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SATURATED AND MONOUNSATURATED LONG-CHAIN HYDROCARBONS OF LIME JUICE SACS

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Abstract—Saturated and monounsaturated long-chain hydrocarbons were determined in juice sacs of two sour lime cultivars, Key and Persian, and one sweet lime, Columbia. The dominant hydrocarbon in the saturated fraction for all limes was linear C_{25}. For the monoene fraction, C_{29} predominated in both Key and Persian, but C_{25} predominated in Columbia. Iso- and anteiso-branched hydrocarbons comprised collectively 38·4–57·8% of the saturated fraction, but only 9·5–20·1% of the monoene group. The major branched isomers found in the saturated group were odd-numbered, iso-branched structures. In the monoene group, both odd-numbered, iso-branched and even-numbered, anteiso-branched structures were prominent.

INTRODUCTION

THE MOST important commercial fruits produced by the United States belong to the genus *Citrus*. Considerable information is available on the chemical constituents of these fruits;[1–3] however, an area which has been neglected is citrus lipids. Citrus lipids are important not only from the nutritional viewpoint, but also they show promise as chemical markers for differentiating several citrus species[4,5] and varieties within any one species.[6–8] Long-chain hydrocarbon profiles are of particular chemotaxonomic interest and have been determined for several varieties of oranges and tangors[9,10] and grapefruit.[11] In the present study we report the saturated and monounsaturated long-chain hydrocarbon profiles for three varieties of lime. Two varieties of acid lime, viz. *C. aurantifolia* Swingle cv. Key and *C. latifolia* Tanaka cv. Persian and one variety of sweet lime, viz. *C. limettioides* Tanaka cv. Columbia were investigated.

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RESULTS AND DISCUSSION

Key lime belongs in the group of small-fruited acid limes thought to have originated in northeastern India.[12] Because of nucellar embryony, the fruit grows true to seed. The major disadvantage of this fruit is that the plant is extremely sensitive to cold and therefore, is grown only in the southern most portion of Florida. Persian lime is a large-fruited acid lime and currently, the most important commercial lime produced in Florida. It is thought to be of hybrid origin with acid lime and citron parents.[12] The origin of Persian, occasionally called Tahiti lime, is unknown. Columbia lime belongs to the sweet lime group. Sweet limes, generally, are not in demand in the United States because of their flat, sweet taste, but apparently are more esteemed in Egypt, India and Latin America. The Columbia sweet lime is the best known clonal selection of the Palestine sweet lime.

TABLE 1. TOTAL LIPID AND HYDROCARBON CONCENTRATIONS OF SOUR AND SWEET LIME JUICE SACS (mg/20 g dry wt)

Variety	Total lipid	Saturated	Hydrocarbon fraction Monoene	Complex
Persian	243·4 ± 6·8	7·2 ± 0·3	0·5 ± 0·2	0·3 ± 0·1
Key	321·9 ± 8·4	9·8 ± 0·4	1·0 ± 0·3	0·5 ± 0·1
Columbia	239·1 ± 7·4	4·9 ± 0·2	0·6 ± 0·1	0·3 ± 0·0

The lipid content and hydrocarbon concentrations of the two acid limes and one sweet lime are shown in Table 1. The total lipids extracted from 20 g of lime sac powder showed that the percentage of lipid in these three limes were: Persian (1·21), Columbia (1·19) and Key (1·60). The total hydrocarbon fraction was separated by argentation TLC into three fractions, viz. saturated, monounsaturated and a complex fraction containing all other components. This latter fraction was obtained by eluting all components found between the monounsaturated fraction and the origin on the TLC plate and contained pigments, polyunsaturated hydrocarbons and undefined hydrocarbons. Saturated hydrocarbons represented 2·0–3·0% of the total lipid while monounsaturated hydrocarbons comprised 0·2–0·3%.

Saturated long-chain hydrocarbon profiles are shown in Table 2. Only hydrocarbons between C_{20} and C_{38} were quantified in this group. Hydrocarbons greater than C_{38} were detected in all limes but always at trace percentages (below 0·01%). For linear chain hydrocarbons (L column), the most prominent hydrocarbon for all limes is C_{25}. The two acid limes, Key and Persian, show C_{27} to be the next most prominent while the sweet lime, Columbia, show C_{23}. Odd-numbered hydrocarbons predominate in this group. The major even-numbered hydrocarbons for all limes are C_{24} and C_{26} with C_{24} predominating in the even-numbered series. Iso-branched hydrocarbons (I column) show C_{25} as dominant, their profiles being similar to those of the linear hydrocarbons. Columbia again differs from the two sour limes in showing C_{23} as the next most prominent iso-branched hydrocarbon after C_{25}. Anteiso-branched hydrocarbons (AI column) show that, in contradistinction to the linear and iso-branched series, even-numbered hydrocarbons predominate. The major hydrocarbons in the AI column are C_{24}, C_{26} and C_{28} with C_{26} predominating. Persual of this table reveals that, in general, odd-numbered, iso-branched hydrocarbons are greater

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in amount than their odd-numbered, anteiso-branched counterparts. Conversely, even-numbered, anteiso-branched paraffins are generally greater than their iso-branched homologs. While Table 2 shows percentages for hydrocarbons between C_{20} and C_{38}, it is evident that 98–99 % of the hydrocarbons fall in the region C_{22}–C_{32}.

