

Analysis of Grapefruit Essence and Aroma Oils

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A comparison was made between oil separated during the preparation of commercial grapefruit essence (essence oil) and oil separated after distillation of the aqueous discharge from grapefruit peel oil centrifuges (aroma oil). Qualitative analysis and quantitative estimation of the main constituents showed the two oils to be similar in composition. The 52 compounds identified from grapefruit essence and aroma oils include nine alcohols, eight aldehydes, 11 esters, two ethers, five ketones, and 17 hydrocarbons. The most volatile fractions of

both oils were analyzed separately by gas chromatography and mass and infrared spectra. Nine of the volatiles identified in this work not previously reported as grapefruit constituents are: pentane, hexane, heptane, toluene, 2-propanol, ethyl vinyl ketone, ethyl propionate, 3-pentanone, and ethoxy-methoxyethane. The latter compound had not previously been reported in citrus. One sesquiterpene hydrocarbon, epi- α -selinene, was also identified as a new citrus constituent.

Utilization of citrus byproducts has become of increasing interest to the citrus industry in recent years. Orange and grapefruit aqueous essences are now recovered in commercial essence recovery units when the respective fresh juices are being concentrated, and can be added back to the concentrated juice to improve its flavor. During the process of collecting aqueous essence, an oily layer (essence oil) floats to the top of the essence solution and is separated from it before aqueous essence is utilized (Byer and Lang, 1964).

Recent publications from this laboratory have reported the composition of orange essence oil (Coleman *et al.*, 1969; Coleman and Shaw, 1971) and of aqueous grapefruit essence (Moshonas and Shaw, 1971). Grapefruit essence oil has an odor similar to that of the aqueous essence, but it has not yet been used commercially to flavor citrus products. However, grapefruit essence oil has been used in experimental packs of gel-coated ready-to-serve grapefruit halves (Rouse *et al.*, 1969).

Distilled grapefruit aroma oils and aqueous aroma solutions have been produced in our laboratory by distilling the aqueous discharge from commercial grapefruit peel oil centrifuges (Veldhuis *et al.*, 1970). Aroma oils are produced from what is currently regarded as waste material in a citrus plant, whereas essence oils are produced from fresh grapefruit juice during its concentration process. Aroma oils are similar to essence oils both in odor and physical appearance, and should be good flavoring agents for citrus products. 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 qualitative and quantitative composition of grapefruit essence and aroma oils. In that study, comparisons of the most volatile fractions were emphasized.

EXPERIMENTAL

Source and Preparation of Samples. Commercial essence oil samples were obtained from Redd Laboratories, Safety Harbor, Fla. Aroma oil samples were prepared from grapefruit centrifuge effluent by the same process described by Veldhuis *et al.* (1970) for orange essence oil. Duncan and Marsh grapefruit blends were used in all cases. All samples were stored at 4°C until analyzed.

Whole Oil Analysis. Samples of oils described above were injected directly into the gas-liquid chromatograph (glc) for quantitative analysis. Quantitative estimation of individual components from whole essence and aroma oils in Table I was made by relating individual peak area to total area under the curve.

Gross Separation Procedures. **DISTILLATION.** The essence and aroma oils were distilled under reduced pressure in a Swissco rotary evaporator with two liquid nitrogen traps in the system between the chilled water condenser and the vacuum pump. One liquid nitrogen trap was placed adjacent to the Swissco evaporator and the other adjacent to the vacuum pump. Three fractions were obtained: pot residue, chilled water (9°C) condensate, and liquid nitrogen trap condensate (in trap adjacent to Swissco). The second trap contained no condensate. Essence oil (415 g) was distilled at 35–42°C and 1.5 mm pressure yielding 25.8 g of pot residue, 373 g of chilled water condensate (mostly D-limonene), and 2.5 g of liquid nitrogen trap condensate. Aroma oil (427 g) distilled at 0.75–1.0 mm with a bath temperature of 39°C maximum yielded 23.0 g of pot residue, 399.2 g of chilled water condensate (mostly D-limonene), and 3.1 g of liquid nitrogen trap condensate.

