Analysis of Tangerine Essence Oil and Aroma Oil

A comparison was made between oil separated during the preparation of commercial tangerine essence (essence oil) from juice and oil separated after distillation of an aqueous slurry from ground tangerine peel (aroma oil). Gas chromatographic analysis of the 20 main constituents showed the two oils to be similar in composition. The most volatile fraction from essence oil was also analyzed by gas chromatography and the separated components were identified from their mass and infrared spectra. This volatile fraction contributed a desirable, essence-like quality when added to single-strength orange juice at a level of 25 ppm.

Distilled citrus essence oils have been recovered for several years as by-products during the production of commercial aqueous citrus essences (Byer and Lang, 1964). With recent emphasis on environmental concerns, citrus processors have been improving the disposal of liquid wastes partly by recovering additional distilled oils (aroma oils) which are sold for their D-limonene content (Veldhuis *et al.*, 1972). The potential for these essence oils and aroma oils as sources for other valuable chemicals or as flavoring agents has been explored for the orange (Coleman *et al.*, 1969; Coleman and Shaw, 1971) and grapefruit (Coleman *et al.*, 1972), but not for tangerine, the third major type of citrus processed in Florida. Tangerine essence oils and aroma oils are colorless, and they possess strong citrus aromas that are mildly tangerine-like in aroma character.

In order to investigate these oils as potential sources for valuable compounds and to provide a background for quality determination, an analytical study was carried out. This paper reports the results of that study undertaken to determine the quantitative and qualitative composition of tangerine essence oil and aroma oil. In that study, analysis and taste evaluation of a water-soluble volatile fraction from essence oil were emphasized.

EXPERIMENTAL SECTION

Samples. Tangerine essence oil is the oily layer separated from aqueous tangerine essence when the vapors from the first stage of an evaporator used in making frozen tangerine concentrate are condensed in an essence recovery unit. These oils from several plants were combined and marketed as tangerine essence oil. Samples of this material were obtained from Redd Laboratories, Safety Harbor, Fla. Tangerine aroma oil was prepared as described by Veldhuis *et al.* (1972) by fractional steam distillation of the aqueous extract from ground tangerine peel and separation of the oily layer from the distillate. All samples were stored at 4° until analyzed.

Whole Oil Analysis. Samples of oils described above were injected directly into the gas–liquid chromatograph (glc) for qualitative and quantitative analysis. Relative estimations of individual components from whole essence and aroma oils in Table I were made by relating individual peak areas to total area under the curve. Whole essence and aroma oils were analyzed on a polar column using a Hewlett-Packard Model 7620A gas chromatograph equipped with a thermal conductivity detector with a block temperature of 285°, an injection port temperature of 280°, and a He flow of 100 ml per min. The 0.20 in. i.d. × 20 ft stainless steel column employed was packed with 20% Carbowax 20M on 60 to 80 mesh Gas Chrom P with temperature programming of: 135° isothermally for 26 min; 200° isothermally for 30 min; and 225° isothermally to the end of the run. Temperature increases were at 30° per min.

Essence Oil Fractionation and Analysis. Tangerine essence oil was distilled under reduced pressure in a Swissco rotary evaporator with two nitrogen traps in the system between the chilled water condenser and the vacuum pump. Distillation of 397 g of tangerine essence oil at 25–30° and 5 mm pressure afforded 7.58 g of material in the first liquid nitrogen trap (volatile fraction no. 1). When this trap was replaced with a clean trap, the pressure then dropped to 2.5 mm and distillation was continued at 50–55° to afford three fractions: 7.40 g of material in the first liquid nitrogen trap (volatile fraction No. 2); 342 g of chilled water condensate (volatile fraction No. 3); and 40 g of pot residue. The second liquid nitrogen trap (nearer the vacuum pump) contained no condensate. The Swissco evaporator (Buchi Rotavapor R, Type KRV 65/45, Rinco Instrument Co., Greenville, Ill.) had been carefully cleaned and dried to avoid solvent contamination of the volatile fractions trapped at liquid nitrogen temperature. The water-soluble volatile fraction (no. 1) condensed at liquid nitrogen temperature was analyzed on both polar and nonpolar columns using an F&M Model 500 gas chromatograph equipped with a thermal conductivity detector with a block temperature of 245°, an injection port temperature of 295°, and a He flow of 100 ml per min. Stainless steel columns 0.20 in. i.d. × 18 ft long were employed. The polar column was packed with 20% Carbowax 20M on 60 to 80 mesh Chromosorb W with temperature programming of: 70° isothermally for 42 min; then raised to 90° at 30° per min; and programmed at 2° per min to 225°. The nonpolar column was packed with 20% UCW-98 on 60 to 80 mesh Chromosorb W with temperature programming of: 70° isothermally for 24 min; then raised to 90° at 30° per min; and programmed at 2.1° per min to 225°. The water-insoluble liquid nitrogen trap condensate (no. 2) and the chilled water condensate (no. 3) were analyzed on the polar column under the above conditions. Relative estimates of individual components in each fraction were made by integrating a typical glc curve and relating individual peak areas to total area under the curve.

