

Quantitative Analysis of the Volatile Constituents of Lemon Peel Oil. Effects of Silica Gel Chromatography on the Composition of Its Hydrocarbon and Oxygenated Fractions

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Fifty-one constituents accounting for $\approx 99.7\%$ of total volatiles common to both Sicilian and California commercial lemon peel oils have been identified and quantified in a single GC capillary run. The quantification was performed by using both internal standard and appropriate response factors, which are necessary for accurate volatile analysis and simultaneous determination of nonvolatiles. Direct comparisons to results obtained by using area percent or internal standard only show the advantage of using response factors. Fractionation of the oils by open-column silica chromatography facilitated identification of constituents and improved the accuracy of the analysis. Yields of the fractions recovered from the column were usually greater than 96%. The reactivity of high-quality silica gel with lemon hydrocarbons was investigated, and while quantitative effects are small, several oxygenates are formed on the column. Use of a cold (3°C) column significantly reduces the amount but not the number of oxygenates formed.

Lemon oil is an essential oil of considerable commercial importance. In the mid 1980s the United States led in lemon oil production with $\approx 25\%$ of total world output (2300 metric tons) followed closely by Italy and Argentina (Lawrence, 1985). In spite of the large domestic production, more lemon oil than any other essential oil except orange is imported into the United States. These imports amount to ≈ 700 metric tons and are third in dollar value only to distilled lime and patchouli oils (Pisano, 1986).

Considering the economic value of lemon oil, it is surprising that many trace constituents that probably contribute to lemon flavor remain unidentified, and in addition, only 37 constituents have been thoroughly quantified (Staroscik and Wilson, 1982a,b). An understanding of the relationship between taste and composition of lemon oil requires that a relatively complete quantitative data base be developed. Some results on the correlation between certain components of cold pressed lemon oil and odor, but not taste, have been published (Drawert and Christoph, 1984; Schieberle and Grosch, 1988).

The most accurate GC analyses of essential oils and flavor volatiles are obtained by using both an internal standard and response factors (RFs). Shaw (1979) has argued convincingly for the adoption of this approach, but it is still not utilized enough. Of some 20 substantive papers concerning the analysis of lemon oil published since 1960, only one group (Staroscik and Wilson, 1982a,b) used both an internal standard and response factors; another group (Selzer and Christoph, 1981) employed an internal standard alone.

A series of papers (Cotroneo, 1986, 1988) has appeared recently on Sicilian lemon oil, which presents processing and seasonal variations for major components, functional group analysis, and GC area percent data on about 40 components. However, internal standard and detector response factors (RFs) were not used. Other recent studies on lemon oils offer either very little quantitative data (Mazza, 1987) or area percent data (Boelens and Jimenez, 1989). A review (Lawrence, 1989) of recent lemon oil analysis has just been published.

Another source of error in quantification of essential oils and flavors is the possible formation of artifacts during isolation and separation of constituents. While flavors are fractionated quite frequently on silica gel or alumina, we find only one relatively recent and comprehensive study/review (Scheffer, 1976, 1977) concerning the quantitative effects of silica gel fractionation on essential oil composition. This work, while reasonable for the time, was done on packed columns and only area percent data were reported. In addition, the column was not checked for potential oxygenated artifacts that may have been formed from hydrocarbon reactions on the column.

We report here a detailed quantitative analysis of commercial lemon peel oils. Sicilian lemon oil, generally considered the industry standard, was chosen for a majority of our work. Some data were also collected from California oil, and pertinent comparisons are made. Using a low-polarity fused silica capillary column and an internal standard with RFs, we have quantified, in a single GC analysis, 51 compounds that occur in both Sicilian and California lemon oils; 23 of these constituents have not previously been quantified in lemon oil. The hydrocarbon and oxygenated fractions of the oils were also analyzed, allowing better resolution and quantification of several peaks that otherwise could not be properly separated in whole oil. Finally, a detailed quantitative study of the effects of silica open column fractionation on lemon hydrocarbons, including an analysis for hydration and oxidation products of the hydrocarbons formed on the silica column, is reported. Some data on the effects of silica gel on the oxygenated fraction of lemon are also given.

EXPERIMENTAL PROCEDURES

Materials. Silica 100–200 μm , active, from Universal Scientific was adjusted to activity III. Commercial lemon peel oil was either Sicilian winter isolated by the "sfumatrice" process or California cold-pressed. B&J hexane UV grade was purified further by passing through an open column packed with equal amounts of silica (Act. I) and Camag basic alumina (Act. I). Tetradecane 99.9% by GC was obtained from Wiley Organics,

