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Composition and Seasonal Variation of the Essential Oil from Leaves and Peel of a Cretan Lemon Variety

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The essential oil of leaves and peel from the Cretan variety Zambetakis (*Citrus limon*) was obtained by steam distillation with a Clevenger apparatus. The essential oil was subjected to GC-MS analysis, and 35 substances were identified. The main component in both essential oils was limonene. β-Pinene, myrcene, neral, geranial, neryl acetate, geranyl acetate, and β-caryophyllene have been identified in the leaf oil. The peel oil contained γ-terpinene, β-pinene, myrcene, neral, and geranial. The quantification of volatile substances was based on the internal standard method, using octyl acetate as internal standard, and expressed in milligrams per kilogram of the essential oil. The high contents of neral and geranial were indicative of the high quality of both essential oils. The aroma profile and quantitative variations among the essential oil components were measured at six different time intervals over a period of greater than one year. Differences between the components of lemon leaves and peel were observed.

KEYWORDS: Lemon Cretan variety; lemon leaf essential oil; lemon peel essential oil; capillary GC-MS

INTRODUCTION

The history of citrus dates back to ancient civilizations. Lemon [*Citrus limon* (L) Burm. f.] originated in Southeast Asia, China, and the Malayan Archipelago. Lemon varieties were first introduced into the Mediterranean countries when Romans navigated through the Red Sea to India. The common Mediterranean type of lemon has not been found growing wild in any part of the Mediterranean region. Lemon is an important crop, grown in coastal sites of southern California, Sicily, Greece, and Spain. Greece is one of the main exporters of citrus to many countries (*1*, *2*).

In addition to juice production, essential oil is one of the main byproducts of citrus processing. Essential oils exported by European producers annually include ~850 tons of lemon, 80 tons of mandarin, and 60 tons of bergamot (*3*). Lemon essential oil is of considerable importance from both economic and scientific points of view. The importance of lemon oil and its widespread use in the flavor and fragrance industry make the acquisition of accurate compositional data highly desirable.

The quality of essential oil depends on different factors. Among them are the chemotype and biotype of the plant as well as the climatic conditions (*4*). A study of the influence of different periods of ripening on the chemical composition of lemon essential oil from leaves and peel is, therefore, considered useful. Staroscic and Wilson (*5*) studied the seasonal and regional variation in the composition of cold-pressed lemon oil. Later, Crescimanno et al. (*6*) measured the variation of the essential oil in the leaves of four lemon varieties.

According to Shaw (*7*) there are several minor unidentified components of lemon oil that may make an important contribution to its flavor. Njoroge (*8*) found that the Japanese lemon leaf oil consists mainly of geranial, followed by limonene and neral. Quantification of the lemon peel oil components appeared in the study of Chamblee et al. (*9*). Volatile constituents of lemon leaf and peel oil were analyzed by capillary GC and GC-MS (*10*). Analyses of peel essential oils of Italian and Japanese

Table 1. Yield (Milliliters per Kilogram of Fresh Weight) of Lemon Leaf and Peel Essential Oil

sampling period	leaves	peels
first (December 1996)	4.2	13.6
second (March 1997)	4.0	12.4
third (May 1997)	3.5	13.4
fourth (June 1997)	4.6	12.9
fifth (November 1997)	5.3	17.4
sixth (April 1998)	4.0	6.5

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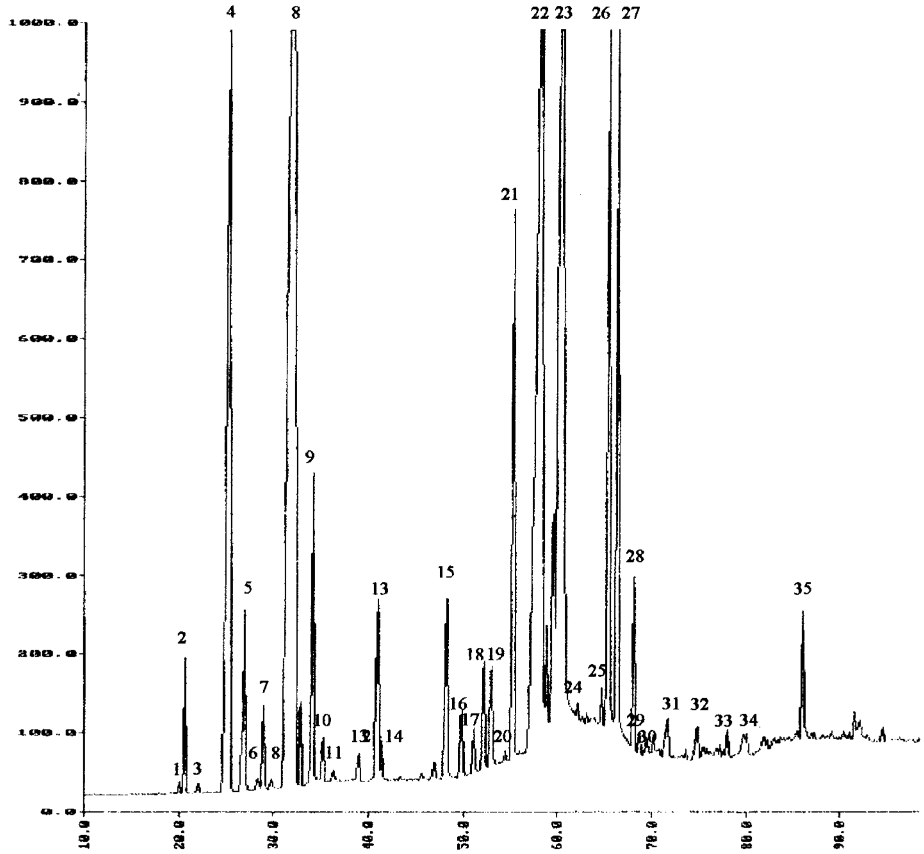

