Analysis of Valencia Orange Essence and Aroma Oils

Richard L. Coleman* and Philip E. Shaw

A comparison was made between oil separated from the aqueous discharge from Valencia orange peel oil centrifuges (aroma oil) and oil separated during the commerical preparation of Valencia orange essence (essence oil). Qualitative analysis and quantitative estimation of the main constituents showed the two oils to be similar in composition. The 42 components identified from aroma oil include diethyl acetal, 12 alcohols, nine aldehydes, two esters, 14 hydrocarbons, and four ketones. The most volatile fractions of these oils were analyzed separately by gas chromatography and mass and infrared spectra. Several compounds possessing strong essence-like odor characteristics were isolated. Odor panel studies indicated these volatile fractions possessed most of the odor characteristic of the whole oils. Three of the volatiles identified in this study that had not previously been reported in citrus were ethyl vinyl ketone, isoprene, and ethyl propionate.

Aqueous orange essence has been increasingly used in recent years to improve the flavor of concentrated orange juice. Orange essence oils have odor profiles similar to that of essence, but they have not yet been utilized commercially to flavor orange products. Much work has been reported on the composition of aqueous orange essence by Kirchner and Miller (1957), Wolford *et al.* (1962, 1965), Wolford and Attaway (1967), Attaway *et al.* (1964), and Schultz *et al.* (1964, 1967a), but comparatively little has been reported on the composition of essence oil (Coleman *et al.*, 1969; Kirchner and Miller, 1957).

Orange essence oil is part of the volatile fraction condensed directly from orange juice by commercial essence recovery units during the process of concentrating the juice. Essence oil is separated from the aqueous layer before aqueous essence is collected (Byer and Lang, 1964; Kelly, 1965; Wolford *et al.*, 1968). This essence oil is currently a byproduct in aqueous essence production, but its essence-like odor quality, its colorless appearance, and its increasing availability make this oil a potentially useful flavoring agent.

Distilled aroma oils and aqueous aroma solutions have been produced in our laboratories using, as feedstock, the aqueous discharge from Valencia orange 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 orange 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 orange products. Since little is known about the composition of these aroma oils, an analytical study was undertaken.

This paper reports the results of that study, which included the analysis of aroma oil and both qualitative and quantitative comparisons between aroma oil and essence oil composition. Analysis and comparison of the most volatile fractions from the two oils are emphasized. This most volatile fraction from essence oil had not been analyzed previously. Odor panel judgments are included that show this fraction to be important to the essence-like character that essence oil possesses.

Fruit and Vegetable Products Laboratory, Southern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Winter Haven, Fla. 33880

EXPERIMENTAL

Source and Preparation of Samples. Four distilled Valencia orange oils were studied, two commercial essence oil samples and two aroma oil samples.

All samples were stored at 4° C until analyzed.

ESSENCE OIL SAMPLE 1. This came from the same source as the Valencia essence oil described previously (Coleman *et al.*, 1969).

ESSENCE OIL SAMPLE 2. This came from a source using an essence recovery unit of different design (Wolford *et al.*, 1968) from that of the first.

AROMA OIL SAMPLES 1 AND 2. Both were prepared from Valencia centrifuge effluent as described by Veldhuis *et al.* (1970). Aroma oil sample 1 had been stored for 1 year prior to whole oil analysis, while aroma oil sample 2 was analyzed the same day it was collected.

WHOLE OIL ANALYSIS. This refers to the method of quantitative analysis by direct injection of whole oils onto the gas–liquid chromatograph (glc). Each of the four oils was analyzed by this method (Table I).

Gross Separation Procedures. DISTILLATION. Essence oil no. 1 and aroma oil no. 1 were each distilled under reduced pressure in a Swissco rotary evaporator (with a liquid nitrogen trap attached) to give three fractions: pot residue, chilled water (9° C) condensate, and liquid nitrogen trap condensate. A 500 ml (420 ± 2 g) sample of essence oil no. 1 was distilled at 31° C under 0.9 mm pressure to give 13.9 g of pot residue, 402 g of chilled water condensate (mostly D-limonene), and 5.0 g of liquid nitrogen trap condensate. Portions of these fractions were recombined for the odor panels discussed below. Aroma oil no. 1 (820 g) distilled at 2.5–1.0 mm with a bath temperature of 45° C maximum yielded 19.5 g of pot residue, 799 g of chilled water condensate (mostly D-limonene), and 1.2 g of liquid nitrogen trap condensate.

