Analysis of Recovered Natural Orange Essence by Gas Chromatography

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Concentrated natural orange essences, commercially recovered in aqueous solutions, have aided in investigations of the volatile flavor of citrus juices. Analyses of chemical components, obtained in organic extracts, were performed by programmed temperature gas chromatography (PTGC). Retention temperature studies of the chemical spectrum employing two principal stationary phases, DEGS and Carbowax 20M, supplied tentative identification and peak assignments for 43 compounds. Application of a subtractive type analysis where the carbonyl constituents were removed chemically provided a less complex material for study. Improved PTGC resolution of the carbonyl-free essence facilitated additional peak assignments.

Recovered natural Florida orange essence has served as a good source material for studies directed toward the ultimate characterization of the flavor of fresh citrus juices and processed citrus products. Certain requirements for such a study were necessary. The recovered material had to originate from a natural source and be recovered under carefully controlled conditions. It was also preferred that the material be from a single variety of fruit to obtain results leading to the flavor characterization of juice from that variety. The recovered orange essences met most of the requirements, in so far as were feasible in a commercial recovery operation. Especially important was the knowledge that the material, which was processed as orange juice essence, originated in the condensate from the low-temperature, high-vacuum concentration of the juice. Volatile components thus recovered by the essence processing system were normally a part of the total losses sustained in concentrating orange juice. Therefore, the essence itself was a concentrated solution of the volatile components from Valencia orange juice which contributed to its distinctive flavor.

The volatile flavor components comprised two broad chemical categories, oil-soluble and water-soluble. The oil-soluble fraction, most of which originates in the peel of the fruit, contributes significantly to the flavor of fresh and processed citrus juices. The volatile flavor incorporated in the juice or water-soluble fraction is, likewise, essential to the characteristic flavor associated with a particular variety of citrus fruit. The oil-soluble and water-soluble constituents together included the following five general classes of compounds: carbonyls, alcohols, esters, terpene hydrocarbons, and volatile organic acids. The organic acids will be discussed in a future article now in preparation. The predominantly aqueous solutions of the volatile components undoubtedly were compatible due to natural solvents, such as ethanol, present in the juice.

Previously published information concerning recovery, isolation, and identification of volatile flavor components of citrus juices is very limited. The work of Kirchner and Miller (*7*), concerning the volatile constituents of California Valencia orange juice, has served as a good source of information. They employed standard techniques of evaporation, extraction, fractionation, and chromatostrip separations, and identified some 25 to 30 volatile components in the above-mentioned chemical classifications. Hall and Wilson (*6*) earlier identified the following components in California Valencia orange juice: ethanol, acetone, acetaldehyde, formic acid, an olefin alcohol ($C_{10}H_{18}O$ constituting 90% of the nonwater-soluble constituents) and amyl (probably isoamyl) alcohol, phenylethyl alcohol, and esters of formic, acetic, and caprylic acids. Geraniol and terpineol were indicated but not positively identified. Blair, Godar, Masters, and Riester (*3*) refer to the flavorful components which give orange juice its distinctive orange character as having their origin in the peel oil. These publications show the results of some excellent chemical work in the identification of volatile flavor components.

Application of gas chromatography to analysis of flavor components of citrus juices, as shown by Wolford and Attaway (*10*), has permitted the development of techniques for separation and isolation of volatile components with a minimum use of classical separation techniques. Therefore, some of the variables, such as temperature, pressure, and contact time, in the handling of these complex flavor mixtures were maintained at optimum levels. Gas chromatographic analyses of citrus volatile components by Bernhard (*2*), Clark and Bernhard (*1*, *5*), and Stanley, Ikeda, Vannier, and Rolle (*8*) have dealt principally with the peel oil fractions of lemon, orange, and grapefruit. A recent publication by Attaway, Wolford, and Edwards (*1*) concerning isolation and identification of some volatile carbonyl components from orange essence presented some earlier findings in these investigations.

This article presents methods of analysis employed in the qualitative determination of a large number of volatile flavor components in recovered natural orange essence. Although the results are concerned with the volatile flavor components of only one variety of Florida oranges, the techniques are applicable to the other varieties of citrus fruits. With the identity and, subsequently, the relative amounts of each of the volatile flavor components determined, it should be possible to ascertain the importance of these components as factors in the flavor of the different varieties of citrus fruits.

Experimental

Apparatus. Gas chromatographic analyses were carried out by using an F & M Scientific Model 500 Linear Temperature Programmed Gas Chromatograph.