Figure 1. Chromatogram of lemon leaf essential oil of Cretan variety Zambetakis.

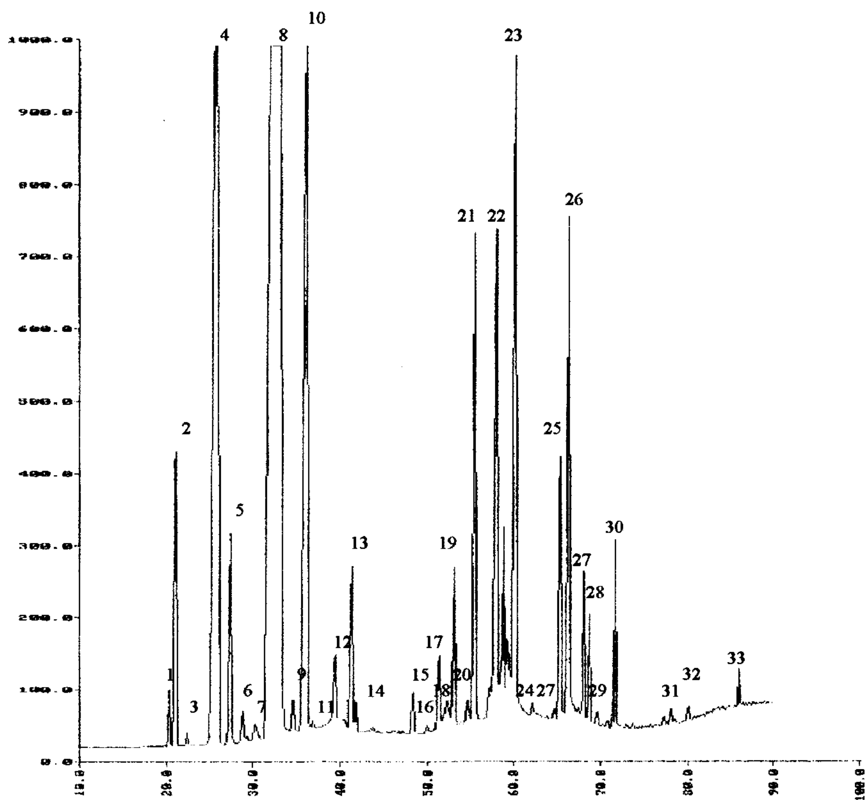

Figure 2. Chromatogram of lemon peel essential oil of Cretan variety Zambetakis.

lemons are described by Sawamura et al. (*11*). Huang (*12*) has thoroughly examined the chemical composition of the leaf essential oil from 110 *Citrus* species of Chinese origin. Although the composition of the volatile fraction of lemon oil has been thoroughly studied by many researchers, data regarding the composition of Cretan lemon oil have not been reported.

The goal of the present work was to study the composition and seasonal variation of the main volatile constituents of the

Table 2. Variation of Lemon Leaf Essential Oil Components (Milligrams per Kilogram of Essential Oil) in Different Periods[a]