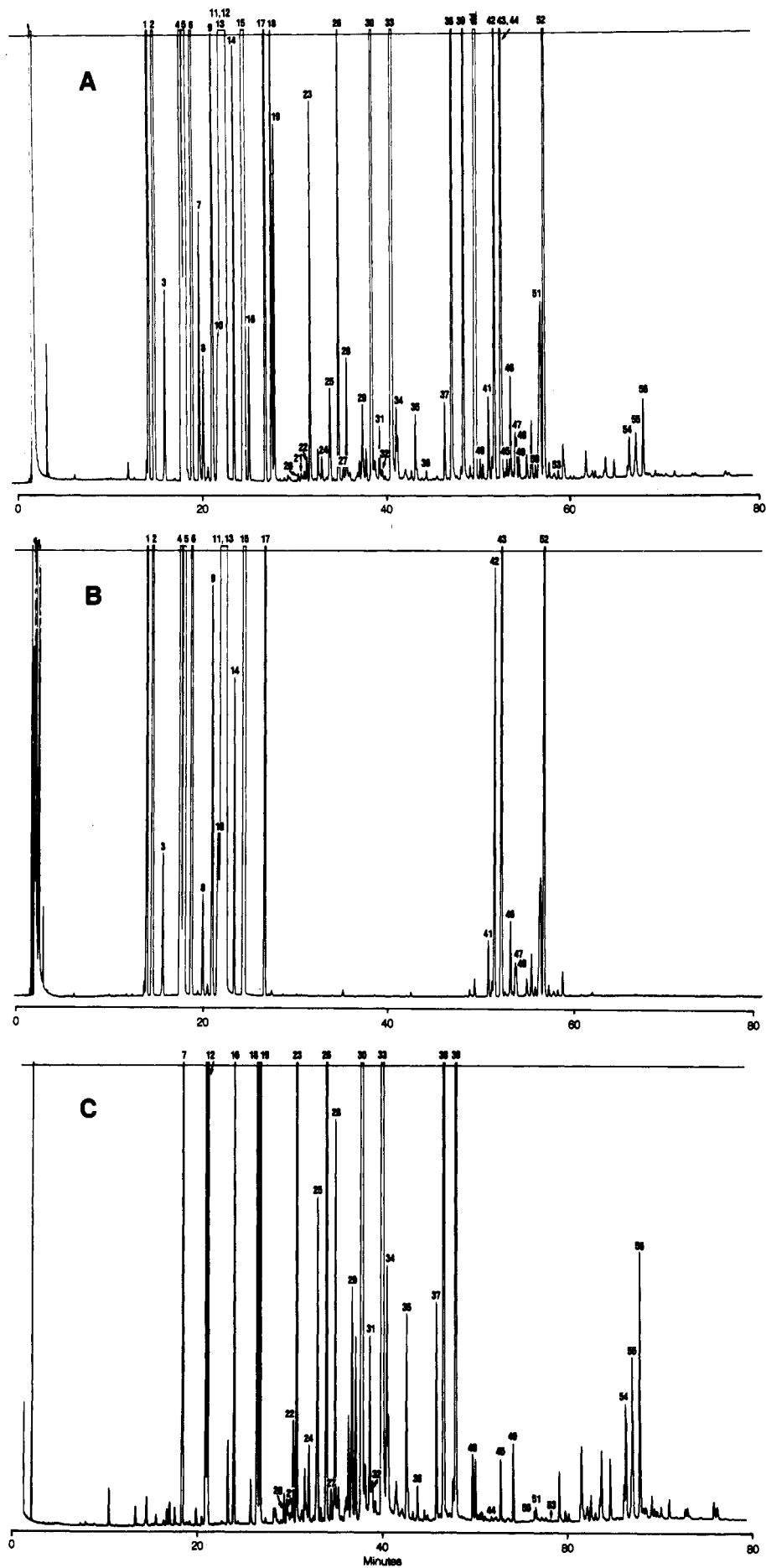


Figure 1. Capillary GC separations of Sicilian lemon oil employing DB-5: (A) whole peel oil; (B) hydrocarbon fraction; (C) oxygenated fraction.

Table I. Quantitative Data for Whole Sicilian and California Lemon Peel Oils, Using Internal Standard and Response Factors