Mass and Infrared Spectral Methods. Mass spectra (ms) were obtained with a Bendix Time-of-Flight Model 3012 spectrometer. Infrared (ir) spectra were obtained on a Perkin-Elmer Model 137A Infracord either in carbon disulfide or as oil films. Spectra were compared with those from authentic samples. Sources for authentic samples were either cited previously (Coleman and Shaw, 1971) or obtained from commercial sources, with the following exception. The authentic sample of α-sinensal was obtained from Valencia orange peel oil (Moshonas and Lund, 1969).

Taste Tests. All taste tests were conducted using single-strength orange juice for the control samples prepared from a high-quality commercial concentrate which contained cold-pressed peel oil, but which contained no added aqueous

Table I. Quantitative Estimation of Whole Tangerine Essence and Aroma Oils

Compound	Retention time, min	Spectra obtained: Essence oil	Spectra obtained: Aroma oil	Area, %: Essence	Area, %: Aroma
Acetaldehyde	3	ms		0.02	
Ethanol	4.5	ms		1.83	
α-Pinene	8	ms	ir	0.98	1.04
Myrcene	12	ir	ir	2.32	2.29
D-Limonene	22	ir	ir	89.74	93.43
γ-Terpinene	24	ir	ms, ir	2.67	2.15
p-Cymene + octanal	24.5	ms, ir	ms, ir	0.23	0.12
Terpinolene	25	ms, ir	ir	0.07	0.04
Nonanal	31	ms, ir	ir	0.06	0.08
Citronellal	34	ms, ir	ms, ir	0.03	0.04
Linalool	35	ms, ir	ir	1.47	0.39
Terpinen-4-ol	39	ir, ms	ir	0.04	0.02
α-Terpineol	43	ir	ir	0.09	0.18
Geranyl acetate + β-elemene	45	ms, ir	ir	0.05	0.01
Citronellol	46	ms, ir	ir	0.05	0.04
Perillaldehyde	52	ir	ir	0.04	0.01
Thymol	79	ir	ir	0.03	0.04
α-Sinensal	102	ir	ir	0.07	0.01

Table II. Composition of Volatile Fractions from Tangerine Essence Oil

Compound	Retention time, min: 20M	Retention time, min: UCW-98	Area % of volatile fraction[a]: No. 1	Area % of volatile fraction[a]: No. 2	Area % of volatile fraction[a]: No. 3
Acetaldehyde	5	2	1.7	0.02	
Acetone	8		0.02		
Methyl acetate	9		0.02		
1,1-Ethoxymethoxyethane	10	12	0.1	0.2	
Tetrahydrofuran	12		0.02		
1,1-Diethoxyethane	13	19	1.0	1.5	
Ethyl acetate	13	8			
Methanol	14	3	3.6		
Ethanol	17	4	79.1	1.7	
Diacetyl	21		Trace		
α-Pinene	32	42	0.5	4.0	0.9
β-Pinene	49	46	2.5	0.8	0.5
m- or *p*-Xylene	53		0.4		
Myrcene	57		0.5	3.6	2.4
D-Limonene	62	50	10.3	87.4	92.5
γ-Terpinene		51	0.8	0.8	3.7

[a] Carbowax 20M column.

essence. Threshold levels were determined using the triangular comparison tests discussed by Boggs and Hanson (1949), with 12 experienced tasters being given two presentations each.