		sampling periods											
component	peak	1	SD	2	SD	3	SD	4	SD	5	SD	6	SD
thujene	1	0.84a	0.11	0.72a	0.01	0.64b	0.03	0.54b	0.01	0.6b	0.04	0.0	
α-pinene	2	10.8a	1.20	9.38b	0.07	8.3 b	0.38	6.97c	0.19	6.9c	0.03	6.1c	0.60
camphene	3	0.78	0.09	0.76	0.04	0.64	0.02	0.50	0.02	0.0		0.0	
β-pinene	4	101.6a	5.87	107.3a	1.72	93.9a	2.20	81.8b	10.7	88.9b	1.20	100.0a	3.34
myrcene	5	17.3a	1.35	12.5b	1.86	13.3b	0.46	14.3g	0.42	12.1b	6.00	11.6b	0.83
α-terpinene	6	1.4a	0.26	1.4a	0.02	0.9b	0.01	0.7b	0.06	0.9b	1.21	0.8b	0.10
δ,3-carene	7	10.4 a	2.81	7.0b	0.01	4.2c	0.10	5.6b	0.04	5.7b	0.14	4.9c	0.05
limonene	8	167.0a	9.58	153.9a	1.43	162.9a	2.17	165.5a	21.4	184.1b	1.29	197.4c	7.89
(Z)β-ocimene	9	4.7a	0.19	3.9b	0.01	5.4a	0.17	5.2a	0.14	4.7a	19.1	4.2b	1.24
(E)β-ocimene	10	23.7a	0.80	19.9b	0.05	26.5a	0.76	24.9a	0.83	22.9a	0.40	20.04b	0.15
γ-terpinene	11	3.7a	0.29	3.2b	0.08	3.3a	0.09	2.6a	0.27	2.7b	2.43	2.2a	0.06
terpinolene	12	2.6a	O.45	2.2b	0.06	1.6c	0.14	1.8c	0.30	1.5c	0.42	1.4c	0.22
linalool	13	12.1a	0.67	14.4b	0.05	10.8a	0.24	8.0a	0.58	24.6c	0.24	13.1a	0.63
C9-al	14	2.1a	0.03	2.8b	0.02	2.9b	0.15	2.0a	8.25	1.5b	0.41	2.1a	0.21
citronellal	15	16.4a	0.30	17.2b	0.02	8.6a	0.23	7.2g	0.41	13.9a	0.24	12.9	0.18
isocitral *cis*	16	3.77	0.13	4.06	0.04	3.27	0.07	4.70	0.20	2.50	1.10	4.04	1.02
isocitral *trans*	17	3.8a	0.30	3.7a	0.04	3.6a	0.03	2.9b	0.19	2.8b	0.37	3.2a	2.57
terpinen-4-ol	18	4.9a	0.28	4.3b	0.12	6.7a	0.08	4.2b	0.60	5.4a	0.40	5.7a	0.30
α-terpineol	19	5.8a	0.29	5.4a	0.27	7.6a	0.10	5.2a	0.35	12.1b	0.33	6.4a	0.28
C10-al	20	0.8a	0.09	0.7a	0.09	0.9a	0.04	0.6a	0.33	0.5b	0.37	1.4a	0.23
octyl acetate	21												
neral	22	121.3a	8.26	108.0a	16.8	120.2a	2.83	128.6a	11.3	148.3a	2.25	134.4a	3.28
geranial	23	109.4a	5.53	103.9a	3.45	115.9a	7.20	131.3a	3.08	156.2b	6.03	164.9c	6.85
C11-al	24	0.9a	0.26	0.8a	0.21	2.5b	0.78	0.4a	0.03	1.9b	3.53	1.4a	2.97
citronellyl/ate	25	2.8a	0.14	2.3a	1.01	4.9a	1.63	1.4a	5.90	3.1a	5.76	2.0a	3.26
neryl acetate	26	55.7a	2.06	40.4b	0.69	32.6b	3.17	40.9b	1.53	55.8a	0.46	61.7a	0.29
geranyl acetate	27	35.4a	1.44	22.5b	0.43	22.8b	1.92	27.0b	0.62	41.3a	0.60	41.8a	0.42
caryophyllene	28	6.9a	0.53	7.3a	0.02	4.9b	0.76	4.7b	3.54	3.4b	3.17	7.1a	2.76
C12-ol	29	0.60	0.10	0.61	0.03	0.65	0.16	0.51	3.21	0.0		0.0	
bergamotene	30	0.77	0.02	0.88	0.02	0.50	0.00	0.0		0.0		1.2	0.13
humulene	31	1.8a	0.06	1.3a	0.03	1.1a	0.01	0.8b	0.04	0.7b	4.33	1.1a	0.37
nerylprop/nate	32	1.21	0.14	1.12	0.11	0.61	0.03	0.88	0.20	1.10	0.67	1.2	0.54
geranylpr/nate	33	0.56a	0.11	1.0b	0.03	0.62a	0.09	1.4b	0.06	3.4c	0.65	1.3b	0.27
farnasene	34	0.0		0.56	0.12	0.61	0.17	1.10	0.07	0.70	0.18	1.0	0.09
caryophyl/xide	35	1.04	0.10	0.0		0.66	0.09	0.97	0.06	1.00	0.10	1.0	0.08

[a] Values within rows followed by the same letter do not differ statistically at $P = 0.05$.

