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SATURATED AND MONO-UNSATURATED LONG-CHAIN HYDROCARBON PROFILES OF SWEET ORANGES

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Abstract—Three varieties of midseason oranges, viz. Jaffa, Homosassa and Queen, were examined for their saturated and mono-unsaturated hydrocarbon composition in juice sacs. Hydrocarbons were isolated by lipid extraction of the juice sac powders followed by column, thin-layer and $AgNO_3$-TLC. After hydrogenation, the mono-unsaturated fraction and the saturated fraction were analyzed by GC. In the saturated fraction, the dominant linear hydrocarbon was C_{23} while C_{25} predominated in the monoene fraction. *iso*- And *anteiso*-branched hydrocarbons comprised between 53 and 63% of the saturated fraction and only 20–26% of the monoene fraction. Queen differed from Homosassa and Jaffa in the accumulation of higher percentages of saturated *iso*- and *anteiso*-branched hydrocarbons and conversely, showed lower percentages for these branched structures in the monoene fraction. Based on the total relative percentages of the three isomeric hydrocarbons, Homosassa could not be differentiated from Jaffa. The overall profiles for these two oranges, however, showed noticeable differences.

INTRODUCTION

ONE OF the great challenges to the plant taxonomist is the genus *Citrus*. Swingle,[1] Hodgson[2] and Tanaka[3] recognize 16, 36 and 157 separate species within this genus, respectively. This disagreement in species recognition has many causes. Through nucellar embryony a hybrid quite different to either of its parents may survive many generations and therefore, in the opinion of some taxonomists, deserves a separate species classification. Further breeding of these 'hybrid species' with recognized pure species has led to a multiplicity of other hybrids. Tracing the parentage of some of these hybrids has proved extremely difficult.

An important system being used with considerable success by citrus taxonomists in determining the purity of a species and elucidating the parentage of a hybrid is chemotaxonomy. During a study of the saturated long-chain hydrocarbon profiles of orange juice sacs,[4] specific patterns emerged which appeared to be intrinsic for each variety. In additional studies on the hydrocarbon composition of grapefruit,[5] limes[6] and lemons,[7] examination of the

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[1] SWINGLE, W. T. (1943) in *The Citrus Industry* (edited by WEBBER, H. J. and BATCHELOR, L. D.), Vol. 1, p. 129, University of California Press, Berkeley, California.
[2] HODGSON, R. W. (1961) *Proc. 2nd Conf. Intern. Organization of Citrus Virologists*, p. 1, University of Florida Press, Gainesville, Florida.
[3] TANAKA, T. (1961) *Citrologia Semi-Centennial Commemoration Papers on Citrus Studies*, Citrologia Supporting Foundation, Osaka Pref. University, Osaka, Japan.
[4] NAGY, S. and NORDBY, H. E. (1971) *Phytochem.* **10**, 2763.
[5] NAGY, S. and NORDBY, H. E. (1972) *Phytochem.* **11**, 2789.
[6] NAGY, S. and NORDBY, H. E. (1972) *Phytochem.* **11**, 2865.
[7] NAGY, S. and NORDBY, H. E. (1972) *Phytochem.* **11**, 3249.

mono-unsaturated hydrocarbon fraction was included and showed that these monoene profiles differed markedly from the saturated fraction. As a result of these differences, the present study was undertaken, to determine whether three varieties of midseason sweet orange, viz. Jaffa, Homosassa and Queen could be differentiated on this basis. These three orange varieties have midseason maturing fruit, i.e. the fruit is at a maturity stage subject to commercial processing between January and March.[8] The Homosassa orange is one of the oldest Florida varieties having originated as a seedling selection in a grove at Homosassa, Florida, around 1865. This orange was granted first-class variety stature in 1877 by the Variety Committee of the American Pomological Society.[8] The Jaffa orange is derived from a clone of the Palestine beledi seedling group being introduced in Florida around 1883. Because of its low seed content, cold resistance and good quality, Jaffa is an important midseason variety. Queen orange originated as a seedling in a grove near Bartow, Florida, sometime prior to 1900.[8] Queen is quite similar to Pineapple orange (the major midseason orange of Florida) but has the added properties of being more vigorous and somewhat more resistant to cold weather.

RESULTS AND DISCUSSION

All three orange varieties were harvested at the same time in mid-March at which time these oranges were at or near an optimum maturity stage. The lipid content and hydrocarbon concentrations of juice sacs from these three midseason oranges are shown in Table 1.

