Quantitative Composition of Cold-Pressed Orange Oils

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One early-season, two midseason, and four late-season cold-pressed orange oils were analyzed quantitatively by gas chromatography (glc) on a nonpolar column where glc response factors and percent nonvolatile material were determined to afford weight percent for each of the 17 main oil components. Late- and midseason oils were similar in composition by this analysis, although late-season oil is considered a better flavoring ingredient for orange products. The early oil had smaller quantities of linalool and of some aldehydes than the others. A synthetic mixture of 15 components prepared for response factor determinations had a citrus-like aroma, but it was not like that of cold-pressed orange oil. The octanal to decanal ratio (measured on a polar column) and the citral to saturated aldehyde ratio in orange oil were comparable to earlier reported results.

Accurate quantitative information on cold-pressed orange oil composition is lacking for many compounds known to be major constituents of the oil (Nursten and Williams, 1967). This information is needed both to evaluate the contribution of individual components to orange flavor and to quantitate differences in oils from different orange varieties to provide a basis for indexes of oil quality.

Several methods for quantitative analyses of cold-pressed citrus oils have been reported. Dougherty and Petrus (1971) measured total aldehyde content in cold-pressed orange and grapefruit oils. Stanley *et al.* (1961) and Naves (1947) measured concentrations of several individual aldehydes in citrus oils, but preliminary separation steps were required. Quantitative gas chromatographic (glc) analyses of cold-pressed citrus oils have not included response factors or percent nonvolatile material not eluted from the glc column (Bernhard, 1960; Ziegler, 1971). Although Lifshitz *et al.* (1970) used the method of Stanley *et al.* (1961) to study aldehydes in the oils from one orange cultivar from different locations, none of the above studies compared compositions of oils from different cultivars.

A method was needed for quantitative analysis of cold-pressed orange oil that would overcome some of the limitations in previous methods. In the current study, oil was injected onto a glc without a prior separation step and determinations were made on glc response factors and percent nonvolatiles not eluted from the column. Thus, weight percent of each main oil component was developed. This procedure was used to compare oils from early-, mid-, and late-season orange cultivars.

EXPERIMENTAL SECTION

Samples. Commercial cold-pressed orange oils included one early-season (mostly Hamlin and Parson Brown), two midseason (mostly Pineapple), and four late-season (mostly Valencia) cultivars. All samples were stored at 4° during the study. For comparison, authentic samples of individual components were purchased commercially with the following exceptions. D-Limonene was obtained as described previously (Shaw and Coleman, 1971). β-Elemene, valencene, and α- and β-sinensals were isolated from cold-pressed Valencia orange oil (Hunter and Brogden, 1965; Moshonas and Lund, 1969). *trans*-β-Farnesene was prepared by a published procedure (Naves, 1966).

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.

Quantitative Glc Analyses. Samples (100 μl) of either cold-pressed orange oil or synthetic mixtures were analyzed on a Hewlett-Packard Model 7620A gas chromatograph equipped with dual thermal conductivity detectors with a block temperature of 275°, on-column injection, and an He flow of 100 ml/min. Stainless steel columns of 0.20 in. i.d. were used. The nonpolar column was 20-ft long and was packed with 20% UCW-98 on 60–80 mesh Gas Chrom P with temperature programming of 120° initially, raised to 150° at 4°/min, then raised to 225° at 2°/min, and held at 225° to the end of the run. The polar column was 18-ft long and was packed with 20% Carbowax 20M on 60–80 mesh Chromosorb W with temperature programming of 120° isothermally for 32 min, then raised to 200° at 30°/min and held there for 10 min, and then raised to 225° at 1°/min.

During cold-pressed oil analysis, the nonpolar column was silanized after each run to remove nonvolatile material from the column and to restore column resolution by injecting 10 μl of Silyl 8 (Chemical Research Services, Inc., Addison, Ill.) onto the column at 225° and holding the column at that temperature for 20 min (Shaw *et al.*, 1971). Resolution on the polar column was not satisfactory unless the column was heated at 225° overnight between runs.