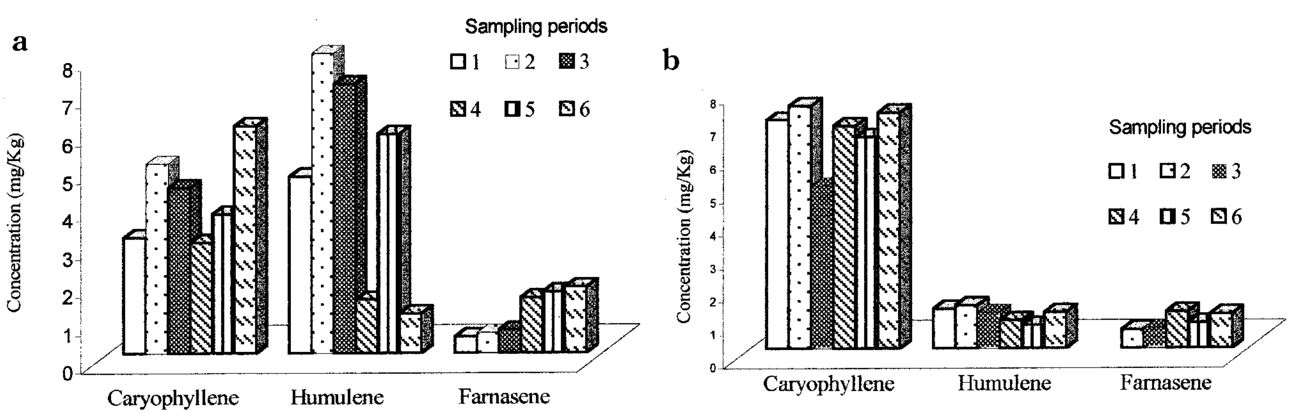

Figure 3. Variation of sesquiterpenic hydrocarbons of Zambetakis variety lemon peel (a) and lemon leaf (b) essential oil in different sampling periods.

most important Cretan lemon variety in order to elucidate its aroma profile. The composition of these oils may be useful in taxonomic studies, as well as in the identification of aromatic compounds of lemon oil obtained commercially by steam distillation.

MATERIALS AND METHODS

Taxonomy of the Variety. The lemon plants used in the present study were of the variety Zambetakis (*Citrus limon*) planted at the Institute of Subtropical Plants and Olive at Chania on the island of Crete. The variety Zambetakis belongs to the Citrus Germaplasm Bank of this Institute and was created by nucelar selection (*2*, *13*). It produces fruits of high quality during summer and is resistant to both cold and warm weather and to malsecco disease (*Phoma tracheiphilla*). It is of high commercial value because of its excellent pomological characteristics and because it is practically seedless. The variety Zambetakis is the most popular and is considered to be the most suitable lemon variety for cultivation in Crete (*2*). To certify this cultivar, an isoenzyme electrophoresis method has been developed. Four isoenzymatic systems were selected for the taxonomy of the lemon varieties: malate

Table 3. Variation of Lemon Peel Essential Oil Components (Milligrams per Kilogram of Essential Oil) in Different Periods[a]