peak no. ^a	compd ^b	Sicilian, ^c wt % (% RSD) ^e	California, ^c wt % (% RSD) ^e	literature citations ^d
monoterpene hydrocarbons				
1	α -thujene	0.42 (0.47)	0.38 (0.36)	f, g, h
2	α -pinene	1.84 (0.49)	1.75 (0.37)	f, g, h
3	camphene	0.05 (0.49)	0.06 (0.64)	f, g, h
4	sabinene	1.79 (0.48)	1.85 (0.38)	f, g, h
5	β -pinene	10.52 (0.48)	11.13 (0.35)	f, g, h
6	myrcene	1.61 (0.50)	1.58 (0.36)	f, g, h
8	α -phellandrene	0.04 (1.38)	0.04 (0.43)	g, h
9	α -terpinene	0.20 (0.57)	0.16 (0.29)	f, g, h
10	<i>p</i> -cymene	0.07 (13.99)	0.14 (7.81)	f, g, h
11, 12, 13	limonene/1,8-cineole/ <i>cis</i> -ocimene ^f	65.16 (0.57)	65.65 (0.30)	f, g, h
14	<i>trans</i> -ocimene ^f	0.11 (0.75)	0.09 (1.07)	f
15	γ -terpinene	8.86 (0.52)	8.32 (0.32)	f, g, h
17	terpinolene	0.39 (0.55)	0.34 (0.31)	f, g, h
	total	91.06	91.49	
alcohols				
16	<i>trans</i> -sabinene hydrate/octanol	0.05 (1.01)	0.01 (16.07)	f, j, k
18	linalool/ <i>cis</i> -sabinene hydrate	0.18 (0.85)	0.13 (0.43)	f, g, h
24	borneol ⁱ	0.009 (2.94)	0.01 (7.19)	f, k
25	terpinen-4-ol	0.05 (0.99)	0.11 (0.64)	f, g, h
26	α -terpineol	0.19 (0.95)	0.16 (0.44)	f, g, h
29	citronellol/nerol	0.03 (0.86)	0.06 (0.74)	f, g, l
31	geraniol	0.02 (1.14)	0.03 (1.05)	f, g, h
	total	0.53	0.51	
aldehydes				
7	octanal	0.10 (0.98)	0.08 (0.66)	f, g, h
19	nonanal	0.12 (1.07)	0.12 (0.71)	f, g, h
23	citronellal	0.13 (0.77)	0.08 (0.53)	f, g, h
28	decanal	0.05 (2.47)	0.05 (1.77)	f, g, h
30	neral	1.26 (0.39)	0.74 (0.40)	f, g, h
33	geranial	2.05 (0.38)	1.18 (0.39)	f, g, h
34	perillaldehyde ^f	0.03 (0.83)	0.03 (0.74)	f, j
35	undecanal ⁱ	0.03 (7.15)	0.03 (0.92)	f, j, k
40	dodecanal/decyl acetate	0.008 (1.35)	0.01 (5.07)	f, h
51	tridecanal	<i>m</i>	<i>m</i>	j, n
	total	3.78	2.32	
sesquiterpene hydrocarbons				
41	<i>cis</i> - α -bergamotene ^f	0.03 (0.54)	0.03 (0.43)	f
42	caryophyllene	0.19 (0.08)	0.22 (0.07)	f, g, h
43, 44	<i>trans</i> - α -bergamotene/citronellyl propionate ^f	0.36 (0.09)	0.38 (0.05)	f, g, h
46	<i>trans</i> - β -farnesene ^f (t)	0.03 (0.26)	0.03 (1.30)	f
47	α -humulene (t)	0.02 (2.19)	0.02 (1.47)	f, g, h
48	β -santalene ^f (t)	0.01 (3.16)	0.01 (1.08)	f
52	β -bisabolene	0.52 (0.24)	0.55 (0.27)	f, g, h
	total	1.16	1.24	
esters				
36	methyl geranoate ^f	0.005 (8.24)	0.007 (15.11)	k
37	citronellyl acetate	0.03 (3.28)	0.03 (1.21)	f, g, h
38	neryl acetate	0.52 (0.41)	0.60 (0.22)	f, g, h
39	geranyl acetate	0.38 (0.35)	0.52 (0.27)	f, g, h
45	neryl propionate ^f	0.007 (3.91)	0.007 (3.82)	f
49	geranyl propionate ^f	0.009 (2.08)	0.01 (3.09)	f
50	undecyl acetate ^f	0.006 (7.10)	0.009 (0.97)	f
	total	0.96	1.18	
ketones and oxides				
20	<i>trans</i> -limonene 1,2-oxide ^f	0.005 (10.16)	0.007 (6.31)	f
21	<i>cis</i> -limonene 1,2-oxide ^f	0.004 (7.63)	0.006 (4.11)	f
22	camphor ^f	0.01 (3.13)	0.008 (1.97)	f
27	<i>cis</i> -4,7-dimethylbicyclo[3.2.1]oct-3-en-6-one (<i>exo</i>) ⁱ	0.008 (8.61)	0.01 (5.76)	o
32	piperitone ^f	0.004 (6.56)	0.004 (4.73)	f
	total	0.03	0.03	
sesquiterpene alcohols				
53	nerolidol ^f	0.004 (4.62)	0.004 (8.88)	j
54	2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol ^f (t)	0.02 (1.88)	0.03 (6.59)	p
55	campherol ^f	0.03 (3.22)	0.03 (2.33)	p
56	α -bisabolol ^f	0.04 (0.87)	0.04 (1.27)	k
	total	0.09	0.10	
	unidentified	0.20	0.29	
	total volatiles	97.81	97.16	

^a Peak numbers refer to Figure 1. ^b Peak identifications are based on MS comparisons with file spectra and relative retention time. If a question remained, a known standard was used for MS and peak enrichment. If a standard was not available, the peak is marked tentative (t). ^c Absolute weight percent using internal standard and response factors, average of six GC runs. ^d Up to three previous literature citations are included. ^e % RSD, percent relative standard deviation = (component standard deviation/average weight percent of component) \times 100. ^f Mazza (1987). ^g Cotroneo et al. (1986). ^h Staroscik and Wilson (1982a,b). ⁱ First time quantified in lemon with internal standard and response factors (for *cis*-ocimene, 1,8-cineole, and citronellyl propionate, see Tables IV and VI). ^j Selzer and Christoph (1981). ^k Fleisher et al. (1987). ^l Cartoni et al. (1986). ^m Cannot be quantified in whole oil due to co-eluting constituents. ⁿ Ikeda et al. (1962). ^o Koepsel and Surburg (1988). ^p Mussinan et al. (1981). These constituents have been identified in lemon juice.

Coshocton, OH. The *cis*-oct-3-en-6-one, peak 27, was obtained as a gift from Dr. H. Surburg, Haarmann & Reimer, Holzmin-den, FRG.

Chromatography and Spectroscopy. GC analyses were performed on a Varian 6000 equipped with a 1075 split/splitless injector and using helium (1 mL/min) as a carrier. A straight-tube, glass, Varian injector insert packed with silanized glass wool was employed with a split ratio of 60:1. A Varian 8000 autosampler equipped with a 10- μ L syringe was used with an injection volume of 0.8 μ L. A J&W, DB-5 fused silica column, 30 m \times 0.32 mm (i.d.), 1- μ m film thickness, was employed with an oven program of 10-min initial hold at 70 $^{\circ}$ C, 2 $^{\circ}$ C/min to 190 $^{\circ}$ C, final hold at 190 $^{\circ}$ C for 45 min. The injector and FID detector were maintained at 190 and 240 $^{\circ}$ C, respectively.

The oils were generally analyzed as 20% solutions in methylene chloride with 4% tetradecane added as an internal standard. Detector response factors were determined for key components relative to tetradecane and assigned to other components on the basis of functional group and/or structural similarity (Table II). In general, standards were >95% pure, and actual purity was checked by packed column GC. Several response factor solutions were prepared that consisted of only three to four components, plus tetradecane, to prevent interference from trace impurities.

