

Quantitative Analysis of a Highly Volatile Fraction from Valencia Orange Essence Oil

A volatile fraction from Valencia orange essence oil that contained most of the essence-like odor of the oil was quantitatively analyzed by a gas chromatograph-digital integrator combination. A synthetic mixture based on this analysis was prepared for odor evaluation and for determining response

factors for correcting peak areas for each component. Based on these response factors, a second synthetic mixture was prepared and its odor was evaluated. Although an odor panel found its odor essence-like, this synthetic mixture was distinguishable from orange essence oil volatiles.

Efforts to produce frozen concentrated orange juice with greater "fresh" orange juice aroma and flavor have led in recent years to the addition of aqueous orange essence to the product (Attaway *et al.*, 1967). Aqueous orange essence is part of the volatile fraction condensed in essence recovery units during the process of concentrating fresh orange juice. An oily fraction is also condensed and is separated from the aqueous fraction before aqueous essence is utilized (Wolford *et al.*, 1968). This oily layer, called essence oil, was previously shown at our laboratories to contain many of the volatile components present in aqueous essence (Coleman and Shaw, 1971). Furthermore, distillation of essence oil at reduced pressure gave a volatile fraction representing less than 1% of the oil by weight, but which contained most of the essence-like odor characteristic of essence oil (Coleman and Shaw, 1971).

This volatile fraction from orange essence oil, wherein the more aromatic components are concentrated, was considered a good source for studying the quantitative relationship of the compounds needed to create an essence-like odor. Unlike aqueous essence, where solvent extraction (Wolford *et al.*, 1962) or preliminary gas-liquid chromatographic (glc) separation (Moshonas and Lund, 1971) is required, this volatile fraction is suitable for direct injection onto a single analytical glc column for quantitative analysis.

Duplication of food flavors has, in the past, been most successful when a combination of the scientific approach involving quantitative analysis and the organoleptic approach involving flavor compounding has been employed (Polak, 1970). However, with recent developments in glc-digital integrator combinations, the strictly scientific approach to duplicating flavors should be more successful.

The current report describes quantitative studies on a volatile fraction from orange essence oil previously shown to be necessary for the essence-like aroma of the oil. The preparation and odor evaluation of synthetic mixtures based on these quantitative studies are also described.

EXPERIMENTAL

Orange Essence Oil Volatiles. Valencia orange essence oil collected from a commercial essence recovery unit (Wolford *et al.*, 1968) was distilled under reduced pressure as described previously (Coleman and Shaw, 1971). The fraction condensed in a trap cooled with liquid nitrogen represented approximately 0.4% of the total essence oil employed.

Analytical Procedures. Samples (100 μ l each) of orange essence oil volatiles were chromatographed on a Hewlett-Packard Model 7620A Research Chromatograph equipped with 20 ft \times 0.20-in. i.d. stainless steel columns packed with either 20% Carbowax 20M or 20% UCW-98 on 60/80 mesh Gas Chrom P. The temperature was 70° C initially and was

raised to 130° C at 1° C per min, then raised to 225° C at 6° C per min, and held isothermally at 225° C to the end of the run. Either dual thermal conductivity detectors operated at 275° C or dual flame ionization detectors equipped with 10:1 splitters were utilized. On-column injection was employed with carrier gas flow rates at 70° C of 100 ml of helium per min. Peaks were identified by comparison both of retention times on polar and nonpolar columns and of mass spectra with those found for authentic samples (Coleman and Shaw, 1971). Quantitative peak area measurements were determined with a Hewlett-Packard Model 3370A Digital Integrator. (Noise suppression, 3; slope sensitivity, 0.03 up, 0.03 down; baseline reset delay, 0.5 min.; front and rear shoulder controls off.)

Preparative glc was carried out using a Nester-Faust Model 850 Prepkromatic Gas Chromatograph equipped with a 12 ft \times 2 in. i.d. (biwall) stainless steel column packed with 20% Carbowax 20M on 30/60 mesh Chromosorb W. The temperature was 100° C initially and raised at 2° C per min to 220° C. Injection port temperature was 190° C, thermal conductivity detector at 255° C, and a helium flow rate at 100° C of 2 l. per min.

Purification of Samples for Synthetic Mixtures. D-Limonene obtained by distillation of cold-pressed Valencia orange oil was redistilled twice, bp 60° C (10 mm) and bp 18–22° C (0.15 mm). Since D-limonene, thus purified, still contained several other components by glc analysis, it was further purified by preparative glc. The D-limonene purified by this procedure, $[\alpha]_D^{26} + 111^\circ \text{C}$ (*c* 1.38 in EtOH), was shown by glc on the analytical Carbowax 20M column to contain 0.12% myrcene as the only detectable impurity.