component	peak	sampling periods 1	SD	2	SD	3	SD	4	SD	5	SD	6	SD
thujene	1	3.6a	0.07	3.4b	0.29	3.12c	0.18	3.08c	0.07	2.11b	0.12	2.9c	0.06
α-pinene	2	14.9a	0.26	16.6b	1.35	15.3a	0.77	15.26b	0.39	8.55b	0.51	15.5b	0.44
camphene	3	0.44	0.03	0.64	0.05	0.56	0.11	0.00		0.00		0.00	
β-pinene	4	70.5a	2.37	108.4b	5.6	91.00a	1.83	107.7b	3.66	57.8a	2.35	138b	3.35
myrcene	5	18.9a	0.30	14.7b	1.04	12.8c	0.50	14.74b	0.66	10.5c	0.67	12.64c	0.05
δ,3-carene	6	2.52	0.58	1.64	0.48	2.43	0.37	1.05	0.12	0.00		2.35	0.25
p-cymene	7	0.52	0.08	1.57	0.63	0.00	0.00	0.42	9.09	0.00		0.84	0.14
limonene	8	270.9a	11.2	262.9a	14.0	220.3b	2.37	289.5a	0.03	229b	6.44	315.5a	7.21
(Z)β-ocimene	9	1.55	0.18	0.24	0.1	0.27	0.09	0.0		0.0		0.0	
(E)β-ocimene	10	1.99a	0.21	4.0b	4.35	3.0b	0.45	2.57b	0.03	1.85a	0.13	1.80a	0.57
γ-terpinene	11	78.32a	2.41	90.2b	0.28	74.2a	0.98	82.23a	2.21	66.8b	1.73	81.98a	1.88
terpinolene	12	4.80a	0.06	5.2a	0.30	4.4b	0.30	4.41b	0.08	3.35b	0.12	4.09b	0.26
linalool	13	4.61a	0.01	7.7b	0.07	5.4b	0.18	10.72c	0.26	3.35a	0.28	13.37b	0.35
C9-al	14	1.25a	0.01	1.1a	0.05	1.5b	0.04	1.91b	0.19	0.54a	0.05	1.78b	0.14
citronellal	15	0.98a	0.03	1.5b	0.03	1.6b	0.01	0.94a	0.11	1.46b	0.13	2.94c	0.27
isocitral *cis*	16	0.75	0.02	0.64	0.05	0.53	0.03	0.0		0.65	0.07	0.0	
isocitral *trans*	17	4.17a	0.04	3.9a	5.67	4.5a	0.02	4.99a	0.09	2.74b	0.12	4.73a	0.10
terpinen-4-ol	18	1.04a	0.05	4.1b	0.03	0.8a	0.01	0.83a	0.03	0.97a	0.08	1.50a	0.58
α-terpineol	19	7.36a	0.04	9.00b	0.00	8.6b	0.09	9.01	0.44	5.63b	0.16	11.87b	0.58
C10-al	20	0.48	0.02	0.53	0.0	0.65	0.04	0.75	0.13	0.0		1.11	0.01
octyl acetate	21												
neral	22	35.3a	0.37	35.0a	0.83	30.3b	0.05	36.1a	0.54	39.91a	3.12	45.45a	2.99
geranial	23	46.6a	1.14	49.9a	0.52	42.3a	5.03	49.29	0.93	55.97b	0.68	65.36b	2.30
citronellyl /ate	24	0.0		0.52	0.07	0.0		0.0		0.66	0.03	0.0	
neryl acetate	25	3.6a	0.08	9.7b	0.35	6.3b	0.23	5.87b	0.16	6.64b	0.96	12.33b	1.69
geranyl/tate	26	3.7a	0.05	13.7b	0.53	11.5b	0.29	6.39b	0.30	5.6b	0.25	24.90b	4.15
C12-al	27	2.1a	0.03	4.0b	0.22	3.5b	0.13	4.45b	0.11	2.8a	0.21	0.0	
β-caryophyllene	28	3.0a	0.05	5.0b	0.28	4.4b	0.15	2.91a	0.09	3.7b	0.19	5.98b	1.25
bergamotene	29	0.4a	0.02	0.7a	3.97	0.6a	0.02	0.59a	0.03	0.63a	0.04	0.0	
humulene	30	4.6a	0.23	7.9a	0.48	7.09a	0.38	1.41a	0.11	5.77a	0.22	1.04a	0.02
neryl prop/ate	31	0.0		0.0		0.0		0.0		0.0		6.57	0.39
geranyl prop/ate	32	0.5a	0.13	5.4b	2.72	0.73a	0.04	3.42b	3.97	1.71b	0.15	0.0	
farnasene	33	0.4a	0.63	0.5a	0.12	0.6a	0.32	1.45b	0.20	1.6b	1.67	1.73b	4.70

[a] Values within rows followed by the same letter do not differ statistically at $P = 0.05$.

dehydrogenase, tetrazolium oxidase, glutamate oxaloacetate transaminase, and esterases (*13*).

Sampling. Samples of leaves and fruits were collected from 16-year-old lemon trees, around the top of the tree at a height of 1.70 m. Care was taken to collect leaves of ∼5−7 months of age, representative of the variety. Samples of 10 leaves from each of 10 different trees were collected at different time intervals over a period of greater than one year. The fruits were collected three months after the anthesis from each tree from the four compass points, to minimize position variables. The sampling periods were December 1996, March 1997, May 1997, June 1997, November 1997, and April 1998.

Essential Oil Extraction and Analysis. For the isolation of the essential oil, the hydrodistillation method with the use of a Clevenger apparatus (*3*) was used. Samples of 150 g were extracted according to the following procedure: The leaves were carefully cleaned and cut, the fruits were cleaned, and the peels were cut and distilled separately for 3 h. Oil samples were collected and stored in vials at −18 °C until use.

For gas chromatographic analysis, a 30 × 0.32 mm i.d., 0.25 μm film thickness, SE-52 capillary column was used. The column was installed in a Perkin-Elmer Autosystem GC equipped with a glass injection splitter with a split ratio 1/20 and flame ionization detector (FID). The oven temperature was programmed from 50 °C (5 min isothermal) to 230 °C at 4 °C/min. The temperatures of the injector and detector were 200 and 240 °C, respectively. Helium (0.6 mL/min) was used as a carrier gas. The injection volume was 0.2 μL. Peak area percentages were calculated automatically by a Hewlett-Packard integrator.

Chromatographic analysis was performed in six replicates. Standards previously identified as constituents of lemon leaves were added to the sample for peak assignment based on peak enrichment. The results were calculated on the basis of an internal standard method (IS). The concentration of the individual compounds was expressed relative to the internal standard octyl acetate. An absolute concentration (grams per milliliter of diluted essential oil with octyl acetate 1/25) of the relevant compound was determined.