Open column chromatographic separations were performed on a 42 cm \times 2.5 cm (i.d.) column, dry packed with silica (40 g), and hexane was added after packing. Lemon oil (4 g) was adsorbed onto the column, and the hydrocarbon fraction was eluted with hexane (\approx 200 mL), until the eluate contained no further material as determined by GC. The oxygenates were eluted with CH_2Cl_2 (500 mL). Finally the column was stripped with 20% CH_3OH in CH_2Cl_2 (70 mL). Only a trace of volatiles was not eluted by CH_2Cl_2 . In one open column separation a jacketed column was used through which chilled water (3 $^{\circ}$ C) was circulated. All other procedures for the cold column were identical with the above except the elution solvent was cooled in an ice bath before use.

The hydrocarbon fraction was analyzed directly by GC after addition of tetradecane standard. The oxygenated constituents were too dilute to analyze directly, and following addition of standard they were concentrated to \approx 3 mL in a Kuderna-Danish evaporative still. After the initial separation, the hydrocarbons, without standard, were concentrated in the K-D still and chromatographed a second time exactly as above except the CH_2Cl_2 elution was omitted. The oxygenated fraction, eluted as noted above from the silica column with CH_2Cl_2 , was concentrated in vacuo and further separated by HPLC. Fifteen subfractions were collected from three silica columns in tandem by using 8% ethyl acetate in a 50/50 mixture of CH_2Cl_2 /hexane as reported previously for lime oils (Chamblee et al., 1985).

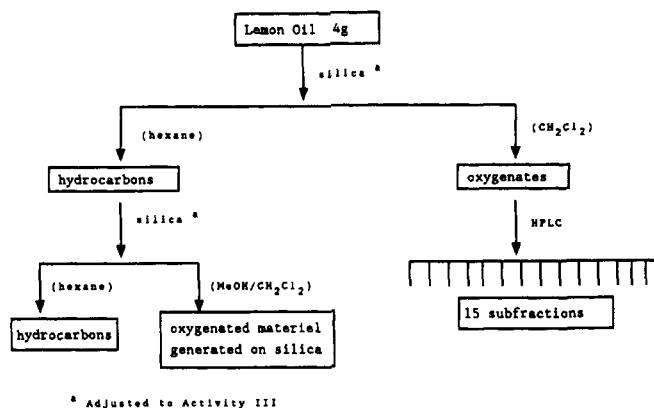
GC-MS analyses were carried out on a Kratos MS 25 RFA equipped with a HP-5890 GC and a 60-m version of the fused capillary column described above.

RESULTS AND DISCUSSION

Quantification of Lemon Oils. A bonded J&W DB-5 thick film, fused silica capillary was chosen for this work. DB-5 is a 5% phenylmethylsilicone similar to SE-54 on which a good overall separation of lemon oil has been reported (Staroscik and Wilson, 1982a,b). A labeled chromatogram of Sicilian lemon peel oil is shown in Figure 1A, and a labeled chromatogram of California oil is available as supplementary material. Quantitative data for both Sicilian and California commercial lemon oils are shown in Table I.

All compounds quantified and listed in Table I have been confirmed by GC-MS analysis of the whole oil. Many of these compounds were initially identified by GC-MS analysis of the oxygenated fraction from the open column chromatography or from the 15 HPLC subfractions of this material. The fractionation is summarized in Scheme I.

Scheme I. Fractionation of Lemon Oil



Once the identification was made from a clean MS in the appropriate fraction, the constituent was confirmed in the whole oil by direct GC-MS and as noted in Table I. Lemon literature citations, up to three, are given for each constituent in Table I. All constituents quantified are present in both Sicilian and California lemon oils, although the concentrations may vary. The Sicilian oil is higher in citral (neral, geranial), total aldehydes, and total oxygenated constituents, while the California oil is somewhat higher in esters. As shown in Table I only 0.2–0.3% of the volatiles in the two oils remain unidentified.

Quantification was based on an internal standard method using tetradecane and RFs which were experimentally determined for most of the major constituents, and some minor ones, by using standards of known high purity. The remainder of the RFs were assigned on the basis of structural similarities and are all noted in Table II. We have used this approach for some time with the FID detector (Clark et al., 1981, 1984, 1987) and find it gives very accurate and reproducible results. For packed columns the RFs change very little even from instrument to instrument. Some variation in RF may be observed with capillary work depending on the type of injector, liner, and column. These changes can be detected by periodic checking of RFs for a few key constituents with a standard solution. The data in Table I show a typical quantification and represent the average of six GC analyses. A percent relative standard deviation (% RSD) below 5% was obtained for all constituents except eight very small constituents, which had higher % RSD.

Comparison of the Sicilian lemon oil data given in Table I (full quantification, internal standard with RF) with those in Table II (partial quantification, either area percent or internal standard alone) shows important differences in the content of several major components such as neral, geranial, and limonene and for the percent total volatiles value. These differences could be even larger when essential oils with greater percentages of nonvolatile material are analyzed. Use of RFs with internal standard gives, in addition to a more accurate volatile analysis, a good value for nonvolatile material in the oil, which generally serves as a check of evaporative methods for nonvolatiles (Wilson and Shaw, 1980). For the Sicilian oil we found 2.3% nonvolatiles by GC and 1.8% by total evaporation and for the California oil 2.9% nonvolatiles by GC and 2.6% by evaporation.