TABLE 2. SATURATED LONG-CHAIN HYDROCARBON PROFILES OF LIME JUICE SACS (wt %)

Carbon	Persian			Key			Columbia		
No.	L*	I†	AI‡	L	I	AI	L	I	AI
20	0·12§	t‖	t	0·10	t	t	0·27	0·01	0·04
21	0·42	0·12	t	0·51	0·13	t	0·57	0·23	t
22	0·97	0·12	0·06	0·89	0·06	0·03	1·49	0·19	0·18
23	6·59	4·86	t	6·51	3·62	t	9·32	7·94	t
24	4·17	1·27	2·72	3·56	0·73	1·59	5·32	2·04	5·30
25	17·26	10·90	1·19	16·56	10·02	0·78	15·21	16·35	1·82
26	3·31	1·31	5·41	3·18	1·06	4·26	2·66	1·34	7·28
27	8·69	5·48	0·95	10·92	5·18	0·74	3·58	5·83	0·88
28	1·49	0·44	2·93	2·04	0·33	2·35	0·77	0·37	2·73
29	5·85	4·81	0·38	8·70	4·39	0·30	1·20	2·10	0·31
30	1·09	0·36	1·68	1·38	0·25	1·09	0·39	0·15	0·80
31	2·79	1·05	0·12	5·76	1·13	0·09	1·21	0·27	0·10
32	0·31	0·07	0·14	0·50	0·05	0·10	0·25	0·12	0·18
33	0·26	0·08	0·01	0·89	0·09	0·01	0·50	0·03	t
34	0·04	0·02	0·04	0·04	0·02	t	0·20	0·06	0·05
35	0·06	0·03	t	0·03	0·03	t	0·16	0·02	0·09
36	t	t	t	t	t	t	t	t	t
37	0·03	—	—	t	—	—	0·09	t	t
38	t	—	—	t	—	—	t	—	—

* Linear chain hydrocarbon.

† Iso-branched, general structure: CH_3–$CH(CH_3)$–$(CH_2)_xCH_3$.

‡ Anteiso-branched, general structure: CH_3–CH_2–$CH(CH_3)$–$(CH_2)_xCH_3$.

§ All values are the mean of 5–7 determinations.

‖ t = Trace, less than 0·01 %.

A composite breakdown of the three isomeric structures found in the saturated hydrocarbon fraction for these three limes is shown in Table 3. Odd- and even-numbered linear structures comprise, totally, the greatest percentage with Columbia and Key manifesting the least and most percentage, respectively. Collectively, the major branched hydrocarbon in all limes is the odd-numbered, iso-branched isomer while the odd-numbered, anteiso-branched isomer is least prominent. For Persian and Key, odd-numbered paraffins show the relationship linear > iso > anteiso while Columbia exhibits approximately equal

TABLE 3. PERCENTAGE COMPOSITION OF NORMAL, ISO- AND ANTEISO-SATURATED HYDROCARBONS

Carbon	Persian			Key			Columbia		
No.	L	I	AI	L	I	AI	L	I	AI
Odd-numbered	41·95	27·33	2·65	49·88	24·59	1·92	31·84	32·77	3·20
Even-numbered	11·50	3·59	12·98	11·69	2·50	9·42	11·35	4·28	16·56
Total	53·45	30·92	15·63	61·57	27·09	11·34	43·19	37·05	19·76

percentages of linear and iso-branched and therefore, the relationship linear $\simeq$ iso > anteiso is manifest. Even-numbered paraffins, on the other hand, show the relationship linear $\simeq$ anteiso > iso for Persian and Key limes and linear > anteiso > iso for Columbia lime.