A Hewlett-Packard model P5890 GC with a split/splitless injection port and split ratio of 1/100, coupled with an HP5970 mass spectrometer system (electron impact mode), was also used with a 25 m × 0.2 mm i.d., 0.2 μm film thickness, HP-1 fused silica capillary column. The temperature program was isothermal for 3 min at 60 °C and then raised to 230 °C at 5 °C/min. The injector temperature was 200 °C, and the transfer line temperature was 250 °C. The column outlet was inserted directly into the ion source block. Ultrapure helium was used as a carrier gas with a flow rate of 1 mL/min. The injection volume was 0.2 μL.

Identification of components was confirmed by comparison of the experimental retention index and mass spectrum with those of authentic reference standards.

For the statistical analysis, an analysis of variance (ANOVA) was used. Duncan's multiple-range test was applied for the calculation of the significant differences of the components among the sampling periods.

RESULTS AND DISCUSSION

Oil Yield. The oil yield was expressed as milliliters of essential oil per kilogram of fresh weight (fw) of the plant material. The yield of the essential oil varied from 3.5 to 5.3 mL/kg (fw) for leaves and from 6.5 to 17.4 (fw) for peel (Table 1). The highest oil yield was obtained from leaves collected during November followed by those of June. This is not in agreement with Crescimanno et al. (*6*), who reported that the maximum yield of the essential oil from lemon leaves was observed during February followed by November and June.

Identification and Quantification of Lemon Essential Oil. In Figures 1 and 2 the chromatograms of lemon leaf and peel

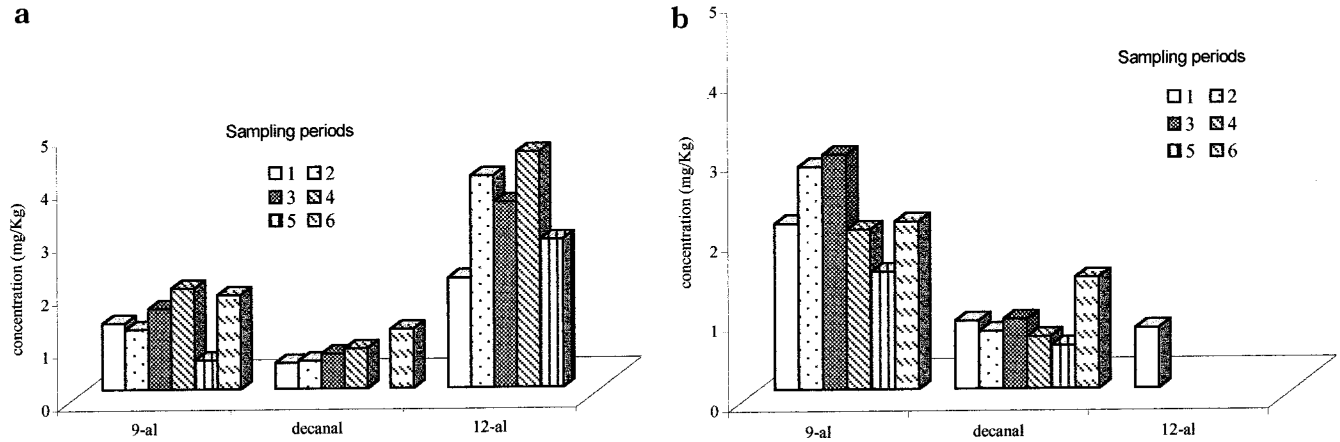

Figure 4. Variation of aliphatic compounds of Zambetakis variety lemon peel (a) and lemon leaf (b) essential oil in different sampling periods.