Analysis of Lemon Hydrocarbons. In addition to facilitating the identification of lemon oil constituents, fractionation (Scheme I) into hydrocarbons and oxygenated constituents on an open silica gel column of medium (III) activity allowed for quantification of each fraction individually. Yields of recovered hydrocarbons and

Table II. Sicilian Lemon: Response Factors (RF) and Comparison of Area Percent vs Internal Standard (No RF)

peak no.	compd	RF	area %	internal std (no RF)
1	α -thujene	0.928	0.45	0.46
2	α -pinene	0.928 ^a	1.94	1.98
3	camphene	0.928	0.06	0.06
4	sabinene	0.928	1.89	1.93
5	β -pinene	0.928 ^a	11.10	11.33
6	myrcene	0.999	1.58	1.61
7	octanal	1.299	0.08	0.08
8	α -phellandrene	0.967	0.04	0.04
9	α -terpinene	0.967 ^a	0.20	0.20
10	<i>p</i> -cymene	0.901 ^a	0.07	0.08
11	limonene ^b	0.947 ^a	67.37	68.80
14	<i>trans</i> -ocimene	0.999 ^a	0.11	0.11
15	γ -terpinene	0.967	8.97	9.16
16	<i>trans</i> -sabinene hydrate ^b	1.134	0.04	0.04
17	terpinolene	0.964 ^a	0.39	0.40
18	linalool ^b	1.222 ^a	0.14	0.14
19	nonanal	1.299	0.09	0.09
20	<i>trans</i> -limonene oxide	1.255 ^a	0.004	0.004
21	<i>cis</i> -limonene oxide	1.255 ^a	0.003	0.003
22	camphor	1.158 ^a	0.008	0.008
23	citronellal	1.193 ^a	0.11	0.11
24	borneol	1.134 ^a	0.008	0.008
25	terpinen-4-ol	1.141	0.04	0.04
26	α -terpineol	1.141 ^a	0.18	0.18
27	<i>cis</i> -4,7-dimethylbicyclo-[3.2.1]oct-3-en-6-one (<i>exo</i>)	1.158	0.007	0.007
28	decanal	1.299 ^a	0.04	0.04
29	citronellol/nerol	1.189 ^a	0.03	0.03
30	neral	1.169 ^a	1.05	1.08
31	geraniol	1.189	0.02	0.02
32	piperitone	1.158	0.003	0.004
33	geranial	1.169 ^a	1.72	1.75
34	perillaldehyde	1.212 ^c	0.02	0.02
35	undecanal	1.212 ^c	0.02	0.02
36	methyl geranoate	1.322	0.004	0.004
37	citronellyl acetate	1.322	0.02	0.02
38	neryl acetate	1.322 ^a	0.38	0.39
39	geranyl acetate	1.322 ^a	0.28	0.29
	tetradecane ^d	1.000		
40	dodecanal/decyl acetate	1.311 ^e	0.006	0.006
41	<i>cis</i> - α -bergamotene ^b	0.979	0.03	0.03
42	caryophyllene	0.979 ^a	0.19	0.20
43	<i>trans</i> - α -bergamotene ^b	0.979	0.36	0.36
45	neryl propionate	1.322	0.005	0.005
46	<i>trans</i> - β -farnesene	0.979	0.03	0.03
47	α -humulene	0.979	0.02	0.02
48	β -santalene	0.979	0.01	0.01
49	geranyl propionate	1.322	0.007	0.007
50	undecyl acetate	1.410 ^a	0.004	0.004
52	β -bisabolene	0.979	0.52	0.53
53	nerolidol	1.350 ^a	0.003	0.003
54	2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol	1.350	0.02	0.02
55	campherenol	1.350	0.02	0.02
56	α -bisabolol	1.350	0.03	0.03
	unidentified	1.000	0.20	0.20
	total		99.92	102.01

^a RFs were calculated from standard solutions. Remaining RFs were assigned according to similarity of chemical structure. ^b Co-eluting with other peaks; see Table I. RF was calculated/assigned for constituent listed. ^c RF determined for tridecanal was assigned. ^d Internal standard. ^e Average of RFs for tridecanal and undecyl acetate.

oxygenated material were checked quantitatively by using internal standard and RFs for several samples chromatographed on the silica column. As shown in Table III the recovery of hydrocarbons and oxygenated material from the column was consistently high.

Comparisons were then made for individual constituents between data obtained from the fractions and from the whole oil. A chromatogram of the hydrocarbons from the

Table III. Yields of Hydrocarbon and Oxygenated Fractions from the Silica Column

	sample no.	% recovery ^a	column temp, °C
Hydrocarbons			
Sicilian	1	96	room temp
	2	97	room temp
	3	100	room temp
	4	102	room temp
	5	99	room temp
	6	101	room temp
	7	96	3
California	1	102	room temp
Oxygenated Constituents			
Sicilian	1	92	room temp
	2	97	room temp
	3	98	room temp
	4	97	room temp
	5	93	3
California	1	98	room temp

^a % recovery = (weight of fraction using quantitative GC, internal standard, and RF/expected weight of fraction as determined by quantitative GC of whole oil) \times 100.

Sicilian oil is shown in Figure 1B, and Table IV shows the quantitative data for Sicilian and California lemon oil hydrocarbon fractions. The hydrocarbon weight percent values for both oils are similar. For the Sicilian oil, which as noted was fractionated several times on silica, an average percent difference between each constituent in the fraction and the whole oil was calculated. A negative percent difference means the peak weight percent in the fraction is less than in whole oil. This can be due to a variety of reasons such as removal of co-eluting oxygenated constituents, reaction on the column, or physical loss. A positive percent difference means the weight percent is higher in the fraction than in whole oil. This could result from generation of material on silica or changes in GC resolution and integration when interfering oxygenated peaks are removed.