The following compounds were purified by glc on the analytical 20 ft \times 0.20 in. Carbowax 20M column prior to addition to the synthetic mixture: hexane; hexanal; sabinene, obtained by distillation of Savin oil, bp 45° C (10 mm); L-carvone, $[\alpha]_D^{29} - 57.5^\circ \text{C}$ (*c* 2.05 in EtOH); L-trans-carveol, $[\alpha]_D^{29} - 175^\circ \text{C}$ (*c* 0.8 in EtOH); L-cis-carveol, $[\alpha]_D^{29} - 36^\circ \text{C}$ (*c* 2.0 in EtOH); D-trans-2,8-p-menthadien-1-ol, $[\alpha]_D^{29} + 66^\circ \text{C}$ (*c* 1.21 in EtOH); D-cis-2,8-p-menthadien-1-ol, $[\alpha]_D^{29} + 150^\circ \text{C}$ (*c* 1.14 in EtOH). The latter two compounds were synthesized by air oxidation of D-limonene using the procedure of Bain *et al.* (1961).

The remaining samples were all purchased from commercial sources and shown to be at least 95% pure by glc analysis.

Synthetic Mixtures. The two synthetic mixtures were each prepared by weighing the appropriate amount of D-limonene in a 1-dram screw-capped vial and adding weighed amounts of each of the other components in succession to the D-limonene. Each sample was introduced by weighing a capillary tube to the nearest 0.001 mg and adding the liquid sample to the tube. As long as a liquid wedge across the tube was present so that no air could flow through the tube,

Table I. Quantitative Analytical Data for Valencia Orange Essence Oil Volatiles and Synthetic Mixtures

Compound	Digital integrator area %		First synthetic mixture		Corrected wt % in essence oil	Second synthetic mixture	
	Essence oil	Synthetic	Mg added	Calcd wt %		Calcd wt (mg)	Mg added
D-Limonene	87.049	91.368	841.63	90.080	85.821	859.22 ^a	861.27 ^b
Octanal	0.511	0.648	5.30	0.567	0.446	4.46	4.42
α -Pinene ^c	1.692	1.279	17.10	1.830	2.421	24.21	24.27
Ethyl butyrate ^c	2.742	2.705	26.50	2.836	2.874	28.74	28.15
Methyl butyrate	0.139	0.109	1.45	0.155	0.202	2.02	1.85
Ethyl propionate	0.028	0.032	0.39	0.041	0.036	0.36	0.37
Ethyl acetate	0.177	0.308	1.99	0.212	0.122	1.22	1.17
Ethanol	1.372	0.818	13.79	1.475	2.474	24.74	24.66
Ethyl vinyl ketone ^c	0.052	0.033	0.74	0.079	0.095	0.95	1.03
Acetone	0.022	0.021	0.42	0.044	0.046	0.46	0.43
Heptane	0.037	0.064	0.62	0.066	0.038	0.38	0.40
Hexane	0.029	0.055	0.51	0.054	0.028	0.28	0.34
Acetal ^c	0.077	0.208	1.61	0.172	0.064	0.64	0.71
Linalool	0.155	0.289	1.82	0.194	0.104	1.04	0.96
Sabinene	0.280	0.173	2.34	0.250	0.405	4.05	4.11
Hexanal	0.750	0.699	7.40	0.792	0.850	8.50	8.48
L- <i>trans</i> -Carveol	0.349	0.238	2.39	0.255	0.374	3.74	3.83
L- <i>cis</i> -Carveol	0.155	0.124	1.24	0.132	0.165	1.65	1.64
L-Carvone	0.679	0.961	7.07	0.756	0.534	5.34	5.37
<i>trans</i> -2,8- <i>p</i> -Menthadien-1-ol	0.441	None ^d	None	None		4.41 ^e	4.34
<i>cis</i> -2,8- <i>p</i> -Menthadien-1-ol	0.448	None ^d	None	None		4.48 ^e	4.38

^a Calculated as 99.88% D-limonene. ^b An additional 2.05 mg added (See Synthetic Mixture). ^c Based on ratio found using UCW-98 column. ^d No synthetic sample was available when first synthetic mixture was made and analyzed. ^e No correction factor could be applied.

weight loss by evaporation was negligible even on the most volatile components used. About 0.3 mg is the minimum amount of a liquid necessary to form such a wedge. The liquid wedge was kept near one end of the capillary tube during the weighing process. That end of the tube was then immersed in the limonene solution five times, softly blowing into the vial each time with an aspirator bulb the wedge of liquid that had been drawn into the tube by capillary attraction. In preparing the first synthetic mixture, a small amount (*ca.* 0.3 mg) of liquid remained on the walls of each capillary tube after the addition. In preparing the second sample, an additional 2 mg of D-limonene more than the calculated amount was added to compensate for this expected loss.

Odor Panels. Six panelists experienced in detecting essence added to orange juice were used in this study. Paired comparison tests were conducted as described previously (Coleman and Shaw, 1971). Triangle comparison tests were conducted with six panelists being given two presentations, each at least 20 min apart, to minimize the fatigue that occurs when oil samples are being tested (Boggs and Hanson, 1949). Ten correct judgments out of 12 presentations are required to achieve a 95% confidence level and 11 out of 12 are required to achieve a 99% confidence level (ASTM Technical Publication, 1968).