Two columns (12 feet × ¼ inch in O.D., aluminum tubing) were employed in the separation of the flavor constituents. One column contained Carbowax 20M, 20% on 40- to 60-mesh Chromosorb P. The other column was packed with DEGS (diethylene glycol succinate) using 30% phase on 60- to 80-mesh Chromosorb P. A third aluminum

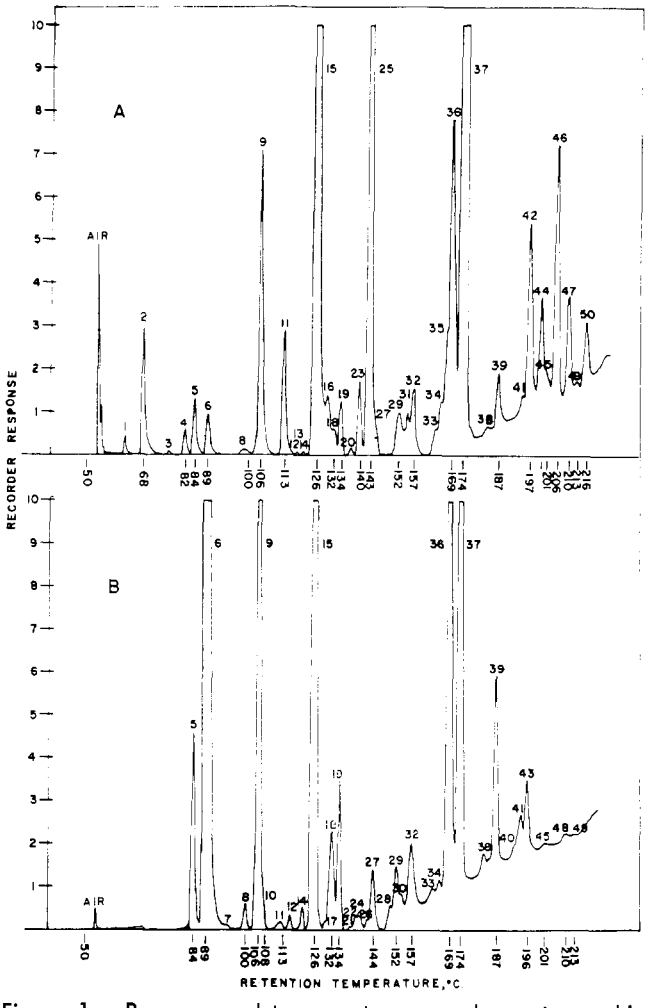

Figure 1. Programmed temperature gas chromatographic analysis of extracts of recovered orange essence

A—analysis of whole extract (VP-41); *B*—analysis of carbonyl-free extract (VP-41). Column: DEGS