The Swissco evaporator had been carefully cleaned and dried to avoid solvent contamination of the volatile fraction trapped at liquid nitrogen temperature. Both liquid nitrogen trap samples were analyzed as taken directly from the trap by injection onto polar and nonpolar glc columns. All essence oil components were identified by retention times plus mass spectral comparison with authentic samples except for myrcene and limonene, where infrared comparison was used. Methods of identification for aroma oil components are described in Table II.

Table I. Quantitative Composition of Whole Essence and Aroma Oils from Valencia Orange

Compound	Retention Time (min)[a]	Spectra obtained	% Area Under Curve: Aroma oils 1[b]	Aroma oils 2	Essence oils 1	Essence oils 2
α-Pinene	12	ir	0.30	0.45	0.30	0.16
Myrcene	16	ir				
D-Limonene[c]	22.5	ir	96.0	96.4	96.0	97.4
Octanal	23.5	ir, ms	0.55	0.44	0.89	0.47
Nonanal	26.5	ir, ms	<0.1	<0.1	0.1	<0.1
Decanal	29.5	ir, ms	0.43	0.65	0.70	0.47
Linalool	30	ir, ms	0.63	1.14	0.62	0.54
α-Terpineol	37.5	ir	0.14	0.15	<0.1	<0.1
Geranial	39	ir, ms	0.12	0.1	0.15	0.1
Carvone	40	ir, ms	0.34	<0.1	<0.1	<0.1
Valencene	40.5	ir, ms	<0.1	<0.1	0.5	0.54
Perillaldehyde	42.5	ir, ms	<0.1	<0.1	<0.1	<0.1
trans-Carveol	43	ir	0.22			
cis-Carveol	44	ir	0.12			
1,8-*p*-Menthadien-9-ol	48.5	ir	0.1			

[a] Carbowax 20M column. [b] Stored 1 year at 40° F. [c] $[\alpha]_D^{29°}$ +117° (*c* 1.15 in EtOH).

Table II. Qualitative Composition of Aroma Oil from Valencia Orange

Compound	Spectra Obtained	Retention Time 20M	Retention Time UCW-98
Acetals			
Diethyl acetal (acetal)	ms	16	4
Alcohols			
Ethanol	ms	18	
Linalool	ir	44	
Octanol	ir, ms	46	
Nonanol	ir	51	
trans-2,8-*p*-Menthadien-1-ol	ir	52	
cis-2,8-*p*-Menthadien-1-ol	ir	54	
α-Terpineol	ir, ms	56	41
Citronellol	ir, ms	56	
trans-Carveol	ir	64	
cis-Carveol	ir	66	
1,8-*p*-Menthadien-9-ol	ir	74	
8-*p*-Menthene-1,2-diol	ir, ms	90	
Aldehydes			
Hexanal	ms	25	
Heptanal	ir	29	
Octanal	ir, ms	34	24
Nonanal	ir, ms	39	28
Decanal	ir, ms	46	
Neral	ir, ms	56	43
Geranial	ir, ms	59	44
Dodecanal	ir, ms	61	52
Perillaldehyde	ir	63	
Esters			
Ethyl butyrate	ms	25	7
1,8-*p*-Menthadien-9-yl acetate	ir	66	
Hydrocarbons			
Hexane	ms	3	
Isoprene	ir, ms	4	
Methyl cyclopentane[a]	ms	5	
Heptane[b]	ms	5	
Octane	ms	10	
Nonane	ir, ms	15	
α-Pinene	ir, ms	25	
Sabinene	ir	31	
Myrcene	ir	33	
D-Limonene	ir	38	
β-Cubebene	ir, ms	50	
β-Elemene	ir	53	48
β-Copaene	ir	55	
Valencene	ir, ms	61	
Ketones			
Acetone	ir, ms	10	
Ethyl vinyl ketone	ms	24	
Carvone	ir, ms	61	43.5
Piperitenone	ir, ms	71	48

[a] Front half of glc peak. [b] Back half of glc peak.

Adsorption Chromatography. Pot residue and chilled water condensate from the aroma oil were further studied after 22.5 g of each was separated on a 5 cm i.d. by 60 cm adsorption chromatography column containing 1 lb of Florisil deactivated by the addition of 6% water. The column was eluted successively with 1 to 2 l. each of hexane, ether, and ethanol, using a Model 5400 liquid chromatography flame detector (Barber-Colman Co., Rockford, Ill.). Elution with each solvent was continued until no more substances were being eluted, as shown by the flame detector. Solvents were removed from each fraction under reduced pressure in a Swissco evaporator. Each fraction was then analyzed by glc and the combined analytical results are listed in Table II.