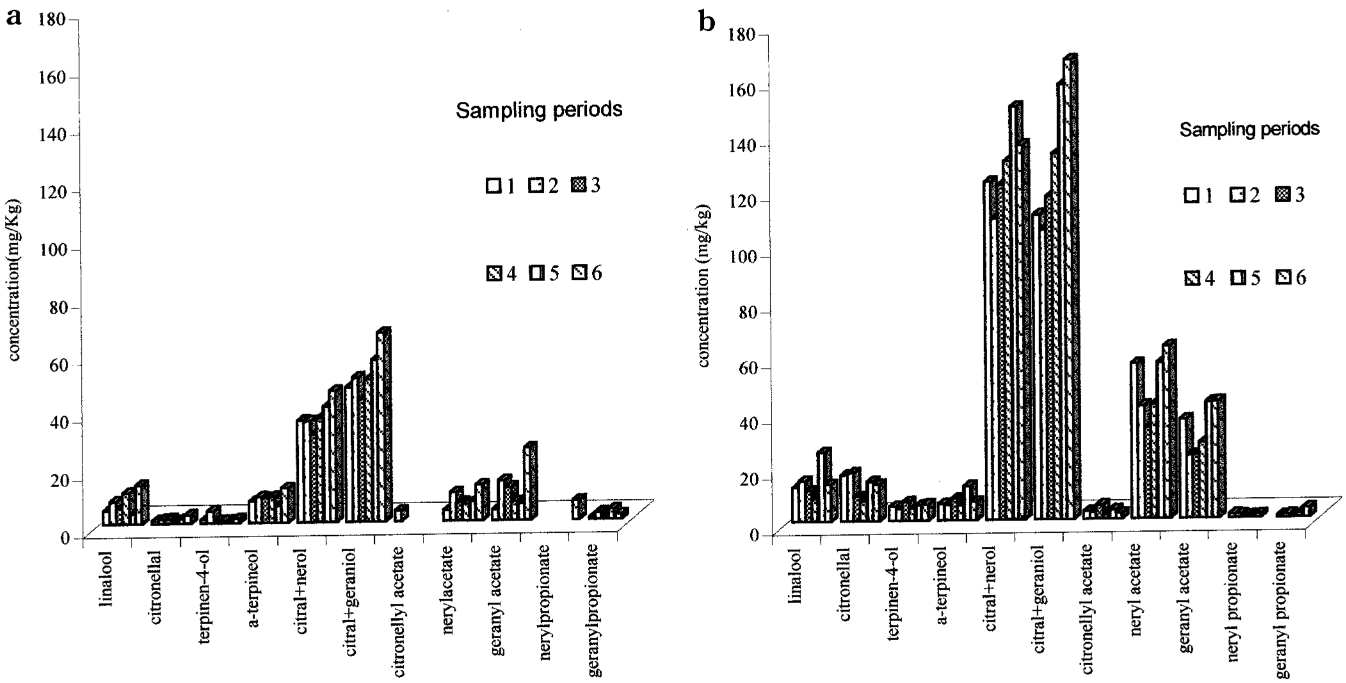

Figure 5. Variation of oxygenated monoterpenes of Zambetakis variety lemon peel (a) and lemon leaf (b) essential oil in different sampling periods.

oil are presented, respectively. For each group and each sampling, the mean value and the standard deviation (SD) have been calculated; these data are reported in Tables 2 and 3. As can be seen, the main common component in the essential oil of both leaves and peel is the monoterpene hydrocarbon limonene followed by β-pinene. Neral, geranial, neryl acetate, geranyl acetate, and β-ocimene (*Z* and *E*) are present in high quantities in leaves but in much lower quantities in peel. It can be seen that most of the constituents belong to the terpene family and may be arranged in two groups. The first is the group of terpene hydrocarbons (terpenes and sesquiterpenes), and the second is the group of oxygenated terpene products. In the essential oil of lemon leaves the second group predominates.

Crescimanno et al. (*6*) studied the essential oil from four varieties of lemon leaves and found that citral (neral and geranial)—and not limonene—was the dominant component followed by limonene, neryl acetate, geranyl acetate, and β- and α-pinene. This is in accordance with our results. Furthermore, Njoroge et al. (*8*) reported that Japanese lemon leaf oil consists mainly of geranial followed by limonene and neral. On the other hand, in the essential oil of peel from Sicilian and Californian lemon varieties, Gramshaw (*14*) reported that the main constituent was limonene followed by β-pinene, γ-terpinene, and small quantities of citral and myrcene. Finally, Staroscic and Wilson (*5*) found limonene as the main constituent of the essential oil from the lemon peel of fruits cultivated in California and Arizona, followed by minor quantities of β-pinene. Relatively high concentrations of monoterpene hydrocarbons in the Japanese lemon peel oil have been found by Njoroge et al. (*8*), with lower quantities of aldehydes and esters. Usai et al. (*15*), in a comparative study of the peel essential oil from 12 lemon varieties, found that monoterpene hydrocarbons were the major components, whereas the aldehydes and sesquiterpene hydrocarbons followed as minor components. Combariza et al. (*16*) reported that limonene was the main constituent of the lemon oil and could be used as a functional index of ripeness. Moreover, citral (neral and geranial) contributes significantly to the quality of lemon flavor and aroma, and its concentration is by far the most important factor in determining the commercial value of a lemon oil (*14*). The studied variety had a high content of citral, indicating that lemons of the variety Zambetakis were of high quality. Also, the presence of relatively