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

Variety	Total lipid	Hydrocarbon fraction		
		Saturated	Monoene	Complex
Homosassa	237·6 ± 8·5	3·6 ± 0·2	0·2 ± 0·1	0·1 ± 0·0
Queen	227·1 ± 7·8	4·4 ± 0·4	0·3 ± 0·1	0·4 ± 0·1
Jaffa	232·9 ± 12·8	4·4 ± 0·2	0·3 ± 0·0	0·3 ± 0·1

The total lipids extracted from 20 g of juice sac powder showed that the percentage of lipid in these three oranges were: Homosassa (1·18), Queen (1·13) and Jaffa (1·16). The complex fraction was composed of pigments, di-unsaturated hydrocarbons and other undefined hydrocarbons. The saturated hydrocarbons represented 1·5–1·9% of the total lipids while monoenes were in the range of 0·08–0·13%.

Table 2 shows the saturated hydrocarbon profiles of three midseason orange varieties. Hydrocarbons have been detected in oranges up to the region C_{40}–C_{45} but always at trace percentages. While the range covered in this paper was between C_{20} and C_{35}, *ca.* 99% of the hydrocarbons were found between C_{21} and C_{31}. Table 2 shows that the two major linear hydrocarbons in the three varieties were C_{23} and C_{25} with C_{23} being dominant. In several early season and late season maturing oranges C_{23} was the predominate linear hydrocarbon.[4] Examination of the *iso*-branched and *anteiso*-branched columns showed that the dominant *iso*-branched structures were C_{23} and C_{25} while C_{24} and C_{26} predominated in the

[8] HODGSON, R. W. (1967) in *The Citrus Industry*, (edited by REUTHER, W., WEBBER, H. J. and BATCHELOR, L. D.), Vol. 1, p. 431, University of California Press, Berkeley, California.

anteiso group. Although odd-numbered hydrocarbons predominated in the normal and *iso*-branched groups, the converse was evident for the *anteiso* group, i.e. even-numbered were prominent. An extension of this observation was the fact that even-numbered *anteiso* hydrocarbons were always found at percentages greater than their *iso*-branched homologs. The Queen orange differed from the other two oranges in showing more *iso*-C_{23} over its linear-C_{23} counterpart and also, more *iso*-C_{25} over linear-C_{25}. Jaffa and Homosassa can be distinguished on the basis of the C_{23} ratios. In Homosassa, *iso*-C_{23} was nearly equal to linear-C_{23} while Jaffa had *ca.* one-third less *iso*-C_{23} than linear-C_{23}. Jaffa had approximately equal percentages of linear-C_{23} and -C_{25} while Homosassa showed a larger percentage of linear-C_{23} over -C_{25}.

TABLE 2. SATURATED LONG-CHAIN HYDROCARBON PROFILES OF SWEET ORANGE JUICE SAC (wt %)

Carbon No.	Homosassa L*	Homosassa I†	Homosassa AI‡	Queen L	Queen I	Queen AI	Jaffa L	Jaffa I	Jaffa AI
20	0·14§	tr‖	tr	0·12	tr	tr	0·17	tr	tr
21	0·92	0·12	tr	0·96	0·15	tr	0·73	0·04	tr
22	2·41	0·41	0·11	2·34	0·72	0·26	2·28	0·23	0·07
23	15·56	15·10	tr	13·49	19·08	tr	15·84	11·17	tr
24	4·74	2·74	9·67	4·38	2·99	11·05	5·37	2·42	8·13
25	12·84	12·74	1·61	10·22	12·71	2·56	15·01	13·16	0·94
26	1·29	0·89	5·95	1·16	0·97	6·28	1·60	0·95	6·71
27	2·64	3·03	0·31	1·90	2·76	0·50	2·74	3·75	0·54
28	0·33	0·17	1·29	0·26	0·18	1·28	0·32	0·25	2·04
29	1·20	0·59	0·12	0·84	0·60	0·10	1·16	0·96	0·15
30	0·33	0·09	0·35	0·20	0·08	0·35	0·27	0·13	0·67
31	1·35	0·15	0·05	0·78	0·16	0·04	1·13	0·28	0·08
32	0·14	0·07	0·10	0·09	0·07	0·13	0·11	0·06	0·16
33	0·24	0·01	tr	0·15	0·03	tr	0·25	0·02	tr
34	0·04	tr	tr	0·02	tr	tr	tr	tr	tr
35	0·06	tr	tr	0·04	tr	tr	0·03	tr	tr

* Linear chain. † *Iso*-branched, $Me-\underset{\underset{Me}{|}}{\overset{\overset{H}{|}}{C}}-(CH_2)_x-Me$. ‡ *Anteiso*-branched, $Me-CH_2-\underset{\underset{Me}{|}}{\overset{\overset{H}{|}}{C}}-(CH_2)_x-Me$.