TABLE 4. MONOUNSATURATED LONG-CHAIN HYDROCARBON PROFILES OF LIME JUICE SACS (wt %)

Carbon No.	Persian L	 I	 AI	Key L	 I	 AI	Columbia L	 I	 AI
20	0·08	0·03	0·10	0·39	t	0·03	0·34	t	0·32
21	0·08	0·07	0·02	0·17	0·05	0·02	0·21	0·19	t
22	0·25	0·03	0·11	0·38	t	0·14	0·40	t	0·20
23	2·55	0·33	0·11	1·82	0·43	0·07	7·36	1·10	0·22
24	1·91	0·08	0·22	1·72	0·04	0·51	3·73	0·12	1·43
25	13·20	1·08	0·53	12·48	1·06	0·27	26·32	2·99	0·93
26	2·35	0·22	1·74	2·06	0·18	0·88	4·04	0·33	2·55
27	17·58	1·62	0·53	23·38	2·28	0·34	20·83	2·53	0·81
28	2·04	0·11	1·80	2·44	0·09	1·20	2·55	0·17	1·71
29	29·34	0·73	0·33	26·10	0·71	0·14	11·60	1·28	0·33
30	1·05	0·03	0·91	1·27	0·03	0·46	0·99	0·15	0·78
31	15·29	0·63	0·15	16·23	0·32	0·05	2·68	0·37	0·10
32	0·35	t	0·63	0·37	t	0·08	0·08	0·01	0·11
33	1·56	0·17	0·07	1·60	0·21	t	0·13	0·01	t
34	t	—	—	t	—	—	t	—	—
35	t	—	—	t	—	—	t	—	—

The monounsaturated hydrocarbon profiles for these three limes are shown in Table 4. These profiles differ markedly from the saturated group. The four most prominent linear monoenes in Persian and Key are odd-numbered and are related in concentration: $C_{29} > C_{27} > C_{31} > C_{25}$. In Columbia the relative amounts of these monoenes are: $C_{25} > C_{27} > C_{29} > C_{23}$. Iso-branched monoenes are present at relatively low percentages. The two major iso-branched monoenes are C_{25} and C_{27} with C_{27} predominating in Persian and Key while C_{25} predominates in Columbia. The very low percentage of even-numbered, iso-branched monoenes in all limes should be noted. The anteiso-branched group show that C_{26} and C_{28} even-numbered monoenes predominate in all limes. In general, odd-numbered, iso-branched monoenes are observed at higher relative percentages than their anteiso-branched homologs. Conversely, even-numbered, anteiso-branched monoenes are always found at higher percentages than their iso-branched homologs.

TABLE 5. PERCENTAGE COMPOSITION OF NORMAL, ISO- AND ANTEISO MONOUNSATURATED HYDROCARBONS

Carbon No.	Persian L	 I	 AI	Key L	 I	 AI	Columbia L	 I	 AI
Odd-numbered	79·60	4·63	1·73	81·78	5·06	0·89	69·13	8·47	2·39
Even numbered	8·03	0·50	5·51	8·63	0·34	3·30	12·13	0·78	7·10
Total	87·63	5·13	7·24	90·41	5·40	4·19	81·26	9·25	9·49

The three isomeric structures found in the monounsaturated fraction are shown in composite form in Table 5. As observed for the saturated group, linear odd- and even-numbered components comprise, collectively, the largest isomeric group; also, Key and Columbia possess the most and least total percentage, respectively. For all limes, odd-numbered monoenes show the relationship linear > iso > anteiso while even-numbered monoenes show linear > anteiso > iso as a rule. The branched isomer found at the least total percentage is the even-numbered, iso-branched monoene. As was also shown for the saturated group, Columbia differs from Key and Persian by the relatively higher total percentage of both iso- and anteiso-branched structures.

The exact mechanism of synthesis of long-chain hydrocarbons in citrus fruits has not been elucidated. In three previous papers,[8–10] the authors have speculated on the possible mechanisms for hydrocarbon synthesis in citrus and generally favor the elongation-decarboxyalation pathway postulated by Kolattukudy[13,14] but have not completely ruled out the head-to-head condensation mechanism of Channon and Chibnall.[15] Citrus synthesizes a multitude of saturated and unsaturated branched-chain fatty acids.[4,5] The elongation-decarboxylation pathway could readily function in converting saturated and monounsaturated branched fatty acids to saturated and monounsaturated branched hydrocarbons.

EXPERIMENTAL

Isolation and purification of lime juice sac lipids. The three lime samples were obtained through the Crops Research Division, U.S.D.A., Orlando, Florida. Key, Persian and Columbia were obtained from U.S.D.A. groves in Homestead, Ft. Pierce and Leesburg, respectively. Key and Persian were harvested in September while Columbia was obtained in October. The three limes were cut in half and the juice sacs carefully separated from core, peel, seeds and carpellary membrane with the aid of a citrus spoon. The juice sacs were freeze-dried to a powder and stored at −18°. Lipids were extracted and purified from 20 g of powder by methods previously described.[6,16] Quadruplicate extractions were run on a single batch of fruit from each variety.

Chromatography and quantitation. Column, TLC and GLC methods for the separation and quantitation of hydrocarbons have been thoroughly described elsewhere.[8–11]

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