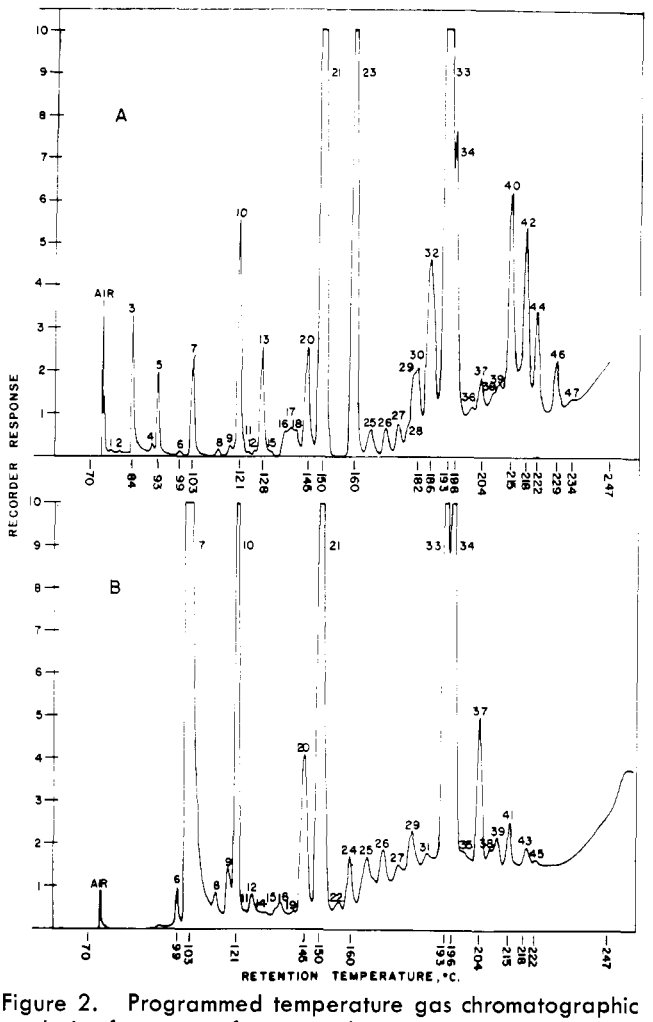

Figure 2. Programmed temperature gas chromatographic analysis of extracts of recovered orange essence

A—whole extract (VP-41); *B*—carbonyl-free extract (VP-41). Column Carbowax 20M

column (12 feet × 1/4 inch in O.D.) packed with Ucon polar (a polyalkylene glycol ether), 30% on 30- to 60-mesh Firebrick, was used for substantiation of peak assignments for a few compounds.

The following operating parameters and procedures were used in these analyses designed for obtaining retention temperatures: The column starting temperature was 50° C. for the DEGS column and 70° C. for the Carbowax 20M column; programmed heat rate was 4.0° C. per minute which was checked by calibration of the reading over the temperature range of operation; the temperature limit on the columns was set at 245° C. for Carbowax and 225° C. for DEGS and Ucon; the two-stage pressure regulator at the helium tank was set at 50 p.s.i. and by controlling the pressure through a Moore differential pressure regulator and needle valve, flow was maintained at 52.6 ml. per minute on the columns. By coincidence, the three columns appeared to have the same back pressure and flow characteristics since no adjustment of pressure and/or flow was necessary when the columns were interchanged. Helium flow to the reference side of the detector was maintained constant at 35 ml. per minute throughout the experiments; block temperature was maintained at 305° C. and injection port temperature at 187° C.; current to the detector filaments was 200 ma.; and the recorder (Minneapolis-Honeywell) −0.2 to 1.0 mv., 1 second, with a 0.25-inch per minute chart speed.

To determine retention temperatures on all separated components in the samples analyzed, 10 μl. were injected using a Hamilton microsyringe. In comparing retention temperatures of knowns and unknowns, 6 μl. of the essence extract and 0.2 to 0.4 μl. of each of the knowns were included in the same injection. An attenuation of 8 was used throughout the study.

Preparation of Samples. Prior to analysis, an organic extract of the predominantly aqueous essence was prepared. Normally, 2 liters of the recovered orange essence, maintained in storage at 32° F., was warmed to 25° C. and saturated with either anhydrous sodium sulfate or sodium chloride. The amount of salt required for saturation was generally variable and may have been dependent upon the concentration of ethanol, which is a normal constituent of orange juice, in the concentrated recovered essence. Following salt saturation, 250 ml. of methylene chloride was added during rapid agitation to cause thorough mixing of the immiscible phases. After sufficient mixing, the solution was transferred to an appropriate size separatory funnel. The organic phase was drawn off and saved. A second extraction of the aqueous layer was carried out using another 250 ml. of methylene chloride. The organic layer was again separated and each extract washed with distilled water to reduce the ethanol content of the extract. The washed extracts were combined and concentrated using a laboratory rotary vacuum evaporator on a water bath at 35° to 40° C. Water aspiration was used as the vacuum source. Although complete removal of residual methylene chloride was not attained, its presence did not materially affect the gas chromatographic results. The resulting organic liquid was straw colored, free of water, and directly analyzable by PTGC.

By using the above extraction procedure, better than 95% of the total volatile components of the essence were isolated. Dual extractions of the same sample proved sufficient as indicated by monitoring successive extractions by PTGC. A third extraction yielded, principally, ethanol. The wash procedure removed approximately 70% of the ethanol content; thus, a greater number of those constituents in very low concentrations were detectable as peaks on the chromatograms.

Application of Subtractive Analysis. The carbonyl components reported in a recent publication (*1*), in the presence of other classes of compounds, lend themselves well to class separation by chemical means. Two procedures have been employed in these investigations. In one method, the organic extract of the aqueous essence was reacted with the Girard-T reagent according to the methods of Teitelbaum (*9*) and Stanley, Ikeda, Vannier, and Rolle (*8*). The results were quite satisfactory for carbonyl removal, but recovered noncarbonyl fractions from the reaction mixtures contained some unreacted carbonyls and formaldehyde used in these methods. The second procedure involved the use of the bisulfite addition reaction to form soluble α-hydroxysulfonate derivatives. This procedure, as employed in the outline below, had the advantage of reactions carried out in dilute aqueous solutions of the essence. The water-soluble addition complexes, furthermore, were not extracted by methylene chloride.

