Analysis of Recovered Natural Orange Flavor Enhancement Materials Using Gas Chromatography

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New information is presented concerning the chemistry of flavor relative to the processing of orange juices. Capillary column–flame ionization (FI) and dual channel FI and electron-capture (EC) detection systems with programmed temperature gas chromatography (PTGC) were used. Analyses were conducted on cold-pressed orange oils, centrifuged juice oils, orange juice emulsions obtained by direct centrifugation of freshly extracted juices, and concentrated aqueous orange essences obtained by vacuum recovery from fresh juices. The complex chemical spectra exhibited by each of these materials showed differences dependent on their sources, whether from the peel or juice of the orange, and also on the method of recovery. Electron-capture responses shown by each flavor enhancement material were related principally to its oxygenated fractions and were more intense in the juice-oriented materials. It was indicated that components giving EC response were related to the fresh aroma factors in the juice, possibly providing secondary flavor characteristics.

Flavor enhancement of concentrated orange juice is essential to the production of a flavorful product. This is accomplished by adding to the concentrated juice from commercial evaporators materials containing the volatile fruit substances. Normally, this includes the addition of cutback juice (*18*), especially prepared from fresh juice, and an appropriate amount of cold-pressed orange oil (*14*). Some other types of recovered natural flavor materials, containing the necessary volatile fruit substances, can be used to augment the flavor further to satisfactory sensory perception levels. More recently, freeze-concentrated cutback juice and aqueous orange aroma essences have been used in the commercial production of frozen concentrated orange juice. Walker (*24*) reviewed the literature on fruit juice essences commercially produced through the recovery of volatile water- and oil-soluble constituents of the juice. Other recent investigations of flavor-enhancement materials have been concerned with the centrifugation of fresh orange juice in the preparation and use of juice emulsion as described by Lawler (*17*) and in the preparation of centrifuged juice oil as described by Thrush (*23*).

The ultimate criteria for supplementing the flavor and aroma of orange juice products, using any one or a combination of these materials containing volatile fruit substances, must be directly related to flavor stability and acceptance by the consumer. These criteria can be determined by organoleptic evaluation. However, a more objective approach to the evaluation of flavor quality in these products has been the subject of investigation for some time. During the past several years, many of the published results on analysis of volatile flavor components of the orange have emphasized the contribution of those components of the peel oil, normally as cold-pressed orange oil (*7, 8, 19, 21*). Blair *et al.* (*5*) referred to flavorful components that give orange juice its distinctive character as having their origin in the peel oil. Kefford (*12*) related the characteristic aroma and flavor of citrus fruits to the aldehydes and esters in the peel oils. The separation, isolation, and identification of volatile components has steadily developed since the advent of gas chromatography as shown in the work of Stanley *et al.* (*22*) and of Bernhard (*4*) in the analyses of California orange oils. Kesterson and Hendrickson (*13*) have reported on the composition of Florida orange oils as influenced by fruit variety and maturity. Hunter and coworkers (*10, 11*) showed identifications for the terpene hydrocarbons, sesquiterpenes, and alcohols in Florida orange oils.

The work of Kirchner and Miller (*16*) employing standard techniques of evaporation, extraction, fractionation, and chromatostrip separations showed identification for some 25 to 30 volatile components in California Valencia orange juice. Some of the earlier efforts in the analysis and identification of the juice volatile flavor components through application of gas chromatography were reported by Wolford and Attaway (*26*). Attaway and coworkers (*1–3*) have reported on the isolation and identification of alcohols and acids, volatile carbonyl components, and the esters in recovered orange juice essences. Determination of chemical components in organic extracts of commercially recovered orange essences by Wolford, Alberding, and Attaway (*25*) using PTGC and two column phases showed tentative identification and peak assignments for 43 components. In the analysis of flavor and aroma constituents of Florida orange juice by gas chromatography, Wolford *et al.* (*27*) showed some compositional differences among varieties of oranges in juices, peel oil–free juices, their respective juice essences, and peel oils. The presence of certain chemical constituents in the juice was related directly to the peel oil. In the systematic analysis of volatile flavor components in orange juices, Wolford, Attaway, and Barabas (*28*) demonstrated that the extreme complexity of this flavor mixture requires considerable diversity in methods of analysis. Through supplementary analytical techniques and multiple detection systems in gas chromatography some degree of specificity for certain types of these compounds was shown.

In all, the many published contributions have pro-

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vided valuable knowledge of the composition and chemistry of the volatile fractions of orange juice. The present paper reports on some new exploratory information for consideration in the total interpretation of characteristics of the aroma and flavor of orange juice. In particular, the analysis of some recovered natural orange flavor-enhancement materials employing capillary column–FI and dual channel FI-EC gas chromatography are presented.