In general, the percent differences are within the range of the RSDs found for the overall analysis. A small consistent loss of strained ring hydrocarbons, particularly pinene and sabinene, was observed in the hydrocarbon fraction. These compounds are susceptible to acid-catalyzed rearrangement and hydration. Additional evidence for these reactions occurring on the silica column is discussed later. *p*-Cymene showed the greatest percent difference of all the hydrocarbon constituents and is difficult to quantify in the whole oil since it is a shoulder on the leading edge of the limonene peak (Figure 1A). It is resolved better in the hydrocarbon fraction, which may be due to removal of co-eluting 1,8-cineole. A small amount of oxidation of γ -terpinene to *p*-cymene on the silica column, or during sample handling, cannot be ruled out since the *p*-cymene peak is so small relative to γ -terpinene and there is a small loss of γ -terpinene.

There is no evidence of hydrocarbon rearrangement as noted for some terpene hydrocarbons on more active silicas (Scheffer, 1976, 1977). The reasons for the relatively large increases in the very small constituents, β -farnesene and α -humulene, are not understood but may simply be due to better integration in the hydrocarbon only fraction. In general, the comparison of results from the hydrocarbon fraction and from the whole oil shows that an accurate quantitative analysis of lemon hydrocarbons can be obtained from GC analysis on the whole oil.

Evaluation of Silica Column Reactivity. It was of interest to identify and quantify any artifacts produced from separation of hydrocarbons to further confirm the

Table IV. Quantitative Analysis^a of Hydrocarbon Lemon Oil Fractions

peak no.	compd	wt % of fraction ^b		wt % calcd as if in whole oil ^c		av % difference of fraction vs whole oil ^d
		Sic ^e	Cal ^f	Sic ^e	Cal ^f	Sic ^e
1	α -thujene	0.45	0.42	0.41	0.39	-2.38
2	α -pinene	1.88	1.87	1.74	1.75	-5.16
3	camphene	0.06	0.06	0.05	0.06	0.98
4	sabinene	1.83	1.96	1.70	1.83	-5.27
5	β -pinene	11.07	11.92	10.25	11.14	-2.07
6	myrcene	1.73	1.69	1.60	1.58	3.97
8	α -phellandrene	0.05	0.05	0.04	0.05	6.27
9	α -terpinene	0.24	0.18	0.22	0.17	3.13
10	<i>p</i> -cymene	0.14	0.27	0.13	0.25	46.85
11	limonene	70.43	70.53	65.16	65.93	0.15
13	<i>cis</i> -ocimene	0.06	0.06	0.05	0.05	
14	<i>trans</i> -ocimene	0.12	0.10	0.11	0.09	6.68
15	γ -terpinene	10.03	8.89	9.28	8.31	-6.17
17	terpinolene	0.44	0.37	0.41	0.35	-5.65
41	<i>cis</i> - α -bergamotene	0.03	0.05	0.02	0.05	-9.71
42	caryophyllene	0.21	0.25	0.19	0.24	0.87
43	<i>trans</i> - α -bergamotene	0.38	0.41	0.35	0.38	-1.61
46	<i>trans</i> - β -farnesene	0.03	0.05	0.03	0.04	15.22
47	α -humulene	0.02	<i>g</i>	0.02	<i>g</i>	17.86
48	β -santalene ^g					
52	β -bisabolene	0.56	0.61	0.52	0.57	1.02
	unidentified	0.25	0.21	0.23	0.17	
	total	100	100	92.51	93.47	

^a Quantitative data were calculated by using RF and internal standard. ^b Weight percent of fraction = (weight of constituent (mg)/total weight of fraction (mg)) \times 100. ^c Weight percent calculated as if in whole oil = (weight of constituent in fraction (mg)/total weight of sample (4000 mg)) \times 100. ^d % difference = (wt % calcd as if in whole lemon oil - wt % in whole lemon oil)/wt % calcd as if in whole lemon oil \times 100. The values in this table are an average of six open column determinations made over a period of several months and cannot be derived by using the values in Table I for whole lemon oil since two additional lemon analyses were used over the period. ^e Average of six open column determinations. ^f One open column determination. ^g Insufficient data for quantification.

high yields recovered from the open silica column and also to confirm the apparent small loss of strained ring hydrocarbons during silica chromatography. A later phase of this work will involve identification of trace oxygenated constituents important to taste, many of which cannot be characterized in the whole oil due to interfering peaks. Thus, it is especially important to identify oxygenated artifacts so they will not be mistaken for trace lemon oil constituents.

To test for trace oxygenated compounds formed on the silica column from hydrocarbon rearrangement, the total hydrocarbon fraction (\approx 3.5 g) from one open column separation of Sicilian lemon oil was concentrated in vacuo and rechromatographed by using conditions identical with those used for the initial separation. After elution of the hydrocarbons, the potential oxygenates from the hydrocarbon reaction were eluted with 20% CH₃OH in CH₂Cl₂. Tetradecane standard was added to this oxygenated fraction, and the solution was concentrated in vacuo. GC analysis showed a total of 0.63 mg of oxygenated material (Table V), which represents 0.3% of total oxygenated lemon constituents. The five largest oxygenated products (Table V) formed on the column were *trans*- and *cis*-sabinene hydrate and terpinen-4-ol, all readily formed from acid-catalyzed rearrangement and hydration of sabinene (Cooper et al., 1973), and the oxidation products *trans*- and *cis*-limonene 1,2-oxide. A variety of additional trace oxidation and hydration products comprising 0.1 mg total is also present, and identification of these products is in progress. The five major oxygenated "artifacts" all occur in unfractionated whole oil (Figure 1A), and only very small additional amounts are produced on the silica column during fractionation.