Retention temperature studies for alcohols, esters, and terpene hydrocarbons were carried out using carbonyl-free essences produced by the following procedure. One liter of aqueous essence at approximately 30° C. was used in each case. The essence, as obtained, had a pH of approximately 6.5. Sufficient buffer, 3 grams of citric acid and 101 grams of disodium phosphate, was added to offset the acidic effect of sodium bisulfite. The pH of the reaction mixture at the time of extraction was approximately 6.0. Following addition of 250 grams of sodium bisulfite to the buffered essence, the reaction mixture was allowed to stand overnight for approximately 18 hours in a sealed, glass container at room temperature. The solution was then extracted, without further treatment, using the methylene chloride extraction method. The aqueous layer retained the water-soluble α-hydroxysulfonate addition compounds, and the other organic constituents were extracted into the organic phase. Residual carbonyl content of the noncarbonyl extract was checked by two principal methods. One method involved the use of the "bubbler technique" with 2,4-dinitrophenylhydrazine (2.4-DNPH) (*1*). There was no indication of any 2,4-DNPH derivatives of condensed, eluted components from the gas chromatographic column. Additional tests on the carbonyl-free essence were made by direct 2,4-DNPH precipitation of the noncarbonyl extract, recovery of any precipitate, and subjection to melting point and/or paper chromatographic analysis (*1*). As a second check on the samples following bisulfite treatment, the carbonyl-free organic extracts were analyzed directly by infrared. No absorption was shown on the infrared charts in the carbonyl region. Although the concentration of any residual carbonyls in the extracts may have been too low for detection, such resulting extracts were considered very satisfactory for additional retention temperature studies.

Results and Discussion

The application of programmed temperature gas chromatography to the analysis of volatile flavor components of citrus juices has generally provided for resolution and indication of 35 to 40 component peaks. This is far in excess of previous results employing isothermal conditions, where normally 18 to 23 component peaks were observed. Continued efforts have been made in these studies toward greater resolution and separation of the full spectrum of chemical components in the extracted essence material. One problem imposed by PTGC has been the limited availability of column stationary liquids, particularly polar ones, which were applicable to the temperature range encountered.

The employment of columns of Carbowax 20M and DEGS in these studies has resulted in resolution and indication of 37 and 38 component peaks, respectively. The lower polarity Ucon column accounted for 25 to 30 component peaks.

The use of a subtractive type analysis for the removal of the preponderant group of carbonyls from the recovered orange essence has opened new approaches for study. The chromatogram of a methylene chloride extract of Florida Valencia orange essence (sample VP 41, Figure 1*A*) represents a typical analysis on DEGS of a sample containing carbonyl constituents. In Figure 1*B* may be seen an analysis, also on DEGS, of the same sample of orange essence from which the carbonyls were removed. By employing retention temperatures and enrichment techniques, both chromatograms (Figure 1) could be superimposed. Retention temperatures throughout the chromatograms correlated very well. There was coincidence of some previously (*1*) identified peaks, such as acetaldehyde, *n*-hexanal, *n*-octanal, geranial, and neral. Also, two previously identified alcohols, ethanol and linaloöl, were employed as a further check on the validity of the method. The same procedure was carried out for those separations made on Carbowax 20M (Figure 2*A*, *B*).

The combined chromatograms, presented for clarity, provide cumulative peak counts based on increasing retention temperatures in both scans. For the purpose of this study, each peak or curve inflection was numbered. The combined chromatograms (Figure 1) show a total count of 50 peaks obtained by separation on DEGS. The chromatograms obtained on Carbowax 20M (Figure 2) show a total of 47 component peaks.

Data are presented in the form of observed retention temperatures with the operating parameters maintained as outlined earlier. All observed temperatures were checked against the calibration of the temperature programmer and recorder chart speed. The gas chromatograph was maintained on a continuous operating basis, with carrier gas pressure, flow, and instrument component temperatures unchanged during off hours. Each column was heated to the maximum temperature of the program schedule daily prior to analyses. Also, flow measurements were checked periodically during the operation throughout the column temperature range employed.