Experimental

Apparatus and Methods. Programmed temperature gas chromatographic (PTGC) analyses were carried out using the Perkin-Elmer Model 226 flame ionization–capillary column system and, also, the Micro-Tek Model 2000R equipped with dual channel flame ionization and electron-capture detector systems.

The Perkin-Elmer Model 226 was equipped with a 300-foot × 0.01-inch i.d. Golay column (Perkin-Elmer Corp.) coated with a liquid phase of 95% Apiezon L, 4% Igepal CO 880 (nonylphenoxypolyethyleneoxyethanol), and 1% DOPC as an antioxidant. The column was operated at two pressures, 40 and 44 p.s.i., using helium as the carrier gas. Sample size was 0.5 μl. split 100 to 1 at the inlet. Nonlinear temperature programming was employed as follows: 60° C. for 10 minutes, 2° C. per minute to 170° C., and isothermal at 170° C. for 60 minutes. A Sargent SR recorder was used in the 5-mv. range with a chart speed of 18 inches per hour.

With the Micro-Tek 2000R the following columns and conditions were used: A 12-foot × 1/8-inch o.d. column of Estrex P4-0 (polyethylene glycol 400 monooleate, Swift and Co., Technical Products Department, Hammond, Ind.), 10% w./w. on 60- to 80-mesh Gas Chrom Z (Applied Science Laboratories, Inc., State College, Pa.) was used with a carrier gas (nitrogen) pressure of 70 p.s.i. and a flow of 28 ml. per minute. The column effluent was split at a ratio of 31 to 5.6 to the flame ionization (FI) and electron-capture (EC) detectors, respectively. Also, a 50-foot × 1/8-inch o.d. column of Carbowax 20M 5% w./w. on 60- to 80-mesh Gas Chrom Z was operated at 60-p.s.i. nitrogen carrier gas with a column flow of 42 ml. per minute. The column effluent was split approximately 1 to 1 to the FI and EC detectors. The EC detector, of plane parallel design with tritium radioactive source, was operated at 18-volt d.c. using nitrogen as a purge gas at a flow of 125 ml. per minute. Columns of Apiezon L, 4%-Igepal CO 710 (nonylphenoxypolyethyleneoxyethanol), 1% w./w. on 60- to 80-mesh Gas Chrom Z, 12- and 50-feet × 1/8-inch o.d., also were employed in retention studies on the dual channel system.

Sample size for all dual channel analyses was 0.5 μl. using the 12-foot columns and 1.0 μl. using the 50-foot columns. Two recording systems were used for the dual channel analyses. In one system two Sargent Model SR recorders, 1 mv., 18 inches per hour, were operated simultaneously using a single drive switch, while the other system employed a dual-channel Westronics Recorder, Model LD11A, 1 mv. each full scale. Methylene chloride and ethyl disulfide were injected for periodic checks on retention values in responses from both detectors.

Preparation of Samples. Representative samples of Valencia cold-pressed orange oil, prepared commercially (*14*), and juice oil, obtained directly from Valencia orange juice using the DeLaval centrifugation process (*23*), were used as received. The Valencia orange juice emulsion, prepared commercially using the Centrico juice centrifugation process (*17*), had to be treated similarly to orange juice for analysis of its volatile flavor components. The emulsion was diluted at the rate of 5 to 95 gallons of distilled water and the aroma-flavor essence removed from the solution in a vacuum system designed to concentrate the volatile components. By using the same vacuum recovery system, the orange juice essences were obtained directly from freshly extracted Valencia orange juice.

The recovered aqueous essences from the diluted emulsion and from fresh juice were extracted with methylene chloride according to the procedure of Wolford, Alberding, and Attaway (*25*). The resulting anhydrous extracts were analyzable by gas chromatography.

The cold-pressed and juice oils were analyzed directly by gas chromatography. Terpeneless oils were prepared from each according to the procedure of Kirchner and Miller (*15*).

Results and Discussion

The chromatograms in Figure 1 represent the capillary column separations of cold-pressed orange oil, its terpeneless fraction, and an extract of recovered aqueous orange essence. More than 200 component peaks have been observed in chromatograms of these recovered orange essences. Comparison of these three fractions, constituting most of the volatile flavor components of the orange, shows that much of the chemical spectrum of each is interrelated. Previous studies (*27*) have shown the contribution of components of the oils to that of the total flavor mixture. Essentially the bulk of the terpeneless oil chromatogram is similar to that of the recovered juice essence with the exception of those components shown in the first 5 to 15 minutes of analysis time in the essence chromatogram. This group represents the alcohols and aldehydes to C-6, both saturated and unsaturated aliphatic, and the esters through ethyl butyrate. The large peak at about 85 minutes is valencene, a sesquiterpene identified by Hunter and Brogden (*10*) in cold-pressed orange oil. Studies made on peel oil–free juices (*27*) have shown this sesquiterpene to be a major juice component. Some 45 of the resolved components with peak heights in excess of 50% of full scale and nine compounds having peak heights between 10 and 50% of full scale in Figure 1 have been identified by Wolford, Attaway, and Barabas (*28*). Other publications (*1–3*, *25*, *27*) have indicated the identification of the compounds listed in Table I. These compounds have received additional confirmation by enrichment for peak coincidence in the present study, using the prescribed coated capillary column.