Samples of tangerine essence oil water-soluble volatiles were added to 1200 ml of single-strength orange juice prepared from the above concentrate in decreasing increments until the flavor threshold was established. In another series of taste tests, tangerine essence oil volatiles at 25 ppm added to orange juice were compared with similar juice without these volatiles in a paired comparison test as described by Boggs and Hanson (1949). A panel of six members experienced in detecting aqueous orange essence added to orange juice was employed. Each was asked to choose the sample that possessed a more essence-like aroma and flavor.

RESULTS AND DISCUSSION

The tangerine essence oils and aroma oils were similar in composition both by qualitative analysis and by relative quantitative estimation. Twenty compounds identified from the analysis of whole essence oil and aroma oil are listed in Table I in order of their glc retention times. Spectral means of identifying each component and quantitative estimates are given. Qualitatively, all but the two most volatile components, acetaldehyde and ethanol, were found in both oils. The most notable quantitative differences were the much larger quantities of linalool and α-sinensal present in the essence oil when compared to the aroma oil sample. Table I lists only a combined area percent for two compounds when their glc peaks are unresolved. Quantitative values in Table I are considered estimates only, because response factors were not determined for individual components (Keulemans, 1959).

Finding significant quantities of ethanol (1.83%) and acetaldehyde (0.02%) present in the tangerine essence oil sample was of special interest. Although these volatile compounds had been found in previous studies on orange and grapefruit essence and aroma oils (Coleman and Shaw, 1971; Coleman *et al.*, 1972), neither of those oils contained the high quantities of these two volatile compounds present in tangerine essence oil. Because almost 2% ethanol was present in this essence oil, a volatile fraction (No. 1) was condensed at liquid nitrogen temperature before any appreciable amount of D-limonene began to distil. This fraction, representing 1.9% of the starting oil, was water soluble and possessed a strong, essence-like aroma.

Continued distillation of the tangerine essence oil sample produced two additional volatile fractions. Volatile fraction no. 2 also condensed at liquid nitrogen temperature, and that fraction representing 1.9% of the starting oil was water insoluble. Volatile fraction no. 3 contained the bulk of the distillate condensed at chilled water temperature. The composition of these two fractions is shown in Table II, as determined by integrating peak areas under the glc curves. Individual peak areas were calculated as half the peak height times the width between inflection tangents at the base (Keulemans, 1959).

Volatile fraction no. 1 from tangerine essence oil was analyzed by glc to afford 16 identified components, and they are listed in Table II in order of their increasing retention times on a Carbowax 20M column. In some cases, as indicated in Table II, retention times on both polar and nonpolar columns were obtained. 1,1-Diethoxymethoxyethane and ethyl acetate were unresolved by glc and accurate individual quantitative estimates could not be made. Two of the components identified from this volatile fraction, methyl acetate and tetrahydrofuran, had not previously been reported as citrus components. Another component, diacetyl, was detected in only trace quantities, but its odor was prominent in this fraction. Either *m*- or *p*-xylene was also a trace constituent. *m*- or *p*-Xylene had been identified in aqueous orange essence by Schultz *et al.* (1964), who considered it a possible artifact. Methanol, β-pinene, and γ-terpinene were the remaining identified volatile compounds that had not been found in either orange or grapefruit essence oils. β-Pinene and γ-terpinene were two of the hydrocarbon components from tangerine peel oil identified by Hunter and Brogden (1965). No single component isolated in this study possessed a characteristic tangerine-like aroma.

Table II also lists the estimated composition of this water-soluble volatile fraction as determined by integrating peak areas under the glc curve. The major component of this volatile fraction from tangerine essence oil was ethanol (79%)

and its abundance accounts for the water solubility of this fraction. The most volatile fractions from both orange and grapefruit essence oils were not water soluble and had D-limonene as the main component in each case (Coleman and Shaw, 1971; Coleman *et al.*, 1972). Lack of water solubility for these fractions had prevented meaningful taste evaluation of them in single strength citrus juices.