The Swissco evaporator was carefully cleaned and dried to avoid solvent contamination of the volatile fraction trapped at liquid nitrogen temperature. Both liquid nitrogen trap samples were taken directly from the trap and injected onto polar and nonpolar glc columns. Essence and aroma oil components were identified by retention times plus mass and/or infrared spectral comparison with authentic samples.

Liquid Adsorption Chromatography. Pot residues from both essence and aroma oils were further studied after 2.2 g

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

Compound	R.T. (min)	Spectra obtained		% Area under curve	
		Essence oil	Aroma oil	Essence	Aroma
Heptane	3.0		ms		<0.01
Acetone	4.2		ms		<0.01
Ethanol	8.4	ms		0.03	
α -Pinene	13.8	ir, ms	ms	0.05	0.15
Myrcene	20.4	ir, ms	ms		
D-Limonene ^a	26.4	ir	ir	99.18	98.50
Octanal	27.6	ir, ms	ir, ms	0.09	0.37
Nonanal	33.0	ir, ms	ir, ms	0.03	0.02
Decanal	38.2	ir, ms	ir, ms	0.05	0.23
Linalool	39.4	ir, ms	ir, ms	0.08	0.18
β -Caryophyllene	45.0	ir, ms	ir	0.09	0.06
α -Terpineol	47.8	ir	ir, ms	0.07	0.07
Geranial	49.8	ir	ir	0.10	0.07
Carvone	51.0		ir		<0.01
Valencene	51.0	ir	ir	0.10	0.06
Δ -Cadinene	52.2	ir	ir	0.02	0.05
Nootkatone	103.0	ir	ir	0.04	0.03

^a $[\alpha]_{25}^{20} + 112^\circ$ (c 1.07 in EtOH) from essence oil; $[\alpha]_{25}^{20} + 130^\circ$ (c 0.46 in EtOH) from aroma oil.

of each was carefully poured into a 50 mm \times 775 mm ice water-jacketed column packed with 65 g of 60–100 mesh Florisil at atmospheric pressure. Each column was eluted successively with four 100-ml portions of hexane, two 100-ml portions of 5% ether in hexane, seven 100-ml portions of 10% ether in hexane, 100 ml of 100% ether, 100 ml of 5% ethanol in ether, and 100 ml of 100% ethanol. Solvents were removed from each fraction under reduced pressure. Infrared spectra were taken of all fractions and those fractions which had nearly identical spectra were combined. Each fraction was then analyzed by glc and the combined analytical results are listed in Table III.

Glc Procedures. The polar column was packed with 20% Carbowax 20M on 60–80 mesh Chromosorb W and the non-polar column with 20% UCW-98 on 60 to 80 mesh Chromosorb W using 0.20-in. i.d. \times 18 ft stainless steel tubing in both cases. The gas chromatograph was an F&M Model 500 with a thermal conductivity detector: block temperature 245°C, injection port temperature 295°C, and He flow of 100 ml per min.

Whole essence and aroma oils and their respective chilled water condensate and pot residue samples were analyzed on the Carbowax 20M column with the temperature programmed as follows: 75°C for 6 min, 90°C for 6 min, 120°C and 2.1°C/min to 225°C, and then the temperature was maintained isothermally at 225°C until the last peak eluted.

Essence oil volatiles were analyzed on the Carbowax 20M column with the following temperature program: isothermally at 60°C for 36 min, then increased to 90°C for 14 min and then raised to 120°C and programmed at 2.1°C/min to 225°C; on the UCW-98 column, the temperature program was 60°C isothermally for 65 min, then 2.1°C/min to 225°C.

Aroma oil volatiles were analyzed on the Carbowax 20M column with the following temperature program: initially 60°C for 24 min, next increased to 90°C and held isothermally for 16 min, then raised to 120°C and programmed at 2.1°C/min to 225°C. On the UCW-98 column the aroma oil volatiles were programmed as follows: initially at 60°C for 26 min, next 90°C for 57 min, then 2.1°C/min to 225°C.

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 for each compound identified were compared with those from authentic samples. Sources for authentic samples were cited previously (Coleman and Shaw, 1971) with the following additions: 2-propanol, pentane, 3-pentanone, and toluene were obtained from commercial sources and ethoxymethoxyethane was prepared as described below.