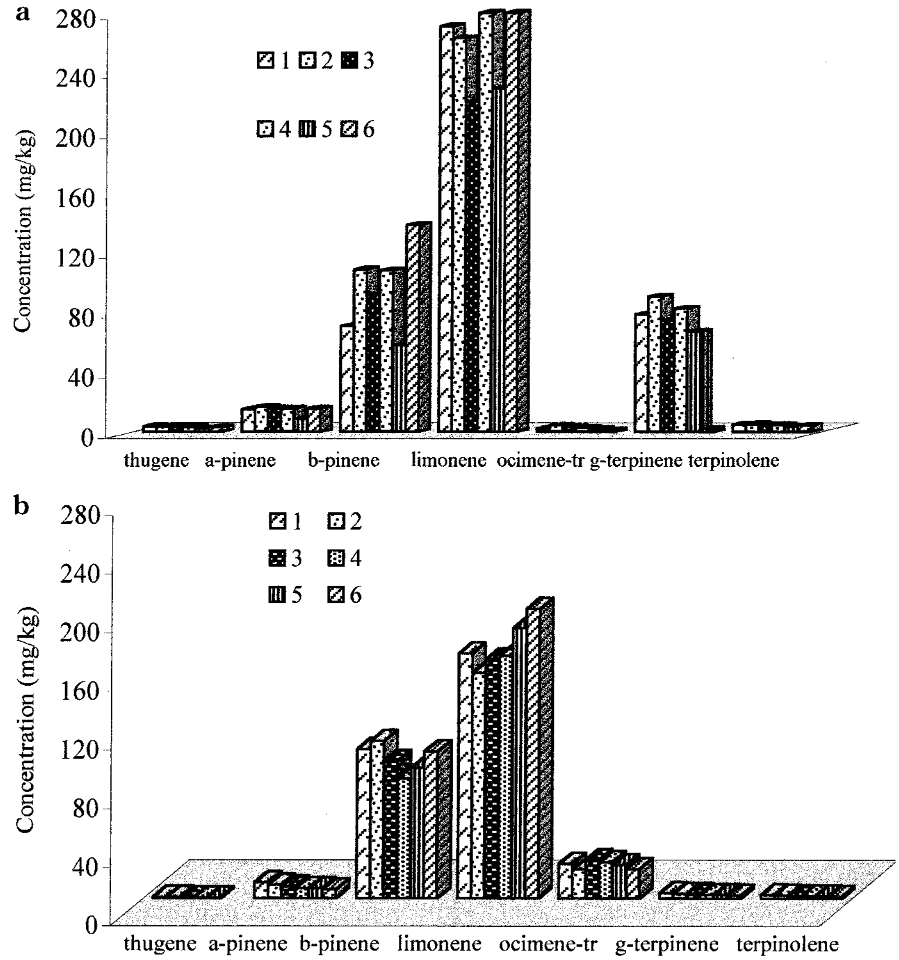

Figure 6. Variation of monoterpene hydrocarbons of Zambetakis variety lemon peel (a) and lemon leaf (b) essential oil in different sampling periods.

high concentrations of the other oxygenated compounds could explain the powerful and characteristic aroma of the essential oil from the leaves.

The quantity of β-ocimene (*Z* and *E*), which is a terpene hydrocarbon, was much higher in leaves compared to that in peel, and in some cases it was not identified in peel. This shows that probably the terpenes of leaves and peel are substances structured by a different metabolic pathway (*17*).

Linalool in leaves was dominant among alcohols, although in peel it was found in lower concentrations. Consequently, the implication that linalool could be the predominant substance in biogenesis of many constituents of citrus aroma is evident (*17*).

Seasonal Differentiation of Lemon Essential Oil Constituents. Comparison of Cretan lemon leaf and peel oil sampled at different time periods for over one year showed significant differences in their composition. Seasonal variation was noticed in the amounts of neryl acetate, geranyl acetate, and citronellal in leaves and peel. The content of *cis*- and *trans*-isocitral was higher during the November sampling in leaves, and the same was observed in peel. According to Crescimanno et al. (*6*) maximum values were measured during the winter harvest followed by that of spring. This is not in agreement with our result; we found maximum values of the main components during the spring.

The mean content of limonene was 171.8 mg/kg in leaves and 264.7 mg/kg in peel. The contents of the rest of the components can be seen in Tables 2 and 3, respectively.

In Figures 3−6 the variation of each component against time for each group of essential oil compounds is presented. The results of the analyses of the essential oil from leaves and peel during a six harvest periods appear in Tables 2 and 3. It can be seen that the different periods of harvesting show significant differences at $P = 0.05$ in most of the compounds. This could be attributed to the changes taking place in the essential oil during ripening. The regression analysis showed that values of the essential oil constituents are generally not linear except for the limonene, geranial, γ-terpinene, and α-pinene in leaves and the geranial and neral in peel. R^2 values varied between 0.72 and 0.79.

In the present study the ripening effect is very important, although the variations noticed may be due, at least to some degree, to the time that elapsed between harvesting and essential oil extraction, a fact that usually affects the chemical composition of essential oils. Apart from the geographical origin of the tree, there were also other variables associated with it, which could not be controlled. Hence, it is difficult to make a thorough and systematic estimation of the compositional differences and further research is needed.

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