Retention temperatures for analyses on both the DEGS and Carbowax 20M columns are shown in Table I. Peak identities were assigned on the basis of agreement between retention temperatures for unknown peaks with those for known compounds added to the essence in the enrichment procedures. In most cases, a requirement was established that the retention temperatures of knowns and unknowns must coincide at identical temperatures. In a few instances, a ±1° C. difference in the observed temperature was considered satisfactory for agreement. Those recorded retention temperatures which fall outside the established limits are shown in parentheses and the compounds corresponding to such temperatures were excluded from the list of tentative identifications shown in Table II. Results show good agreement of observed retention temperatures between the two columns employed for the compounds listed. Some 56 known compounds were studied of which 43 were tentatively identified as occurring in recovered orange essence.

A third column, Ucon polar, was employed for substantiating tentative identification of the following compounds: *n*-decanal, *n*-nonanal, α-ethylbutyraldehyde, and 2-octenal. No positive correlation was achieved for 2-octenal on the DEGS-Carbowax 20M system. However, its presence in the orange essence received some substantiation in the DEGS-Ucon polar system.

The compounds listed with a question mark (Table II) were either not sepa-

Table I. Observed Retention Temperatures of Components in Recovered Orange Essence on Two Stationary Phases

Peak	Retention Temperatures, °C. Unknown	Known	Compound	Peak	Unknown	Known	Compound
			DEGS				
1	61	...	...	29	152	152	3-Hexenol
2	68	68	Acetaldehyde				2-Octenal
3	78	...	...	30	154	...	...
4	82	82	Acetone	31	155	156	*n*-Nonanal
5	84	84	Methanol	32	157	157	Ethyl-*n*-caprylate
		84	Acetal	33	164	164	2-Nonanol
		87	Ethyl acetate	34	166	166	2-Octenol
6	89	89	Ethanol	35	167	167	*n*-Decanal; citronellal
7	97	96	α-Pinene	36	169	169	*n*-Octanol
8	100	(102)	α-Ethylbutyraldehyde	37	174	174	Linaloöl; citronellol
9	106	...	...			(180)	*n*-Dodecanal
10	108	(111)	2-Methyl-2-pentanol	38	183	183	1-Nonanal; *n*-undecanal
11	113	113	*n*-Hexanal			(186)	Undecylenic alcohol
12	115	115	Δ^3-Carene	39	187	187	α-Terpineol
			n-Butyl alcohol			188	Citronellyl acetate
13	118	118	β-Myrcene	40	192	192	Citronellal
14	121	121	α-Terpinene			(193)	*n*-Decanol
15	126	126	2-Hexenal; *d*-Limonene	41	195	195	Geraniol; terpinyl acetate
16	130	(129)	Ocimene	42	196	196	Nerol
17	130	(131)	2-Ethylhexanal	43	197	197	*d*-Nerolidol
		(131)	γ-Terpinene	44	200	200	Geranial
18	132	132	*n*-Amyl alcohol	45	201	...	...
19	134	...	...			(203)	Undecanol
20	137	137	Terpinolene	46	206	206	Neral
21	138	...	...			207	Methyl-*N*-methyl anthranilate
22	139	139	*p*-Cymene				
23	140	140	2-Hexenal	47	210	210	*L*-Carvone
24	141	...	...	48	210	...	...
25	143	143	*n*-Octanal; 2-Octanone			(211)	*trans*-Carveol
26	143	...	...	49	213	214	*cis*-Carveol
27	144	144	*n*-Hexanol	50	216	...	...
28	150	...	...				
			Carbowax 20M				
1	77	...	...	25	165	165	*n*-Hexanol
2	80	...	...	26	170	170	3-Hexenol
3	84	84	Acetaldehyde			(173)	2-Octenal
4	91	91	Acetone	27	175	175	*n*-Nonanal
5	93	...	...	28	178	...	...
6	99	99	Ethyl acetate; acetal	29	180	(179)	Ethyl-*n*-caprylate
7	103	103	Ethanol; methanol	30	182	(181)	2-Octenal
8	113	...	...			(184)	Furfural
9	118	118	α-Ethylbutyraldehyde	31	186	186	2-Nonanol
10	121	...	...	32	186	186	*n*-Decanal
11	123	123	α-Pinene			(187)	Citronellal
12	126	...	...	33	193	193	Linaloöl
		(127)	2-Methyl-2-pentanol	34	196	196	*n*-Octanol
13	128	128	*n*-Hexanal			(197)	Citronellyl acetate
14	130	...	...	35	200	...	...
15	131	...	...	36	202	...	...
16	136	...	...	37	204	204	*n*-Undecanal
17	138	138	*n*-Butanol	38	208	208	1-Nonanol; citronellol
18	140	...	...	39	211	...	...
19	142	142	β-Myrcene			(214)	*d*-Nerolidol
		(142–3)	Δ^3-Carene	40	215	215	Geranial
20	145	145	α-Terpinene	41	215	215	α-Terpineol
21	150	150	2-Hexenal; *d*-limonene			(217)	*n*-Dodecanal
				42	218	218	Neral; methyl-*N*-methyl anthranilate
		150	*n*-Amyl alcohol				
		(152)	Ocimene	43	219	219	*n*-Decanol; nerol; geraniol; terpinyl acetate
22	157	157	γ-Terpinene				
23	160	160	*n*-Octanal; 2-Octanone				
24	160	160	Terpinolene; *p*-cymene	44	222	222	L-Carvone
				45	222	...	...
		163	2-Hexenal	46	229	229	*trans*-Carveol
						232	*cis*-arcveol
				47	234	...	...