Preparation of Ethoxymethoxyethane. A solution of 2.36 g (20 mmol) of acetal (Eastman Kodak Co.) and 0.84 g (29 mmol) of methanol was treated with a trace of para-toluene-sulfonic acid and stirred 18 hr at room temperature. The reaction products were separated by injecting the reaction mixture onto the Carbowax 20M glc column described above. With the temperature held isothermally at 75°C, the following compounds, as listed in increasing order of retention times, were identified by mass spectra, and by infrared spectra where indicated: ethoxyethylene, 1,1-dimethoxyethane, 1-ethoxy-1-methoxyethane: ir (CS₂), 2910 (m), 2850 (m), 1375 (m), 1330 (w), 1200 (w), 1135 (s), 1100 (s), 1080 (s), 1060 (m), 1050 (m), 995 (w), 940 (w), 927 (w), 855 (m), 815 (w); methanol, acetal, and ethanol.

RESULTS AND DISCUSSION

Seventeen components identified from the analysis of whole oils are listed in Table I in order of their glc retention times. Spectral means of identifying each component and quantitative estimates are given. Myrcene appeared as a shoulder on the D-limonene peak in both oils, and it could not be estimated quantitatively. The quantitative values given in Table I are considered estimates only, because response factors were not determined for individual components (Keulemans, 1959).

The essence and aroma oils were similar in composition both by quantitative estimation and by qualitative analysis. In order to make a more complete qualitative analysis a combination of distillation, liquid adsorption chromatography and glc separation was used. All 52 compounds identified by this more thorough analysis of essence and aroma oils are

Table II. Volatiles from Grapefruit Essence and Aroma Oils

Compound	Spectra obtained		R.T. (min)	
	Essence oil	Aroma oil	UCW-	
			20M	98
Acetaldehyde	ms		4	4
Pentane	ms		2	
Hexane	ms	ms	3	
Heptane	ms	ms	4	27
Acetone	ms	ms	8	6
Ethoxymethoxyethane	ms, ir	ms	10	20
Ethyl acetate	ms	ms	13	14
Diethoxyethane (acetal)	ms		14	33
Ethanol	ms	ms	17	5
2-Propanol	ms		17	7
3-Pentanone		ms	22	
Ethyl propionate	ms		20	
Methyl butyrate	ms		24	
Ethyl vinyl ketone	ms	ms	28	27
Ethyl butyrate	ms		35	51
α -Pinene	ms	ms	35	95
Toluene		ms	35	33
Sabinene	ir	ms	40	
Myrcene	ir	ms	42	
Limonene	ir	ir	48	
Octanal	ms	ms	49	

listed in Tables II and III. Only minor differences are seen in the composition of the two oils.

From both essence and aroma oils, the distillation fraction trapped at liquid nitrogen temperature was judged by the authors to possess most of the essence-like odor characteristic of these oils, and was therefore carefully examined. Table II lists the compounds identified in this volatile fraction from each oil. Nine compounds were identified in this study that had not previously been reported as grapefruit constituents. Four of these, hexane, heptane, ethyl propionate, and ethyl vinyl ketone, had been found in the corresponding volatile fraction from Valencia orange essence oil (Coleman and Shaw, 1971). A fifth compound, 3-pentanone, had been re-

ported by Schultz (1970) as a constituent of orange juice head-space volatiles. The remaining three new grapefruit constituents were traces of the hydrocarbons pentane and toluene, and a mixed ketal, ethoxymethoxyethane. Schultz *et al.* (1964) suggested that toluene and other volatile hydrocarbons found in orange juice could have come from solvent used to apply resin to the fruit. Ethoxymethoxyethane had been found previously in strawberries (Teranishi *et al.*, 1963) and in grapes (Stevens *et al.*, 1966), but not in citrus. The ethoxymethoxyethane could have been formed from acetal and methanol in the acidic grapefruit juice medium. The authentic sample for comparison was formed in this manner by treating acetal and methanol with a trace of *p*-toluenesulfonic acid (Jansson, 1963). Glc separation of the crude reaction mixture revealed the expected products, *i.e.*, the three possible ketals, dimethoxymethane, ethoxymethoxyethane, and acetal, plus methanol and ethanol. Ethoxyethylene, the most volatile compound identified, was an unexpected product and is probably an artifact formed from acetal during the glc separation (Evans and Williamson, 1970).