Triplicate runs were made for each analysis and glc peak areas were calculated as half the product of peak height times width given by inflection tangents at the base (Keulemans, 1959). Average percent deviation from the mean for individual glc peaks was generally less than 10%.

Synthetic Mixture Preparation. Samples of individual compounds for addition to the synthetic mixture were purified, when necessary, by glc on the nonpolar column so that all were >99% pure. Using the technique previously described (Shaw and Coleman, 1971), the synthetic mixture was prepared by adding the 14 other compounds in succession to D-limonene using proportions determined from glc peak area percentages for late-season oil, sample A. For three components, response factors for other components of similar structure were used. Thus, an authentic sample of β-copaene was not available and so the response factor for β-caryophyllene was used since the two eluted together as one peak. The response factor for geranial also was used for neral because the synthetic mixture neral peak was poorly shaped for integration. The response factor for valencene was used for β-farnesene because a sufficient sample of the latter was not available for accurate weighing.

Percent Nonvolatiles Determination. Valencia orange oil sample B (855 g) was distilled at 38–43° and 1.65–2.55 mm in a rotary evaporator (Buchi Rotavapor R, Type

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Table I. Quantitative Composition of Cold-Pressed Orange Oils

Compound	Identity	Response factor	Corrected weight percent in oils: Late-season A	Late-season B	Late-season C	Late-season D	Mid-season A	Mid-season D	Early-season D
α-Pinene	ir, ms	1.19	0.54	0.62	0.51	0.50	0.46	0.54	0.54
Myrcene	ir, ms	0.84	1.78	2.18	1.94	2.01	2.02	2.05	2.08
D-Limonene	ir	1.00	95.24	94.71	95.07	94.77	95.20	94.87	95.37
Linalool	ir	1.21	0.46	0.48	0.46	0.56	0.48	0.62	0.25
Citronellal	ir, ms	1.23	0.20	0.14	0.12	0.14	0.12	0.10	0.10
Decanal	ir, ms	0.98	0.35	0.41	0.44	0.46	0.30	0.39	0.24
Neral[a]	ir		0.09	0.06	0.08	0.05	0.05	0.08	0.06
Geranial	ir, ms	1.54	0.17	0.17	0.14	0.12	0.14	0.17	0.12
Perillaldehyde	ir	1.25	0.02	0.02	0.02	0.03	0.02	0.03	0.02
Dodecanal	ir, ms	0.82	0.06	0.05	0.07	0.07	0.04	0.06	0.03
β-Elemene	ir, ms	1.25	0.05	0.04	0.06	0.05	0.06	0.05	0.05
β-Caryophyllene + β-copaene	ir, ms	2.20	0.07	0.07	0.09	0.09	0.07	0.07	0.04
β-Farnesene[b]	ir		0.02	0.02	0.02	0.27[c]	0.02	0.02	0.01
Valencene	ir	1.33	0.08	0.07	0.09	0.15	0.12	0.09	0.04
β-Sinensal	ir	1.33	0.07	0.10	0.10	0.13	0.07	0.07	0.07
α-Sinensal	ir	1.29	0.02	0.04	0.04	0.06	0.02	0.04	0.02

[a] Geranial response factor was used. [b] Valencene response factor was used. [c] Mostly butylated hydroxyanisole.

KRV 65/45, Rinco Instrument Co., Greenville, Ill.) to afford 826.2 g of distillate and 19.7 g of pot residue. This pot residue was distilled in a 2-in. molecular still (Rota Film Model 50-2, Arthur M. Smith, Rochester, N. Y.) at 170–180° and 0.6 mm to yield 8.8 g of viscous nondistilled material (1% of the starting oil sample).

Aroma Evaluations. Five panelists, experienced in evaluating citrus oils by aroma, were each asked to compare the above synthetic mixture to late-season oil, sample A, for orange-like aroma. Samples were presented singly in screw-capped 1-dram vials with paper blotter strips to be used if desired and with the orange oil sample being presented first. Panelists were asked to judge the aroma of the synthetic mixture for its similarity to orange oil aroma, and, if not similar to orange aroma, to citrus aroma.