Glc Procedures. The polar column used was packed with 20% Carbowax 20M on 60- to 80-mesh Chromosorb W. The nonpolar column was packed with 20% UCW-98 on 60- to 80-mesh Chromosorb W. Quarter inch stainless steel tubing, 18 ft long, was used with one exception, noted below. The gas chromatograph was an F & M Model 500 instrument equipped with a thermal conductivity detector. The detector block temperature was 245° C, the injection port temperature was 295° C, and a He flow rate of 100 ml per min was employed.

Temperature programming for analysis on the polar column of whole essence oils, whole aroma oils, and adsorption column chromatography fractions from aroma oil separation was as follows: 75° C initially, raised to 90° C at 6 min, raised to 120° C at 12 min, and programmed at 2.1° C per min to 225° C, then isothermal until completion of the analysis.

Temperature programming for liquid nitrogen trap condensate for essence oil on the polar column was isothermally at 75° C for 29 min, then increased to 90° C and programmed at 2.1° C per min to 225° C. For the corresponding fraction

for aroma oil, an initial temperature of 75° C was held isothermally for 14 min, then increased to 90° C and programmed at 2.1° C per min at that point. Nonpolar column temperature for both essence and aroma oil liquid nitrogen trap condensates was held isothermally at 75° C. A 0.25 in. by 6 ft nonpolar column was used for the aroma oil liquid nitrogen trap condensate.

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.

Mass and Infrared Spectral Methods. Mass spectra (ms) were obtained either with a Bendix-Time-of-Flight Model 3012 mass spectrometer or with a Bell and Howell Model 21-490 mass spectrometer. Infrared (ir) spectra were obtained on a Perkin-Elmer Model 137A Infracord either in carbon disulfide or as oil films, with one exception; the isoprene ir was obtained with a gas cell (10 cm path length). Spectra for each compound identified were compared with those from authentic samples.

For all but the following exceptions, authentic samples were obtained from compounds purchased commercially. For the following compounds, spectra of authentic samples had been obtained previously at our laboratories by the authors indicated: *trans*- and *cis*-2,8-*p*-menthadien-1-ols, *cis*-carveol, 1,8-*p*-menthadien-9-ol, and 8-*p*-menthene-1,2-diol by Hunter and Moshonas (1965); perillaldehyde and 1,8-*p*-menthadien-9-yl acetate by Moshonas and Lund (1969); piperitenone by Moshonas (1967); sabinene and β-elemene by Hunter and Brogden (1965); and β-cubebene and β-copaene by Veldhuis and Hunter (1968).

Odor Panel Methods. The odor panel consisted of six members who were experienced in detecting essence added to orange juice. Panel members were first asked to compare, for essence-like quality, essence oil sample no. 1 with an oil reconstituted from 4.02 g of chilled water condensate and 139 mg of pot residue (see RESULTS AND DISCUSSION). Thus, the liquid nitrogen trap condensate was excluded from this reconstituted oil. The panel then was asked to compare, for essence-like quality, essence oil sample no. 1 with an oil reconstituted from 50 mg of liquid nitrogen trap condensate, 4.02 g of chilled water condensate, and 139 mg of pot residue.

To minimize the fatigue that readily occurs with an odor panel involving oil samples, only paired comparison tests were used (Boggs and Hanson, 1949) and at least 20 min was allowed between the two presentations made to each panelist. Ten correct judgments out of 12 presentations were required to achieve a 95% confidence level (ASTM Technical Publication, 1968).

RESULTS AND DISCUSSION

Two samples each of whole essence and aroma oils have been analyzed, and their main components are listed in Table I in order of their glc retention times. Spectral means of identifying each component and quantitative estimates are also given. The quantitative values are considered estimates only since response factors were not determined for individual components (Keulemans, 1959). Quantitative estimates are only listed for compounds identified by retention time and spectral data. When no quantitative value is listed, the compound was present in quantity too small to be trapped and positively identified. The one exception was myrcene, which appeared as a shoulder on the D-limonene peak and could not be estimated quantitatively. These oils were amenable to glc analysis as received, because they had each been distilled during their preparation. Thus, the carotenoids, waxes, and other higher boiling components that make glc analysis of cold pressed oils difficult had already been separated from these distilled oils in their preparation.

