol, 1-*p*-menthen-9-ol, *p*-cymen-8-ol, 1,8-*p*-menthadien-9-yl acetate, α -cubebene, α -copaene, α -selinene, farnesene, and β -elemene. Caution was taken to prevent rearrangement or decomposition of compounds by using low temperature reduced pressure distillation and cold water-cooled columns for liquid adsorption chromatography.

A quantitative estimation made from glc peak areas showed the two major constituents to be limonene (92%) and thymol (6%). Geranial and neral, which are important flavor constituents of most lemon oils, were found only in trace quantities. Since the whole oil had a strong odor characteristic of thymol and since its presence presumably had a great impact on the oil flavor, its taste threshold was determined by a trained panel, as shown in Table II. This panel noted the unusual taste of thymol and felt it would have an objectionable taste in citrus products if present in high quantity.

Analytical results of this study on Meyer lemon oil have shown that the off-flavor produced in lemon juice whenever small quantities of this oil are present (Wenzel *et al.*, 1958) is undoubtedly due to the large amount of thymol present. Taste threshold studies on thymol have indicated that a relatively small amount of Meyer lemon oil in a juice product could cause off-flavor. Furthermore, one of the factors that contributes most to the value of a lemon oil, namely a high level of citral (Wenzel *et al.*, 1958), is lacking in Meyer lemon oil. The differences found in the composition of Meyer lemon oil and the true lemon oil may be explained by noting that the Meyer lemon is considered a hybrid of lemon and sweet orange (Swingle, 1967) [*Citrus limon* \times *C. sinensis*]. If this oil is to be a commercially valuable product, use should be made of its thymol-like odor and flavor rather than its potential to produce a lemon-like flavor.

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 References to brand names are for identification and do not imply endorsement.