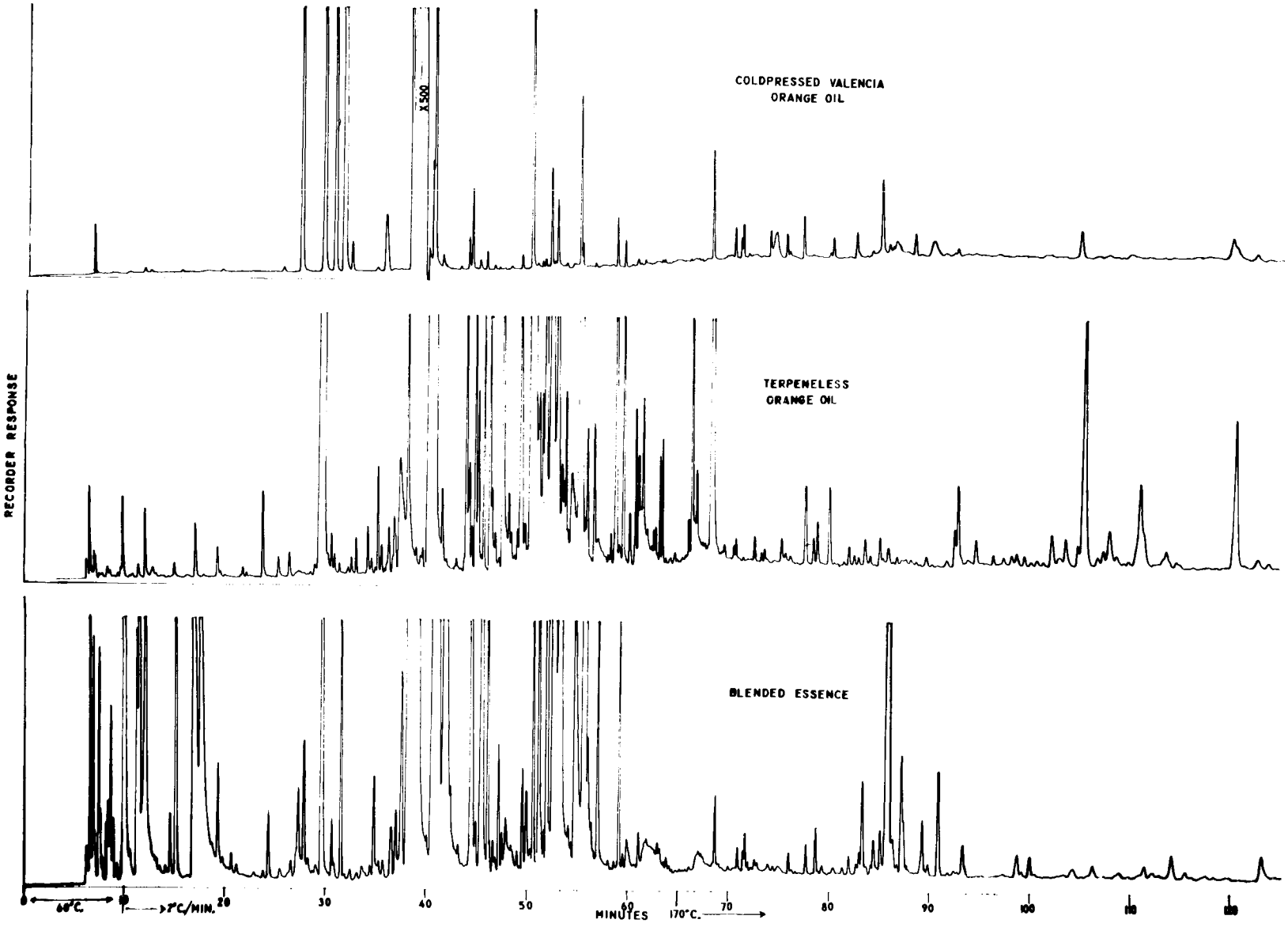

Figure 1. Programmed temperature capillary column–flame ionization gas chromatograms

Cold-pressed Valencia orange oil, terpeneless orange oil, and recovered orange essence extract. Attentuation. × 10 except where indicated. Helium carrier pressure. 40 p.s.i. Sample size. 0.5 μl., split 100 to 1