The taste threshold of the sample of water-soluble volatile components from tangerine essence oil and its flavor quality were determined in single-strength orange juice. Triangular taste tests were employed for taste threshold determinations with an initial level sufficiently high (83 ppm) for panel members to become acquainted with the flavor being evaluated. The concentration was presented in successive tests at 41, 25, 8, and finally 17 ppm. The lowest level at which a significant difference was detected was 25 ppm (16 correct of 24 judgments or >99% significance; Krum, 1955). Flavor quality was then evaluated using a paired comparison test with the experimental sample containing 25 ppm of the water-soluble volatiles. Panelists experienced in tasting aqueous orange essence added to orange juice were employed in this study. The panel judged these tangerine essence oil volatiles to have a desirable essence-like flavor when added to single-strength orange juice (10 correct of 12 judgments or 95% significance; Krum, 1955). Thus, a potent water-soluble fraction of volatile components with a desirable essence-like aroma and flavor can be separated from a citrus essence oil and used to impart a fresher flavor to orange juice.

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Richard L. Coleman
Philip E. Shaw*

Citrus and Subtropical Products Laboratory
Southeastern Marketing and Nutrition Research Division
Agricultural Research Service
U. S. Department of Agriculture
Winter Haven, Florida 33880

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Determination of Sulfur in Plant Material Using a Leco Sulfur Analyzer

A method is described for the determination of sulfur in plant material using a Leco Sulfur Analyzer. A 0.05-g finely ground dried (at 80°) plant tissue sample is weighed into a combustion cup containing a small amount of iron accelerator. Magnesium oxide is added to cover the tissue sample. The combustion cup containing the sample is muffled at 500° for 1 hr. Iron and tin accelerator are added in layers and the combustion cup is placed into the induction furnace of the Leco Sulfur Analyzer. The combustion gases are passed over antimony before entering the titration chamber of the titrator. Sulfur determinations were made on five different plant tissues and compared with results obtained by other laboratories. The precision of the method was determined (σ 0.0088) by repeated analyses of NBS Standard 1571, Orchard Leaves.

Sulfur is becoming increasingly important in the production of various field and vegetable crops in Georgia. Analysis of plant tissue from suspected sulfur-deficient plants offers a means of verifying the deficiency. The Georgia Soil Testing and Plant Analysis laboratory purchased a Leco Sulfur Analyzer in hopes of using the instrument to determine the sulfur in plant tissue on a routine basis. A number of procedures were considered based on the review of methods for the determination of sulfur in agricultural samples prepared by Beaton *et al.* (1968). Of all the procedures available, the Leco Sulfur Analyzer offered the best alternative based on simplicity of operation and speed. Personal experiences from several who had successfully used the Leco Sulfur Analyzer for plant tissue analyses were encouraging (Castenson, 1970; Ferrara, 1969; Trowbridge, 1969). However, the results obtained with a Leco Sulfur Analyzer for total sulfur analyses in soils had not proven to be entirely acceptable (Bremner and Tabatabai, 1971). The problem seems to be related to sample preparation. Although a Leco method for the determination of total sulfur in soil has been published (Tabatabai and Bremner, 1970), there is no published procedure using the Leco Sulfur Analyzer for plant material.

A sulfur analysis procedure for plant material has been developed in this laboratory which gives sulfur results comparable to those determined by other laboratories and methods.

APPARATUS

The sulfur analyzer is manufactured by the Leco Laboratory Equipment Corp., St. Joseph, Mich., and consists of: an induction furnace, (Model 521-500), with the "L" modification, combustion tube (Leco No. 519-4), and ignitor (Leco No. 519-5); sulfur titrator, (Model 532-000); gas purifying train; source of pure oxygen, and sample crucibles (Leco No. 528-14). The gas train is modified to pass the combustion gases over antimony prior to entering the reaction chamber of the titrator. The principle of the combustion method is given in detail in Leco Form 100(10-66) (Leco Laboratory Equipment Corp., 1966).