The essence and aroma oils were similar in composition, both by qualitative analysis and by quantitative estimation. A major quantitative difference was the relatively high percentage of valencene (0.5%) found in the two essence oil samples, while the aroma oils both had less than 0.1% valencene. Amounts of carvone and *trans*-carveol were less than 0.1%, except for aroma oil sample no. 1, which had 0.34% carvone and 0.22% *trans*-carveol. This aroma oil sample had been stored for 1 year at 40° F prior to whole oil analysis, whereas the other three oil samples listed in Table I were all analyzed within a month after their preparation (which may account for this observation). Carvone and *trans*-carveol amounts should increase in these oils during storage, since orange peel oil shows increased amounts of carvone and carveol after storage (Strausz, 1947).

Table II is a composite list of the 42 components identified in aroma oil sample no. 1 from the three portions separated above. It includes results from glc analysis of all liquid chromatography fractions, as well as those from the liquid nitrogen trap condensate (see Gross Separation Procedures). The compounds are listed by functional group classes in order of their retention times on a polar column. Listing of an additional retention time on a nonpolar (UCW-98) column indicates that the sample had to be collected from a polar column and rechromatographed on a nonpolar column to be adequately purified.

Comparison of the qualitative analysis of Valencia aroma oil in Table II with that reported earlier from this laboratory (Coleman *et al.*, 1969) for midseason and Valencia essence oils shows the aroma oil and essence oils to be qualitatively similar. Quantitative estimation of minor components isolated by adsorption column chromatography was not made because of the likelihood of error due to (1) losses of material on the column and (2) required corrections for large solvent peaks still present after individual fractions were concentrated to small volume and analyzed by glc (usually most of the sample injected was solvent). Quantitative estimates for main components as given in Table I are more meaningful, since introduction of solvents and adsorption column chromatography were avoided.

Volatiles identified in the liquid nitrogen trap condensate from both aroma oil no. 1 and essence oil no. 1 are listed in Table III. In each case this fraction was judged to possess most of the essence-like odor characteristic of essence and aroma oils, and was therefore worthy of careful examination. Finding ethyl vinyl ketone as a component of both essence and aroma oil volatiles was significant because it had not previously been reported as a constituent of citrus, nor has it been reported in other fruit essence volatiles (Flath and Forrey, 1970; Scanlan *et al.*, 1970; Stinson *et al.*, 1969; Do *et al.*, 1969; Schultz *et al.*, 1967b; Heinz and Jennings, 1966). Ethyl vinyl ketone was recently reported by Wilkins and Lin (1970) as a soybean milk volatile. Its strong acetylene-like odor suggests that ethyl vinyl ketone probably contributes to the essence odor character of essence and aroma oils.

Isoprene is another component of aroma oil volatiles possessing an acetylene-type odor. It was not found in essence oil volatiles and had not previously been reported in citrus. Stinson *et al.* (1969) tentatively identified isoprene in cherry essence and suggested it was an artifact in their sample resulting from thermal degradation of heat sensitive materials. A third compound not previously reported in citrus was ethyl

Table III. Volatiles from Essence and Aroma Oils of Valencia Orange

Compound	Found in: Essence oil	Found in: Aroma oil	Retention Time[a]: 20M	Retention Time[a]: UCW-98
Acetaldehyde	X		4	2
Hexane		X		
Isoprene		X		
Heptane	X	X	4	12
Methyl cyclopentane		X		
Acetone	X	X	6	
Ethyl acetate	X		8	7
Acetal		X		
Ethanol	X	X	10	3
Ethyl propionate	X		13	12
Methyl butyrate	X		16	13
Ethyl vinyl ketone	X	X	20	10
Ethyl butyrate	X	X	24	22
α-Pinene	X	X	24	
Hexanal		X		
Myrcene	X	X	47	
D-Limonene	X	X	58	
Octanal	X	X	60	

[a] When isolated from essence oil. Table II lists retention times for these compounds isolated from aroma oils.

propionate, which was found in essence oil volatiles, but not in aroma oil. It has been found by Flath and Forrey (1970) in pineapple volatiles, and by Schultz *et al.* (1967b) in apple essence.

As indicated in Table III, the hydrocarbons hexane, heptane, and methyl cyclopentane were present in aroma oil volatiles, but of these only heptane was found in essence oil volatiles. Methyl cyclopentane was shown by glc to be the main contaminant in hexane used as a solvent in this laboratory. However, hydrocarbon solvents were vigorously excluded from the Swissco distillation apparatus used in collecting liquid nitrogen trap volatiles, and such solvents were not used with the equipment in which aroma oils were prepared from centrifuge effluent.