Table I. Volatile Flavor Components in Recovered Orange Essence

Confirmed identifications unless noted[a]

Aldehydes and Ketones	Alcohols	Acids
Acetone	Methanol	Formic
Acetaldehyde	Ethanol	Acetic
n-Hexanal	1-Propanol	Propionic
2-Hexenal	2-Butanol	Butyric
1-Octanal	1-Butanol	Caproic
1-Nonanal	2-Pentanol	Capric
1-Decanal	1-Pentanol	Isovaleric[a]
Neral	1-Hexanol	Valeric
Geranial	*cis*-3-Hexen-1-ol	Isocaproic[a]
Carvone	Methyl heptenol[a]	Caprylic[a]
2-Octenal	Linalool	
Methyl heptenone	3-Hepten-1-ol[a]	
Undecanal	1-Octanol	
Citronellal	Terpinen-4-ol	
α-Ethyl butyraldehyde[a]	1-Nonanol	
	α-Terpineol	
	1-Decanol	
	Citronellol	
	Nerol	
	Geraniol	
	Carveol	

Terpene Hydrocarbons	Esters	Oxides
α-Pinene	Ethyl butyrate	*cis*-Linalool oxide
β-Pinene	Ethyl caproate	*trans*-Linalool oxide
D-Limonene	Ethyl caprylate	*cis*-Limonene oxide
Myrcene	Linalyl acetate	*trans*-Limonene oxide
γ-Terpinene	Terpinyl formate	
α-Terpinene	Citronellyl butyrate	
δ-3-Carene[a]	Ethyl propionate[a]	
Terpinolene[a]		
p-Cymene[a]		
α-Phellandrene[a]		
β-Phellandrene[a]		

[a] Strong tentative identification—lacks confirmation.

In Figure 2 are shown the comparative chromatograms of cold-pressed orange oil, centrifuged orange juice oil, and the essence from centrifuged juice emulsion. These flavor enhancement materials show a distinct compositional relationship to each other. The juice emulsion, although originating from the juice, will range between 2 and 5% recoverable oil by the Clevenger oil procedure (*6*). One of the characteristics in the emulsion essence is seen in the major peak for valencene, at about 85 minutes, similar to the quantity found in juice oil and juice essence. Other characteristics of the juice volatiles found in the juice emulsion essence are the high concentration of α-terpineol, the *cis*- and *trans*-limonene oxides, a significant citronellol content, and a lower decanal content than in the oil fractions. Reference to the chromatogram for orange essence in Figure 1 will show the composition of the juice emulsion to be more or less intermediate between the predominantly oil-soluble and water-soluble flavor-enhancement materials. As in the cold-pressed and juice oils, there is an apparent absence of components in the early region of the chromatogram for the emulsion essence. However, deterpenated emulsion essence extracts have shown some representation of those early eluting components found in the juice essence.

During the study of the chemical characteristics of orange juice, efforts were made to correlate the findings to the aroma of the juice as perceived by olfactory responses. However, throughout the many phases of these investigations it appeared that the analyses to this point had not indicated all the factors involved. In view of the work of Oaks, Hartmann, and Dimick (*20*) on dual channel electron-capture–flame ionization analyses of sulfur compounds, the use of a similar method in the analysis of orange juice flavor was investigated.

Preliminary results were obtained using dual-channel FI and EC detectors with PTGC in the analyses of these several natural flavor-enhancement materials. Virtually nothing is known at present regarding the nature of the chemical compounds, which show selective response to the electron-capture detector and are deemed