RESULTS AND DISCUSSION

Table I lists the data used in carrying out the following procedures. A volatile fraction from Valencia orange essence oil has been quantitatively analyzed by glc. A synthetic mixture based on this analysis was prepared and this synthetic mixture was used to determine glc response factors for correcting peak areas. Then a second synthetic mixture based on these response factors was prepared whose odor closely resembled that of the volatile orange fraction.

Column one of Table I lists quantitative results obtained by glc analysis of the volatile fraction from orange essence oil that contained most of the essence-like aroma of the oil. Unless otherwise noted, data were obtained on the Carbowax 20M analytical column. Using the quantitative results thus obtained, a synthetic mixture was prepared as listed in column

three. The weight percent composition of this synthetic mixture is listed in column four. Weight percent rather than mole percent was used because area percent, as determined with a thermal conductivity detector, more closely approximates true weight percent rather than mole percent composition (Dal Nogare and Juvet, 1962).

Glc quantitative analytical data for this first synthetic mixture are presented in column two of Table I. These data were then used to calculate the weight percent composition of essence oil volatiles by the method of internal normalization (Keulemans, 1959). Thus, area % in essence oil (column 1)/area % in synthetic mixture (column 2) \times calcd wt % (column 4) = corrected wt % in essence oil (column 5).

The same information was collected utilizing dual flame detectors, but these data are not listed because they were not used to calculate the composition needed for the second synthetic mixture. Correction factors were much greater for acetone, ethyl acetate, ethanol, and ethyl propionate with a flame ionization detector compared to a thermal conductivity detector. In each of these four cases, the flame was much less responsive to a given quantity of compound than were the other components of the mixture, or the same compounds with a thermal conductivity detector. Overall, the uncorrected response to each compound was closer to the actual weight percent present with thermal conductivity than with flame detectors. Since an assumption in the method of internal normalization is that variation in response is linear for a given compound (Keulemans, 1959), the more accurate results should be those obtained using the thermal conductivity detector with this mixture.

Using the corrected weight percent for each compound identified from essence oil volatiles, the quantities of compounds needed to prepare a synthetic mixture to duplicate the composition of essence oil volatiles are listed in column 6 of Table I. This synthetic mixture was prepared as listed in column 7 and then odor panels were utilized to evaluate the similarities of the synthetic mixtures to essence oil volatiles in producing an essence-like odor. In a triangular test involving this second synthetic mixture *vs.* essence oil volatiles, the odor panel was

able to distinguish a difference in 11 out of 12 judgments (99% confidence level). However, there was no definite preference for one sample over the other. (Six of 11 judgments preferred the synthetic mixture.)

Paired comparison tests were run with the same odor panel using either the first synthetic mixture (no response factors utilized) *vs.* essence oil volatiles or the second synthetic mixture *vs.* essence oil volatiles. In each case the odor panel was asked to choose the sample with more "essence-like" odor. In the first case, 10 out of 12 judgments favored essence oil volatiles (95% confidence level). In the second case, no significant preference for either the synthetic mixture or essence oil volatiles was found (eight of 12 judgments favored the synthetic mixture).

These results showed that by use of glc response factors in addition to relative peak areas, a synthetic mixture could be constructed whose odor more closely resembled the natural essence-like odor than was the case when response factors were not utilized. Although an odor panel found its odor essence-like, this synthetic mixture was distinguishable from orange essence oil volatiles.

ACKNOWLEDGMENT

The authors wish to thank Eric D. Lund for the samples of *cis*- and *trans*-2,8-*p*-menthadien-1-ol used in this study.

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., Dougherty, M. H., Edwards, G. J., *J. AGR. FOOD CHEM.* **15**, 688 (1967).
Bain, J. P., Gary, W. Y., Klein, E. A., (to Glidden Co.) U.S. Patent 3,014,047 (Dec 19, 1961).
Boggs, M. M., Hanson, H. L., *Advan. Food Res.* **2**, 219 (1949).
Coleman, R. L., Shaw, P. E., *J. AGR. FOOD CHEM.* **19**, 520 (1971).
Dal Nogare, S., Juvet, R. S., Jr., "Gas-Liquid Chromatography," Wiley, New York, N.Y., 1962, p 258.
Keulemans, A. I. M., "Gas Chromatography," Reinhold, New York, N.Y., 1959, p 35.
Moshonas, M. G., Lund, E. D., *J. Food Sci.* **36**, 105 (1971).
Polak, E. H., *Flavor Ind.* **1**, 442 (1970).
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).

Philip E. Shaw*
Richard L. Coleman

Fruit and Vegetable Products Laboratory
Southern Marketing and Nutrition Research Division
Agricultural Research Division
U.S. Department of Agriculture
Winter Haven, Florida 33880

Received for review April 5, 1971. Accepted May 24, 1971. Mention of brand names does not constitute endorsement.