§ Mean of 5–7 determinations. ‖ Trace, less than 0·01%.

While it was not unusual for an orange[4] to show more *iso*-C_{25} than linear-C_{25}, it had not been previously shown that an orange could accumulate in juice sacs more *iso*-C_{23} than linear-C_{23}. The total percentages for the three isomeric structures in this saturated fraction were:

Homosassa—linear (44·33), *iso* (35·86), *anteiso* (19·56).
Queen —linear (36·95), *iso* (40·50), *anteiso* (22·55).
Jaffa —linear (47·01), *iso* (33·42), *anteiso* (19·57).

It was evident that Queen accumulated a relatively higher per cent of *iso*- and *anteiso*-branched hydrocarbons than the other two midseason varieties.

The percentage composition of the mono-unsaturated hydrocarbon fraction is shown in Table 3. For the linear hydrocarbon group, C_{23}, C_{25} and C_{27} were prominent with C_{25} predominating in all cases. In comparison to the percentage of C_{23} for Jaffa and Homosassa, the Queen orange showed *ca.* twice as much for this hydrocarbon. Jaffa had larger percentages of linear-C_{25} and -C_{27} and a lesser amount of C_{31} than the Homosassa variety. The *iso*-branched group showed a relationship similar to the linear group, i.e. C_{23}, C_{25} and C_{27} were prominent with C_{25} predominating. For the *anteiso*-branched group, C_{24}, C_{26} and C_{28} were prominent with C_{26} the major hydrocarbon. A breakdown of the total percentages showed the following for the three isomeric mono-unsaturated structures:

Homosassa—linear (73·12), *iso* (13·44), *anteiso* (13·44).
Queen —linear (79·34), *iso* (10·45), *anteiso* (10·21).
Jaffa —linear (73·57), *iso* (13·76), *anteiso* (12·67).

The mono-unsaturated group possessed a lower percentage of branched hydrocarbons than the saturated group. Approximately equal amounts of *iso*- and *anteiso*-branched monoenes were formed. This was in opposition to the saturated group where there was 1·5–2·0 times as much *iso* as *anteiso*. While the Queen orange showed a higher percentage of saturated branched hydrocarbons (63·05) than either of the other two oranges (55·42 and 52·99) the reverse was true for the monene fraction where it showed the lowest percentage (20·66).

TABLE 3. MONO-UNSATURATED LONG-CHAIN HYDROCARBON PROFILES OF SWEET ORANGE JUICE SACS (wt %)

Carbon No.	Homosassa			Queen			Jaffa		
	L	I	AI	L	I	AI	L	I	AI
20	1·88	0·25	0·65	0·44	0·15	0·27	2·36	0·16	0·52
21	0·42	tr	0·56	0·18	tr	0·09	0·44	tr	0·49
22	2·25	0·20	0·69	0·98	0·12	0·24	2·20	0·25	0·62
23	12·16	2·48	0·46	20·48	2·56	0·33	10·52	1·94	0·38
24	4·55	0·47	2·82	5·00	0·42	3·09	4·70	0·38	1·98
25	24·76	6·06	1·08	28·24	4·60	0·63	27·75	5·56	0·82
26	3·28	0·36	4·21	3·00	0·27	3·63	3·78	0·34	4·33
27	11·63	2·62	0·62	9·65	1·65	0·36	13·69	3·26	0·70
28	1·22	0·14	1·42	0·75	0·08	0·93	1·35	0·12	1·68
29	4·92	0·66	0·35	5·09	0·38	0·24	3·64	0·91	0·17
30	0·57	0·04	0·29	0·47	0·05	0·18	0·81	0·11	0·52
31	4·46	0·15	0·05	4·31	0·15	0·02	1·89	0·60	0·11
32	0·23	tr	0·21	0·31	0·02	0·20	0·21	0·02	0·33
33	0·76	tr	tr	0·44	tr	tr	0·19	0·11	0·02
34	0·03	—	0·03	tr	—	—	0·04	tr	tr
35	tr	—	—	tr	—	—	tr	—	—

In sweet oranges, saturated *iso*-branched fatty acids comprise between 0·4 and 0·6% and saturated *anteiso*-branched between 0·05 and 0·1% of the total fatty acids.[9] In this study saturated branched hydrocarbons show a range between 33 and 40% for saturated *iso*

[9] NORDBY, H. E. and NAGY, S. (1971) *Phytochem.* **10**, 615.

structures and between 19 and 22% for *anteiso* components. Correspondingly, mono-unsaturated *iso*- and *anteiso*-branched fatty acids show ranges of 0·03–0·12 and 0·01–0·02%, respectively. This study shows that mono-unsaturated *iso*- and *anteiso*-branched hydrocarbons are both in the range 10–13%. The reason for this large difference in the percentage of branched structures found in fatty acids and hydrocarbons is not known. However, since long-chain hydrocarbons are one of the end-products of fatty acid metabolism, there is no reason to assume some qualitative and/or quantitative relationship between a precursor (branched fatty acid) and its end-product (branched hydrocarbon).