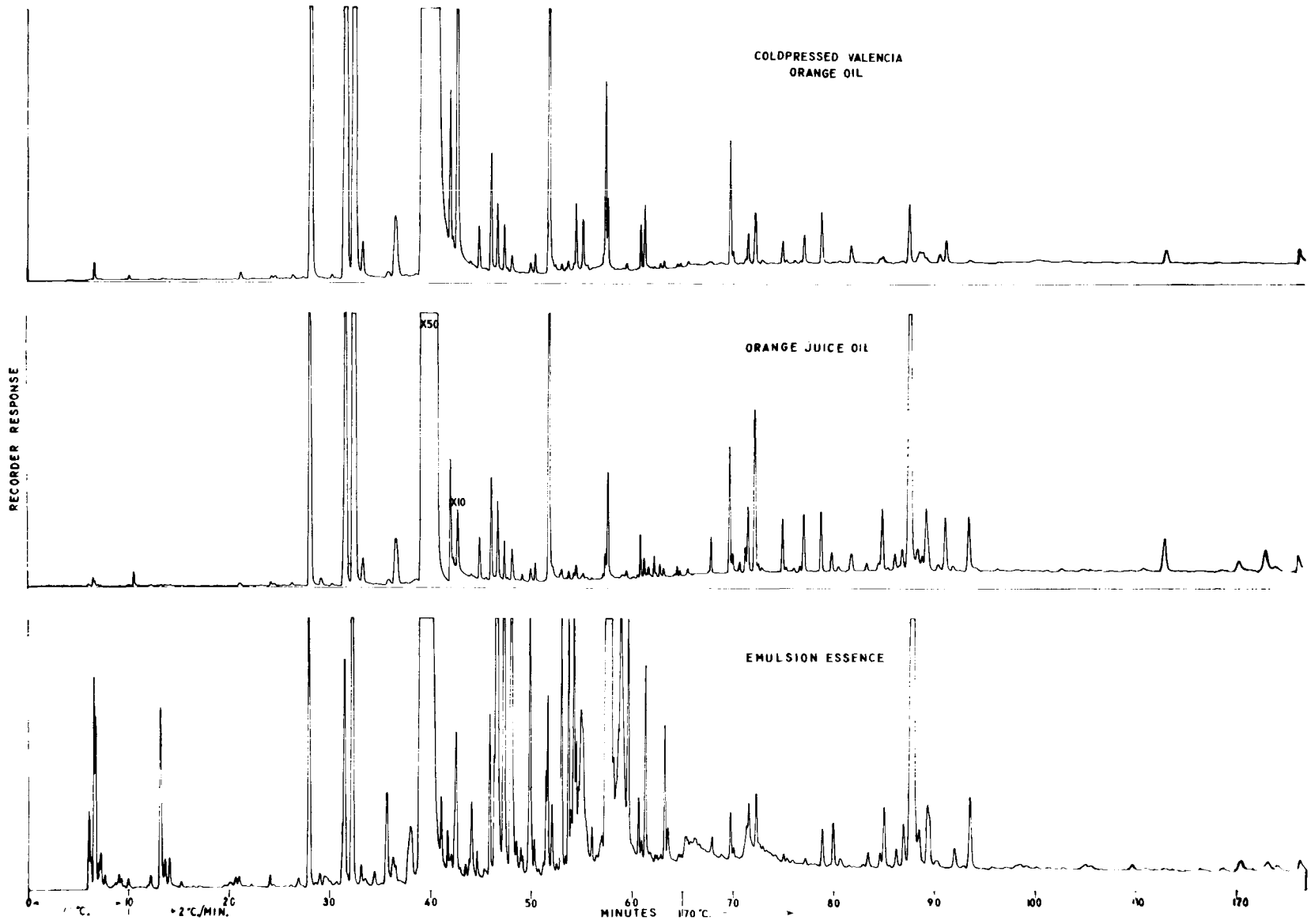

Figure 2. Programmed temperature capillary column–flame ionization gas chromatograms

Cold-pressed orange oil, juice oil, and juice emulsion essence. Attenuation. × 10 except where indicated. Helium carrier pressure. 44 p.s.i. Sample size. 0.5 μl., split 100 to 1

as having some degree of electron affinity. Throughout the study there was little retention coincidence among the compounds responding to EC and FI as shown for juice emulsion and juice essence in Figure 3. This is difficult to justify since the first assumption would be that C-H compounds are present and should be detectable by both systems regardless of the selectivity of EC response. Presumably, concentration factors and the considerable differences in sensitivity between the two detectors could account for the divergent chromatographic readout obtained. However, certain specific "fingerprints" of the various materials studied have shown orientation with the juice or oil fractions, and with the combination of the two fractions in materials such as orange juice emulsions and recovered orange essences.

The chromatograms in Figure 4 show comparison of the EC responses for the terpeneless or oxygenated fractions of the cold-pressed and juice oils with juice emulsion and juice essence. Distinctive differences among all four of the materials are shown in analyses conducted on the Estrex column. The major peak in the juice emulsion chromatogram at 80° C. column temperature would appear related to the same peak in the cold-pressed oil. Exclusive of concentration factors, the reasonably good qualitative comparison of EC components tends to orient the emulsion to the oil fraction. Similar comparisons between the oxy-fraction of juice oil and the juice essence can be made. However, the major peaks in the EC chromatogram for the essence at 120–22° C. column temperature show only slight qualitative comparison with the same region in the juice oil.