RESULTS AND DISCUSSION

Early-, mid-, and late-season cold-pressed orange oils were quantitatively analyzed by glc on a nonpolar column (Table I) to see if differences in flavor quality were reflected in the quantitative data. A polar column was not used because high-boiling material could not be removed easily between glc runs (Shaw *et al.*, 1971). Components in Table I were identified by comparison of retention times with known compounds and by comparison of spectra as indicated in column 2. Glc response factors and percent nonvolatiles were determined to afford weight percent of each of the 17 main oil components listed.

Response factors in column 3 of Table I were determined as described previously for orange essence oil (Shaw and Coleman, 1971) by preparing a synthetic mixture of cold-pressed orange oil components in known proportions using glc area percent to approximate weight percent values and chromatographing the synthetic mixture under the same conditions used for orange oil. For each component, known weight percent in the synthetic mixture divided by area percent in the synthetic mixture afforded the response factor. This factor multiplied by area percent of that component in the orange oil sample afforded weight percent, assuming all of the injected oil sample eluted from the glc column. Since orange oil distillation showed that 99% of the oil was volatile enough to elute from the column during analysis, 99% of the above calculated weight percent values were listed in Table I as corrected weight percent values for each oil component.

Comparison of results in Table I shows marked similarities and differences in the oils examined. All four late-season oils were similar in composition except for sample D, which was the only one containing an antioxidant, 0.27% butylated hydroxyanisole (BHA). Since sample D was stored at higher temperature (10–13°) than the other late season oils (0–4°), BHA probably had been added to that oil to protect it from oxidation during storage. Midseason oils generally are regarded as slightly less aromatic than late-season oils for orange flavor use. In the oils studied, however, the midseason oils were quite similar in composition to the late-season oils. Compositional differences may have been too slight or of such nature that they were not detected by this analytical procedure. The early-season oil showed lower linalool, decanal, and dodecanal than late- and midseason oils. Generally, the carbonyl compounds, which are believed important to orange flavor (Moshonas and Lund, 1969), were relatively low in the early-season oil.

Since many of the compounds reported here had been quantitated in orange oil by other methods, our results were compared with those reported previously. The octanal:decanal ratio, stated by Stanley (1962) to be an indication of oil purity, could not be measured on the nonpolar column used in our studies because octanal is not separated from D-limonene under these conditions and thus an accurate octanal content cannot be determined. Therefore, a polar Carbowax 20M column was used to measure an octanal:decanal ratio of 0.70 in late-season oil sample A. This is near previously reported values. Lifshitz *et al.* (1970) found a ratio of 0.85 in Florida and 0.62 in California late-season orange oils.

Our results for late-season oil sample A from Table I, considering octanal as 70% of the decanal value listed, show a citral to total aldehyde ratio of 0.2. This is in general agreement with previously found values. The ratio of citral (neral + geranial) to total aldehydes in orange oils is approximately 0.11–0.14, as reported by Lifshitz *et al.* (1970), and 0.25, calculated from data of Ziegler (1971).

One compound not quantitated in this study whose presence is an indication of orange oil hydration is α-terpineol (Blair *et al.*, 1952). On the nonpolar column under the conditions used, α-terpineol appeared as a small shoulder following the decanal peak on the chromatogram and could not be accurately quantitated. Glc response factors as listed in Table I were generally close to 1, as expected. However, the response factor for β-caryophyllene at 2.20 was higher than anticipated and was significantly different from that of β-elemene and valencene, two compounds of similar structure. The β-caryophyllene value

was checked for accuracy by adding it singly to a sample of D-limonene and was again approximately 2.2.

Except for our earlier study on Persian lime oil (Shaw *et al.*, 1971), the percentage of material considered too high boiling to be eluted from a glc column had not been considered in quantitative glc analyses of citrus oils (Bernhard, 1960; Ziegler, 1971). We determined by distillation that approximately 1% of cold-pressed orange oil would remain on the column (nonvolatiles) at the end of a normal glc run. Persian lime oil contained a much higher percentage (7.5%) of nonvolatiles than did orange oil (Shaw *et al.*, 1971).