Table II. Tentative Identification of Volatile Flavor Components in Commercially Recovered Florida Valencia Orange Essence

Hydrocarbons	Carbonyls
d-Limonene	Acetaldehyde[a]
β-Myrcene	Acetone
Terpinolene	α-Ethylbutyraldehyde?
α-Terpinene	*n*-Hexanal[a]
γ-Terpinene	2-Hexenal[a] (2-isomers)
α-Pinene?	*n*-Octanal[a]
p-Cymene?	2-Octanone?
Δ^3-Carene	2-Octenal (2-isomers)?
Alcohols	*n*-Nonanal
	n-Decanal
Methanol	Citronellal
Ethanol	*n*-Undecanal?
n-Butanol?	Geranial[a]
n-Amyl alcohol	Neral[a]
1-Hexanol	L-Carvone[a]
3-Hexenol	**Esters**
1-Nonanol	
2-Nonanol	Ethyl acetate?
1-Octanol	
Linaloöl	Ethyl *n*-caprylate
Citronellol	Terpinyl acetate
1-Decanol?	Methyl *N*-methyl anthranilate
α-Terpineol	
Geraniol	
Nerol	
Carveol?	

[a] Previously identified as 2,4-DNPH derivatives (*1*).

rately resolved or deviated from the required ±1° C. differential on either one of the columns.

Figure 3 shows a chromatogram of an organic extract of Valencia orange essence (sample V2) separated on DEGS. This particular sample, a little less concentrated than the VP-41 sample, provides a clear example of the effect of bisulfite treatment on the essence for carbonyl removal. In zone *B*, the dotted line indicates the location of the 2-hexenal (isomer) at 126° C. prior to its removal. The peak remaining, identified as *d*-limonene by the enrichment technique, was masked in the analysis of the whole essence extract. In zone *C*, the two component peaks, 2-hexenal (isomer) at 140° C. and octanal at 143° C., were removed. The peak at 144° C. then corresponded to 1-hexanol. This component appeared as a small shoulder on the octanal peak previous to carbonyl removal. The dotted line in zone *D*, representing the peak as it appeared in the original chromatogram of the whole essence extract, shows the possible presence of one or more carbonyl components mixed with linaloöl. These studies have failed to show a carbonyl with a retention temperature on DEGS to correlate with that in zone *D*. It remains to be determined whether any carbonyls are involved with the linaloöl peak on Carbowax 20M as evidenced by a lower relative concentration in peak 33 (Figure 2*B*) after carbonyl removal. Also in zone *D*, the dotted line at 167° C. substantiates the presence of some carbonyls, probably *n*-decanal and/or citronellal, since they had the same retention