EXPERIMENTAL

Sources and preparation of juice sac lipids. Three midseason orange varieties of *C. sinensis*, Queen, Homosassa and Jaffa, were obtained at a mature stage from Whitmore Experimental Farm (Plant Science Research Division, U.S.D.A., Orlando, Florida). The fruit were cut in half and the intact juice sacs (vesicles) separated from core, peel, seeds and carpellary membrane with the aid of a citrus spoon. The juice sacs were freeze-dried to a powder possessing a moisture content no greater than 4% and stored at −18°. Lipids were extracted and purified from 20 g of juice sac powder by a method previously described for total juice sac powder.[10] 5–7 extractions were run on each of the three orange variety powders.

Chromatography and hydrogenation methods. The total purified lipid (*ca.* 150–200 mg) was dissolved in $CHCl_3$ and percolated onto a 0·9 × 30 cm column containing 10 g Merck, 70–325 mesh silica gel (Brinkmann Instruments, Westbury, N.Y.). The neutral lipids were eluted with 200 ml $CHCl_3$, glycolipids with 200 ml acetone and phospholipids with 200 ml MeOH. The glycolipid and phospholipid fractions were not subjected to any further analysis during this study. The neutral lipid fraction was concentrated to a small volume and streaked on percoated silica gel G plates (20 × 20 cm, 500 μ, Analtech, Inc., Wilmington, Del.) These non-activated plates were developed in hexane–ethyl ether (9:1) at room temp. in chambers lined with filter paper. The plate upon drying was sprayed with Rhodamine 6G and viewed under UV light. The band corresponding to the long-chain hydrocarbon fraction was scraped from the plate and eluted with ethyl ether (30 ml). The hydrocarbon fraction was, in turn, streaked on a silver nitrate-impregnated silica gel G plate[9] and developed in 2% ethyl ether in light petrol. (30–60° boiling range). Upon drying, the plate was sprayed with Rhodamine 6G, viewed under UV light and the saturated, monoene and complex (everything between the origin and the monoene band) fractions detected. These fractions were eluted with Et_2O and dry wts taken. The monoene fraction was dissolved in 1 ml hexane and hydrogenated in a Parr apparatus with 10 mg 10% Pd-C catalyst under 5 kg/cm² at room temp. for 1 hr.

Quantitative analyses. The hydrocarbons were analyzed by gas chromatography with an F & M Model 5750 gas chromatograph equipped with flame ionization detectors. The glass column (3·05 m in length and 4 mm i.d.) contained 3% SP-1000 (Superco, Inc., Bellefonte, Penn.) on 100/120 mesh, Gas Chrom Q. The injection port and detector were at 275° and the helium flow rate was 80 ml/min. The sample was injected on-column at 165° and programmed for 5 min at 4°/min and then 2°/min up to 270° and finally held isothermally at this upper limit until the C_{35} hydrocarbon eluted from the column. A hydrocarbon standard containing linear, *iso*- and *anteiso*-branched structures from C_{16} to C_{36} was prepared from a long-chain fatty acid mixture as previously described.[4] The long-chain, saturated and monoene hydrocarbons of three varieties of orange juice sacs were determined by comparing the GLC R_fs against the above standard as well as by plots of retention times versus equipalent carbon numbers. Mass spectra of citrus long-chain, branched hydrocarbons were previously determined by Hunter and Brogden[11] from this laboratory. Quantitative measurements were obtained with the aid of a disc integrator and by triangulation.

Each value shown in Tables 2 and 3 represents the mean of 5–7 determinations. The coefficient of variation (CV) determined for several mean ranges (MR) showed the following: MR 0·01–0·10; CV 10–35%; MR 0·1–1·0; CV 5–10%; MR 1·0–5·0; CV 3–5%; MR above 5·0; CV less than 2%.

[10] NAGY, S. and NORDBY, H. E. (1970) *J. Agric. Food Chem.* **18,** 593.
[11] HUNTER, G. L. K. and BROGDEN, W. B. (1966) *Phytochem.* **5,** 807.