A possible reason for the observed column reactivity is that, without the oxygenated constituents present, the hydrocarbons would be more exposed to silica during rechromatography. Consequently, the chromatography of the hydrocarbons was repeated after addition of citro-

Table V. Hydrolysis Products and Artifacts from Silica Column Separation of Lemon Hydrocarbons

peak no.	compd	wt retrieved from column, mg	
		room temp	3 °C
11	limonene	0.041	0.016
16	<i>trans</i> -sabinene hydrate	0.186	0.043
18	<i>cis</i> -sabinene hydrate	0.211	0.052
20	<i>trans</i> -limonene 1,2-oxide	0.009	0.003
21	<i>cis</i> -limonene 1,2-oxide	0.007	0.004
25	terpinen-4-ol	0.081	0.014
	unidentified	0.097	0.042
	total	0.632	0.174
	starting material (approx), g	3.50	3.50
	% of starting material	0.02	0.005
	% of total oxygenated constituents in lemon	0.30	0.08

nellyl acetate at a level of \approx 5%, which is equivalent to the total amount of oxygenated constituents in whole lemon oil. It was found that the same amount of artifacts is produced as when the hydrocarbons alone are chromatographed. Therefore, these artifacts are almost certainly being formed on first chromatography of whole lemon oil. While it was certain that the hydration products were forming on the column, it was not clear whether the oxidation products were also forming on the column or during storage or manipulation of the fraction. The hydrocarbons were found to be extremely susceptible to oxidation on storage as confirmed by GC-MS.

To further understand and possibly minimize the changes occurring during the silica open column chromatography of lemon oil, the complete open column chromatography was repeated for both whole oil and the hydrocarbons with a jacketed column cooled to 3 °C. On rechromatography of the hydrocarbons, only one-fourth as much oxygenated material was produced (Table V). The decrease in limonene oxide indicates that a considerable

Table VI. Quantitative Analysis^a of Oxygenated Lemon Oil Fractions

peak no.	compd	wt % in fraction ^b		wt % calcd as if in whole oil ^b		av % difference of fraction vs whole oil ^b	
		Sic ^c	Cal ^d	Sic ^c	Cal ^d	room temp, Sic ^c	cold, ^e Sic ^d
7	octanal	1.83	1.91	0.10	0.08	0.38	7.41
12	1,8-cineole	0.92	0.95	0.05	0.04		
16	<i>trans</i> -sabinene hydrate/octanol	1.14	0.40	0.06	0.02	20.61	10.78
18	linalool/ <i>cis</i> -sabinene hydrate	3.51	3.17	0.20	0.14	7.47	3.99
19	nonanal	2.34	3.07	0.13	0.13	-0.62	1.83
20	<i>trans</i> -limonene oxide	0.09	0.17	0.005	0.007	14.81	12.77
21	<i>cis</i> -limonene oxide	0.06	0.12	0.003	0.005	-6.03	2.94
22	camphor	0.40	0.33	0.02	0.01	57.11	32.06
23	citronellal	2.01	1.80	0.11	0.08	-15.94	2.63
24	borneol	0.17	0.25	0.009	0.01	6.87	f
25	terpinen-4-ol	0.93	2.58	0.05	0.11	13.01	4.25
26	α -terpineol	3.31	3.77	0.19	0.16	-0.26	0.59
27	<i>cis</i> -4,7-dimethylbicyclo[3.2.1]oct-3-en-6-one (<i>exo</i>)	0.07	0.12	0.003	0.005	-143.23	-78.0
28	decanal	0.88	1.22	0.05	0.05	-0.46	-2.25
29	citronellol/nerol	0.62	1.32	0.03	0.06	-1.59	-3.51
30	neral	22.45	16.95	1.25	0.73	-0.71	0.11
31	geraniol	0.37	0.69	0.02	0.03	0.42	1.43
32	piperitone	0.07	0.10	0.004	0.004	-0.89	4.65
33	geranial	36.15	26.53	2.02	1.14	-2.44	-3.07
34	perillaldehyde	0.35	0.62	0.02	0.03	-48.46	-81.18
35	undecanal	0.43	0.73	0.02	0.03	-14.03	-15.23
36	methyl geranoate	0.08	0.16	0.004	0.07	-9.38	2.17
37	citronellyl acetate	0.56	0.75	0.03	0.03	-1.67	0.31
38	neryl acetate	9.18	13.42	0.51	0.57	-0.13	-1.32
39	geranyl acetate	6.78	11.35	0.38	0.49	-0.98	-0.03
40	dodecanal/decyl acetate	0.14	0.30	0.008	0.01	-3.69	0.00
44	citronellyl propionate	0.01	0.03	0.001	0.001		
45	neryl propionate	0.15	0.20	0.009	0.008	28.66	29.07
49	geranyl propionate	0.16	0.25	0.009	0.01	6.74	f
50	undecyl acetate	0.03	0.11	0.002	0.005	-368.11	f
51	tridecanal	0.04	0.06	0.002	0.002	f	f
53	nerolidol	0.01	0.12	0.001	0.005	-455.41	-437.50
54	2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbornanol	0.40	0.60	0.02	0.03	6.28	11.97
55	campherenol	0.51	0.68	0.03	0.03	16.37	32.37
56	α -bisabolol	0.62	0.80	0.03	0.03	-6.82	-1.40
	unidentified	3.21	4.38	0.18	0.19		
	total	100	100	5.56	4.35		

^a Quantitative data were calculated by using RFs and internal standard. ^b Refer to Table IV footnotes for explanation of table headings. ^c Average of four open column determinations. ^d One open column determination. ^e Cold column: 3 °C. ^f Insufficient data.

portion of the oxidation is occurring on the column when the sample of hydrocarbons is held for less than 24 h at 5 °C under argon before rechromatography; otherwise, oxidation can also occur on storage.