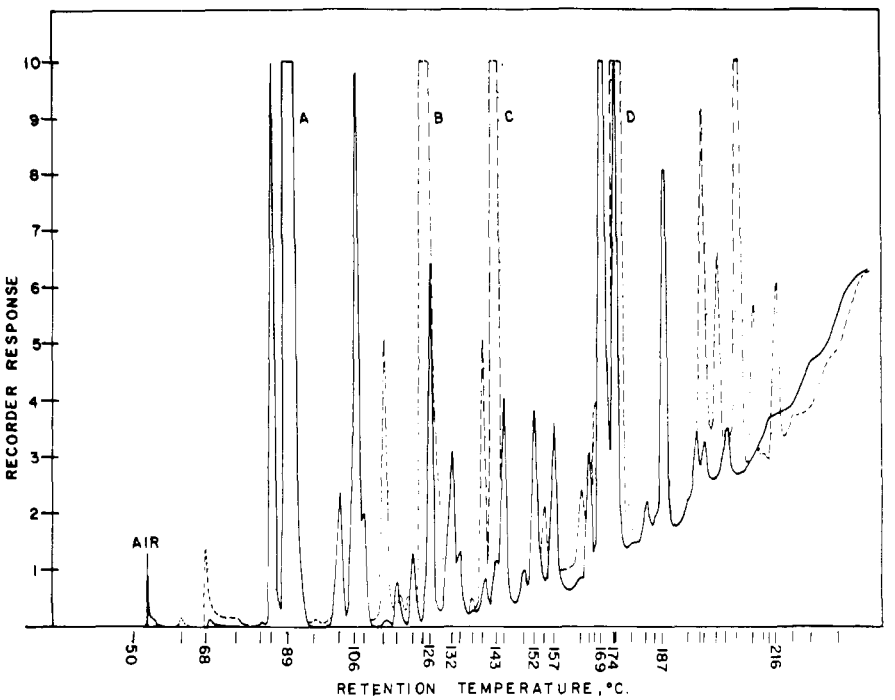

Figure 3. Programmed temperature gas chromatographic analysis of carbonyl-free essence extract

Removed carbonyls indicated by dashed lines. Column: DEGS

temperatures in each of the two columns employed. Neither DEGS nor Carbowax 20M made possible the separation of these carbonyls. However, Ucon polar showed a retention temperature for *n*-decanal above linaloöl where it occurred as a shoulder on the tailing edge of the linaloöl peak. Additional column studies will be required to obtain true separation of *n*-decanal and citronellal. Others requiring additional studies are ethyl acetate, 1-decanol, 2-octanone, and carveol.

The peak at 89° C. in zone *A* contains both ethanol and methylene chloride which was employed in the extraction procedure. The disproportionate concentrations of peak 6 (Figure 1*B*) and peak 7 (Figure 2*B*) are, likewise, due to methylene chloride residue mixed with ethanol.

It was interesting to note (Figures 1 and 2) a reversal in the order of elution and retention temperatures of 1-octanol and linaloöl. In the separation on DEGS, 1-octanol (Figure 1*A*, peak 36) had a lower retention temperature than linaloöl, peak 37, while on Carbowax 20M, linaloöl (Figure 2*A*, peak 33) had the shorter elution time and a lower retention temperature than 1-octanol, peak 34.

The application of a subtractive method for the quantitative removal of the carbonyl components as a class provided a much less complex complex system. Elimination of carbonyls from the orange essence sample and, therefore, from the chromatogram of the essence extract served several purposes. By virtue of their elimination, further confirmation of retention temperatures of the carbonyl components was obtained. Also, at least five additional carbonyls were tentatively identified. Because of similar partition coefficients many non-carbonyls were masked and unobserved, except upon removal of the carbonyl constituents. By subtractive analyses some conception of the importance of a class of chemical components to the natural flavor may be realized. The removal of carbonyls left a material virtually void of the odor of orange juice.

The regeneration of the carbonyls from their α-hydroxysulfonate addition compounds has received some intensive study. Also, regeneration from the Girard-T reaction has been carried out. However, a quantitative regeneration is desired with a maximum purity. Work has been continued, particularly in the regeneration following the bisulfite reaction, in the hope of obtaining positive identifications for the five additional carbonyls indicated in this study.

Many terpene hydrocarbons (Table II) are known to be associated with cold-pressed orange oil. However, in studying recovered natural orange essences, identification of the terpene hydrocarbons is more difficult than may be the case with peel oil. Kirchner and Miller (7) identified *d*-limonene and β-myrcene along with three other terpenes which lacked absolute identification. Certainly, a separate study of terpene hydrocarbons in recovered orange essence will be required.

Additional confirmation of retention temperatures of some of the tentatively identified components is needed. By use of additional liquid stationary phases, studies of some components (Table II) would lend more credence to the assignment of peak identities. Nevertheless, results of these investigations indicate the presence of several volatile flavor components not previously identified.

Acknowledgment

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