Some interesting comparisons between EC chromatograms of essence and emulsion are shown in Figure 5. The left-hand top and bottom chromatograms of juice essence and juice emulsion are qualitatively very similar. However, the juice essence components responsive to EC are indicated to be more concentrated at the sensitivity of detection. This particular essence was prepared from fresh extracted orange juice using the rotary-type extractor which is known to cause a large concentration of peel oil to be expressed into the juice. Thereby the resulting recovered aqueous essence, containing 6 to 8% alcohol by volume, probably assumed a higher concentration of solubilized oil. Following the line of reasoning for the significant orientation of juice emulsion to the oil fraction, this particular essence appears similarly more oil-oriented in its EC response. The right-hand top chromatogram in Figure 5 shows the EC analysis for essence obtained from present-day commercially extracted orange juice. Normally, juices of lower oil content are obtained using these extractors and, subsequently, the recovered juice essence contains a lower percentage of oil components. The differences in EC response characteristics for the two essences, shown in the top chromatograms, were consistent in repeated analyses and have been attributed to the methods of extraction. The bottom right-hand chromatogram in Figure 5 was included to show the corresponding qualitative EC response to that of the top-right chromatogram. The dilute essence, taken during the essence recovery operation at a concentration approximately one half that of the concentrated essence, shows a generally weaker quantitative EC response.

A more definitive comparison of EC responses for

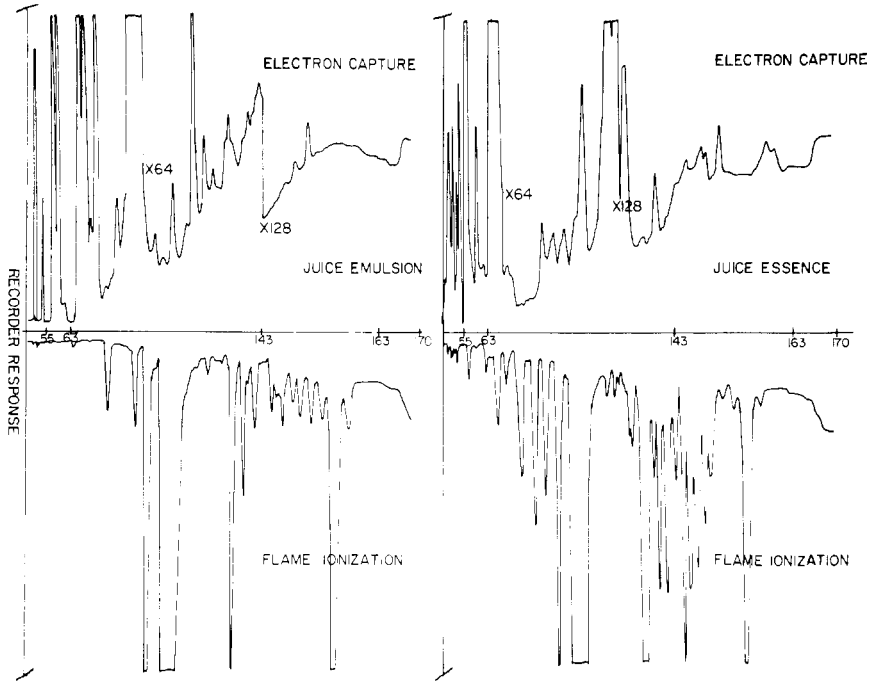

Figure 3. Programmed temperature dual channel electron-capture–flame ionization gas chromatograms

Juice emulsion and juice essence. Sample size. 0.5 μl., column exit split 31 to 5.6, FI-EC, respectively. Attentuation. $10^2 \times 32$, except where noted. Column. Estrex