Analysis of Lemon Oxygenates. The oxygenated fractions from both Sicilian and California oils were also analyzed after recovery from the silica column (Table VI). Since the oxygenates only make up $\approx 5\%$ of the whole oil, the fraction was too dilute for direct capillary analysis and had to be concentrated before analysis. Internal standard was added, and the fraction was concentrated on a Kuderna-Danish evaporative still. A chromatogram of the oxygenates from the Sicilian oil is shown in Figure 1C, and the weight percent values are compared for Sicilian and California oxygenates in Table VI. It is interesting to note that neral and geranial make up almost 60% of total oxygenates in Sicilian lemon, and the seven most abundant constituents account for over 80% of total oxygenates.

The amount of each oxygenated constituent is calculated as if in lemon oil and compared to a whole Sicilian oil analysis (Table VI). Of these constituents, only 1,8-cineole and citronellyl propionate could not be quantified in the whole oil due to co-elution of hydrocarbon constituents. As observed with the hydrocarbon fraction (Table IV), a positive percent difference means greater concentrations found in the silica column fraction than in whole oil; negative percent difference denotes less material found in the fraction than in whole oil.

As expected from the analysis of possible oxygenated artifacts generated from the hydrocarbon fraction on silica, *trans*- and *cis*-sabinene hydrate, terpinen-4-ol, and *trans*-limonene oxide show increases in the oxygenated fraction which compare well with values found in Table V. The inconsistency in *cis*-limonene oxide may be due to its poor GC resolution. An increase in camphor in the fraction compared to whole oil is presently unexplained. A decrease is observed for citronellal, which is very susceptible to acid-catalyzed cyclization and subsequent hydration (Clark et al., 1984; Scheffer, 1977), and for undecyl acetate and nerolidol, which is probably due to removal of interfering sesquiterpene hydrocarbons. The decrease in the bicyclooctenone is also due to removal of a co-eluting hydrocarbon, and the decrease in undecanal is unexplained. Perillaldehyde is a poorly resolved doublet on the tail of the geranial peak and consequently was hard to integrate; otherwise, its decrease is unexplained.

Comparison of the quantitative data from the oxygenated fraction of lemon oil obtained through cold column chromatography to those from room temperature separation (Table VI) demonstrated that citronellal is stabilized at the lower temperature. In addition, lower levels of sabinene hydrate and terpinen-4-ol were generated during low-temperature chromatography. These types of comparisons can only show substantial changes in composition relative to whole oil. One cannot determine trace oxygenated artifacts formed from oxygenated

constituents as was done for the hydrocarbons since they could not be separated. Overall, the values found for the oxygenated fraction from the silica column provide a better analysis than can be obtained from whole oil. This is in contrast to hydrocarbons, which gave very similar analyses for the fraction and whole oil. For quantification it is not necessary to employ the cold column if very inert silica is used, as it was in this case; however, for organoleptic studies and identification of trace constituents, the cold column should be used to minimize artifacts.

Supplementary Material Available: GLC of California lemon peel oil (1 page). Ordering information is given on any current masthead page.

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Registry No. α -Thujene, 3917-48-4; α -pinene, 80-56-8; camphene, 79-92-5; sabinene, 3387-41-5; β -pinene, 127-91-3; myrcene, 123-35-3; octanal, 124-13-0; α -phellandrene, 4221-98-1; α -terpinene, 99-86-5; *p*-cymene, 99-87-6; 1,8-cineole, 470-82-6; *cis*-ocimene, 27400-71-1; *trans*-ocimene, 27400-72-2; γ -terpinene, 99-85-4; terpinolene, 586-62-9; linalool, 78-70-6; nonanal, 124-19-6; camphor, 464-49-3; citronellal, 106-23-0; borneol, 464-43-7; α -terpineol, 10482-56-1; *cis*-4,7-dimethylbicyclo[3.2.1]oct-3-en-6-one (*exo*), 116764-38-6; decanal, 112-31-2; citronellol, 106-22-9; neral, 106-26-3; geraniol, 106-24-1; piperitone, 89-81-6; geranial, 141-27-5; perillaldehyde, 2111-75-3; undecanal, 112-44-7; methyl geranoate, 2349-14-6; citronellyl acetate, 150-84-5; neryl acetate, 141-12-8; geranyl acetate, 105-87-3; dodecanal, 112-54-9; *cis*- α -bergamotene, 23971-87-1; caryophyllene, 87-44-5; *trans*- α -bergamotene, 13474-59-4; citronellyl propionate, 141-14-0; neryl propionate, 105-91-9; *trans*- β -farnesene, 18794-84-8; α -humulene, 6753-98-6; β -santalene, 511-59-1; geranyl propionate, 105-90-8; undecyl acetate, 1731-81-3; tridecanal, 10486-19-8; β -bisabolene, 495-61-4; nerolidol, 142-50-7; 2,3-dimethyl-3-(4-methyl-3-pentenyl)-2-norbormanol, 98205-40-4; campherol, 18530-03-5; α -bisabolol, 23089-26-1; nerol, 106-25-2; decyl acetate, 112-17-4; octanol, 111-87-5; limonene, 138-86-3; *trans*-sabinene hydrate, 17699-16-0; *trans*-limonene 1,2-oxide, 6909-30-4; *cis*-limonene 1,2-oxide, 4680-24-4; terpinen-4-ol, 562-74-3; *cis*-sabinene hydrate, 15537-55-0.