Notable differences were seen in the qualitative composition of the two liquid nitrogen trap fractions from essence and aroma oils despite their similar strong essence-like odors. Fewer compounds were found in aroma oil volatiles. Only one (ethyl acetate) of the four esters present in the essence oil volatiles was identified in aroma oil volatiles. Neither acetaldehyde nor acetal were found in aroma oil volatiles, although both had been identified in the corresponding fraction from essence oil.

After distillation of essence or aroma oils, higher boiling constituents remaining in the pot residue were analyzed by liquid adsorption chromatography followed by glc of the eluted fractions. Table III lists the compounds identified according to their increasing retention times on the Carbowax 20M column and grouped according to the solvent by which they were eluted.

All but two compounds in Table III had previously been reported as constituents of cold-pressed grapefruit oil (Hunter and Brogden, 1965; Hunter and Moshonas, 1966; Kirch-

Table III. Composition of Pot Residue from Grapefruit Essence and Aroma Oils

Compound	Spectra obtained		Compound	Spectra obtained	
	Essence oil	Aroma oil		Essence oil	Aroma oil
Ether-hexane eluate			Hexane eluate		
Nonane	ir, ms	ir	D-Limonene	ir	ir
Heptanal	ms		α -Copaene	ir	ir
Octanal	ir, ms	ir	β -Cubebene	ir	ir
Nonanal	ir, ms	ir	β -Elemene	ir	ir
Octyl acetate	ir, ms	ir	β -Caryophyllene	ir	ir
Linalool	ir	ir	α - and β -Humulene	ms	ir
Decanal	ir, ms	ir	Valencene	ir, ms	
<i>trans</i> -2,8- <i>p</i> -Menthadien-1-ol	ir	ir	Δ -Cadinene	ir, ms	ir
<i>cis</i> -2,8- <i>p</i> -Menthadien-1-ol	ir	ir	epi- α -Selinene	ir, ms	
Neral		ir			
Geranial		ir	Ethanol-ether eluate		
Undecanal	ir, ms	ir	Octanol	ir	
Nonanol	ir		α -Terpineol	ir	ir
Citronellyl acetate	ir, ms		Nootkatone	ir	ir
Decyl acetate	ir, ms	ir			
Neryl acetate	ir, ms	ir			
Geranyl acetate	ir, ms	ir			
Carvone		ir			
<i>trans</i> -Carveol		ir			
<i>cis</i> -Carveol		ir			
<i>trans</i> -Carvyl acetate	ir				
1,8- <i>p</i> -Menthadien-9-yl acetate	ir				

ner and Miller, 1953; Moshonas, 1971). One of these, nonane, was found in both essence and aroma oils.

The other newly reported grapefruit constituent separated from essence oil pot residue was epi- α -selinene, previously isolated as an unknown sesquiterpene hydrocarbon at our laboratories from both grapefruit juice oil and peel oil (Hunter and Brogden, 1965). The mass, ir, and nmr spectra of this compound were recently published when it was reported as an unknown sesquiterpene hydrocarbon from Valencia orange peel oil (Lund *et al.*, 1970). Rouillier (1970) provided an ir spectrum of authentic epi- α -selinene reported by Klein and Rojahn (1970) which was identical to that of the unknown hydrocarbon from grapefruit essence oil.

Glc analysis of 250- μ l portions of essence oil chilled water condensate showed it to contain the following constituents in order of their increasing retention times (area percent of each component in parentheses): α -pinene (0.47), D-limonene (98.17), octanal (0.62), nonanal (0.07), decanal and linalool (0.60). Aroma oil chilled water condensate showed similar composition except an additional component, octanol, was present with a slightly larger retention time than the decanal-linalool peak. All compounds were identified by retention times and ir and/or ms comparison with authentic samples. Thus, grapefruit essence and aroma oils are similar in composition, with the principal difference being a greater amount of the most volatile constituents present in essence oil.

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