An odor panel judged the liquid nitrogen trap condensate separated from essence oil to be necessary for the original oil to have a full essence-like odor. The panel judged an oil reconstituted from pot residue and chilled water condensate (omitting liquid nitrogen trap condensate) to have less essence-like odor than the original oil. A reconstituted oil that included the proper proportion of liquid nitrogen trap condensate plus pot residue and chilled water condensate was judged to have more essence-like odor than the original oil. Both judgments were at the 95% confidence level. Thus, the most volatile components in essence oil are important contributors to essence-like odor of the oil.

Essence and aroma oils can be qualitatively analyzed and quantitative estimates of their main components can be made by the procedures described herein. These samples are basically similar in odor and composition, so that subtle odor differences are probably due to variation in minor constituents. Odor panel judgments showed the most volatile compounds to be important to the essence-like odor of the whole oil. A trapping technique employed here permits isolation of the most volatile essence oil components so that they are free from the organic solvents commonly used when aqueous essence solutions are analyzed.

LITERATURE CITED

ASTM Special Technical Publication No. 434, p 67, American Society for Testing and Materials, Philadelphia, Pa., 1968.
Attaway, J. A., Wolford, R. W., Alberding, G. E., Edwards, G. J., J. AGR. FOOD CHEM. **12,** 118 (1964).
Boggs, M. M., Hanson, H. L., *Advan. Food Res.* **2,** 219 (1949).
Byer, E. M., Lang, A. A. (to General Foods Corp.) U.S. Patent 3,118,776 (Jan. 21, 1964).
Coleman, R. L., Lund, E. D., Moshonas, M. G., *J. Food Sci.* **34,** 610 (1969).
Do, J. Y., Salunkhe, D. K., Olson, L. E., *J. Food Sci.* **34,** 618 (1969).
Flath, R. A., Forrey, R. R., J. AGR. FOOD CHEM. **18,** 306 (1970).
Heinz, D. E., Jennings, W. G., *J. Food Sci.* **31,** 69 (1966).
Hunter, G. L. K., Brogden, W. B., Jr., *J. Food Sci.* **30,** 1 (1965).
Hunter, G. L. K., Moshonas, M. G., *Anal. Chem.* **37,** 378 (1965).
Kelly, E. J. (to Libby, McNeill and Libby), U.S. Patent 3,223,533 (Dec. 14, 1965).
Keulemans, A. I. M., "Gas Chromatography," Reinhold Publishing Corp., New York, N.Y., 1959, pp 32–33.
Kirchner, J. G., Miller, J. W., J. AGR. FOOD CHEM. **5,** 283 (1957).
Moshonas, M. G., *J. Food Sci.* **32,** 206 (1967).
Moshonas, M. G., Lund, E. D., *J. Food Sci.* **34,** 502 (1969).
Scanlan, R. A., Bills, D. D., Libbey, L. M., J. AGR. FOOD CHEM. **18,** 774 (1970).
Schultz, T. H., Black, D. R., Bomben, J. L., Mon, T. R., Teranishi, R., *J. Food Sci.* **32,** 698 (1967a).
Schultz, T. H., Flath, R. A., Black, D. R., Schultz, W. G., Teranishi, R., *J. Food Sci.* **32,** 279 (1967b).
Schultz, T. H., Teranishi, R., McFadden, W. H., Kilpatrick, P. W., Corse, J., *J. Food Sci.* **29,** 790 (1964).
Stinson, E. E., Dooley, C. J., Filipic, V. J., Hills, C. H., *J. Food Sci.* **34,** 544 (1969).
Strausz, H. J., *Perfum. Essent. Oil Rec.* **38,** 200 (1947).
Veldhuis, M. K., Hunter, G. L. K., *J. Food Sci.* **32,** 697 (1968).
Veldhuis, M. K., Berry, R. E., Wagner, C. J., Jr., "Proceedings of the 1970 Conference on Citrus Chemistry and Utilization," Winter Haven, Fla., Oct. 9, 1970.
Wilkins, W. F., Lin, F. M., J. AGR. FOOD CHEM. **18,** 333 (1970).
Wolford, R. W., Attaway, J. A., J. AGR. FOOD CHEM. **15,** 370 (1967).
Wolford, R. W., Alberding, G. E., Attaway, J. A., J. AGR. FOOD CHEM. **10,** 297 (1962).
Wolford, R. W., Atkins, C. D., Dougherty, M. H., MacDowell, L. G., *Trans. Citrus Eng. Conf.* **14,** 64 (1968).
Wolford, R. W., Attaway, J. A., Barabas, L. J., *Proc. Fla. State Hort. Soc.* **78,** 268 (1965).

Received for review November 4, 1970. Accepted January 22, 1971. References to specific products of commercial manufacture are for illustration only and do not constitute endorsement by the U.S. Department of Agriculture.