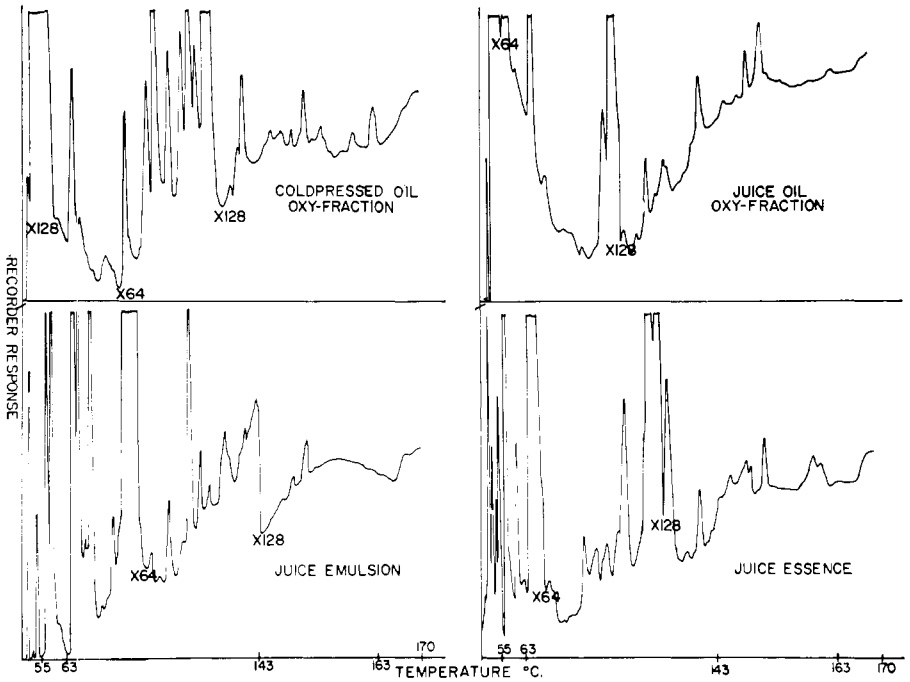

Figure 4. Programmed temperature electron-capture gas chromatograms

Oxygenated fractions of cold-pressed and juice oils, juice emulsion, and juice essence. Sample size. 0.5 μl., column exit split 31 to 5.6, FI-EC, respectively. Attenuation. $10^2 \times 32$, except where noted. Column. Estrex

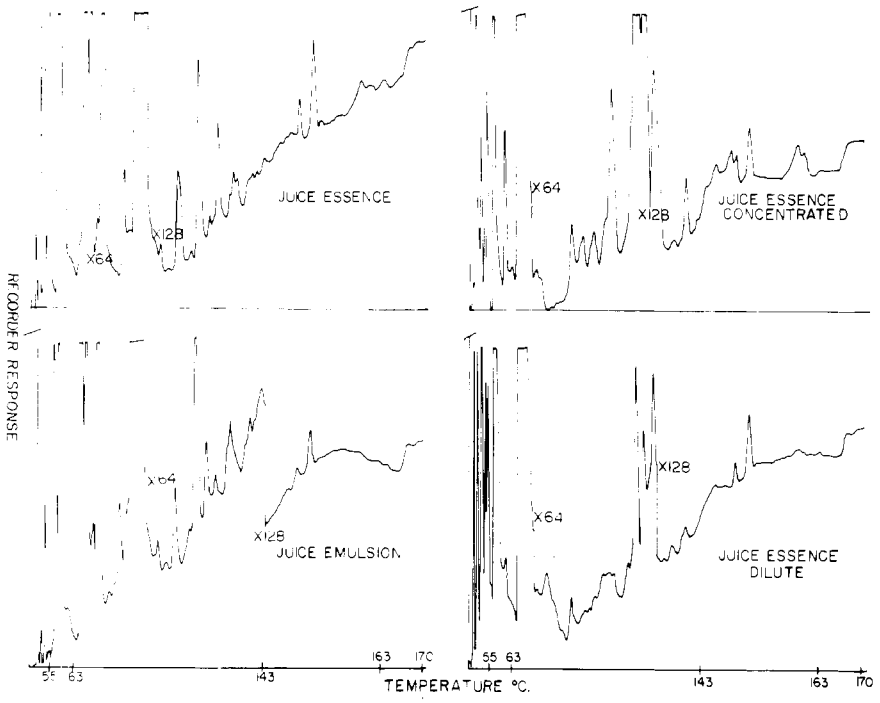

Figure 5. Programmed temperature electron-capture gas chromatograms

Juice essences and juice emulsion. Comparison of EC responses related to different methods of juice extraction. Sample size. 0.5 μl., column exit split 31 to 5.6, FI-EC, respectively. Attenuation. $10^2 \times 32$ except where noted. Column. Estrex

these materials may be seen in Figure 6 from chromatograms using the 50-foot Carbowax column. The analyses show the very close association between the terpeneless fractions of cold-pressed and juice oils with a carry-over of some EC characteristics shown by component peaks 15 through 27 in the top chromatogram of the whole oil. Similarly, the chromatogram for juice emulsion shows those EC components derived from the oil, particularly peaks 28 and 30, in the terpeneless fractions. In addition, some characteristic EC response in the juice essence is shown in the early part of the chromatogram for the emulsion indicating some interrelationship between the oil- and water-soluble fractions. The bottom chromatogram for orange juice essence shows a minimum response for the EC components in the oils and emulsion. Apparently the EC response for component peaks 11, 16, and 23 in the juice essence are derived strictly from the juice. The major