A panel evaluated the aroma of the synthetic mixture of 15 compounds prepared in approximate proportions to that found in orange oil. The synthetic mixture was judged to have an aroma that was different from late-season orange oil sample A, but it was citrus-like in character. This synthetic mixture is being subjected to more extensive and thorough flavor and aroma evaluations.

The quantitative analytical glc procedure described herein should prove useful for monitoring cold-pressed orange oils for changes in main components. Since late-season orange oil is used at a level of about 0.020% in orange juice as a primary flavoring ingredient, the quantity of each of the 17 main oil components in orange juice can be approximated from these results. However, allowance should be made for those components such as valencene which are already present in juice oil (Hunter and Brogden, 1965) before peel oil addition.

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Free Amino Acids, Sugars, and Organic Acids in Aqueous Beef Extract

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The identified free amino acids, peptides, amines, sugars, sugar amines, sugar phosphates, and organic acids account for 0.55% of the fresh weight of bovine *semimembranosus* muscle. Of 27 ninhydrin-positive compounds, 23 were identified and quantified (mg/100 g fresh weight): phosphoserine, 1.84; taurine, 0.09; aspartic acid, 0.95; threonine, 3.17; serine, 3.60; glutamic acid, 11.60; proline, 0.94; glycine, 3.10; α-alanine, 18.17; cystine, 0.60; valine, 6.91; methionine and methionine sulfoxide (as methionine), 6.48; isoleucine, 5.07; leucine, 9.73; tyrosine, 5.44; phenylalanine, 6.04; β-alanine, 0.96; glucosamine, 3.04; hydroxylysine, 0.59; lysine, 2.40; anserine, 29.43; and arginine, 1.53. In the sugar fraction, glucose, fructose, ribose, inositol, glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, and adenosine monophosphate were quantified. Lactic acid accounted for 44.5% of the organic acid fraction; succinic acid was a minor component.

Studies on post-mortem changes in beef (Bodwell *et al.*, 1965; Colombo and Gervasini, 1955; Fredholm, 1960; Grau *et al.*, 1960; Niewiarowicz, 1956; Pavlovskii, 1965; Sharp and Rolfe, 1958) have identified amino acids, peptides, amines, sugars, and sugar phosphates in beef muscle. These compounds contribute to flavor through the nonenzymatic browning reaction (Hodge, 1967; Reynolds, 1965). Their relationship to tenderness of beef has also been considered (Field and Chang, 1969; Locker, 1960; Ma *et al.*, 1961; Parrish *et al.*, 1969). Moreover, these same water soluble, low molecular weight compounds have been reported in studies on an index of incipient spoilage, the effects of heat and irradiation on proteolysis, and the variation of composition among different tissues from the same animal (Gardner and Stewart, 1966; Thompson *et al.*, 1961; Walker, 1952). Numerous other studies have indicated that these soluble compounds may have important effects on meat flavor and palatability (Disney *et al.*, 1967; Dryden *et al.*, 1969; Gunther and Schweiger, 1966; Macy *et al.*, 1964a,b; Tonsbeek *et al.*, 1969; Wasserman and Gray, 1965; Wood, 1956, 1961; Wood and Bender, 1957).

Most of the research cited above pertained to studies with aims somewhat different from the present work; therefore, some of the techniques used were not suitable for this study. The use of heat during extraction or later in the separation is evident in the procedures of Gardner and Stewart (1966), Ma *et al.* (1961), Pavlovskii (1965), Sharp and Rolfe (1958), Walker (1952), Wood (1956), and Wood and Bender (1957). It was shown by Macy *et al.* (1964b) that heat caused substantial losses of sugars and amino compounds in meat extracts. Some studies reported only qualitative results (Locker, 1960; Tonsbeek *et al.*, 1969; Wood, 1961). Other studies quantitated only specific compounds (Bodwell *et al.*, 1965; Disney *et al.*,

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