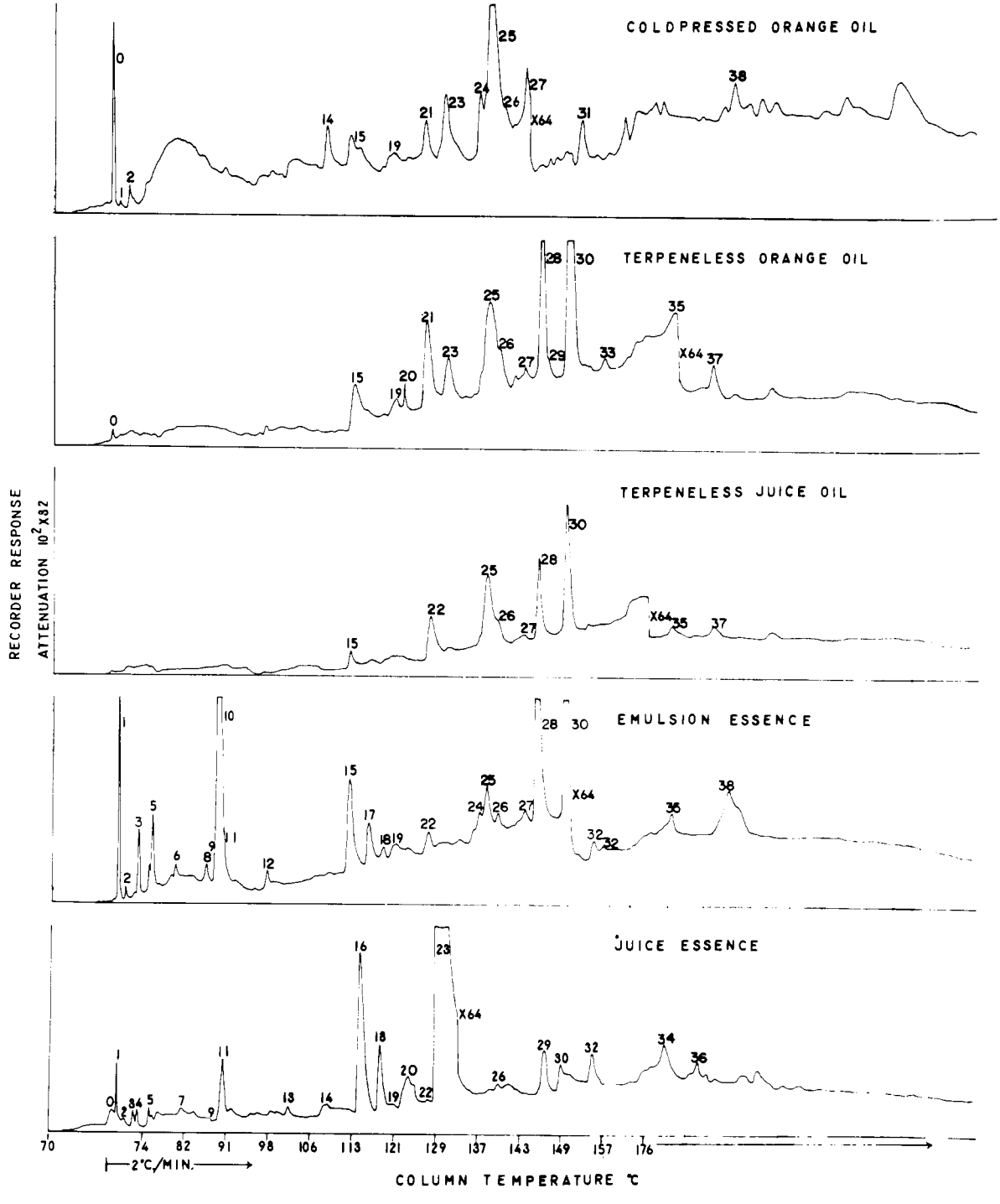

Figure 6. Programmed temperature electron-capture chromatograms

Cold-pressed orange oil, terpeneless fractions of orange oil, juice oil, emulsion essence, and juice essence. Sample size. 1.0 μl., exit split 1 to 1. Attenuation. $10^2 \times 32$ except where noted. Column. Carbowax 20M

EC responses for the juice essence have been characteristic of essences prepared both experimentally and commercially, which have been tested from three entirely different essence recovery procedures.

Among several methods used in efforts to classify and identify the individual EC components was a subtractive method employing basic lead acetate reported by Wolford, Attaway, and Barabas (*28*). In that study several compounds were removed from the EC chromatogram but showed no loss of components as detected by flame ionization. Following the method of Gumbmann and Burr (*9*), mercuric chloride complexes were prepared from aqueous essence in attempts to ascertain the presence or absence of suspected volatile sulfur compounds. However, regeneration according to their procedure was unsuccessful. The material was insoluble in 6*N* HCl. Ultimately, regeneration by vaporization at 195° C. into the gas chromatograph was achieved. No positive indication of sulfur compounds was obtained. Following a quiescent 12-hour reaction of the essence with mercuric chloride at room temperature in sealed containers to form the complexes, very little orange aroma was detected compared with the untreated essence used as a control. Apparently, some aroma-producing compounds were complexed out of solution. However, no change in the FI chromatograms of the treated essence was indicated. Investigations are continuing in the identification of these new unknown compounds considered to have some influence on the aroma of fresh orange juice.

Although these results are empirical with respect to the chemistry of aroma and flavor in orange juice, the specific dual-channel EC-FI chromatograms have aided in the use of these natural recovered flavor enhancement materials in product development.

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