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FATTY ACIDS OF TRIGLYCERIDES AND STEROL ESTERS FROM DUNCAN GRAPEFRUIT, DANCY MANDARIN AND THEIR TANGELO HYBRIDS

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Key Wor Index—*Citrus paradisi*; *C. reticulata*; Rutaceae; grapefruit; mandarin; tangelo hybrids; triglycerides; sterol esters; fatty acid profiles; chemotaxonomy.

Abstract—The fatty acid composition of the triglycerides and sterol esters of juice sac lipids were determined in Duncan grapefruit, Dancy mandarin and three of their tangelo hybrids. The fatty acid profiles of Orlando and Minneola tangelos resembled those of the Dancy mandarin parent more than those of the grapefruit. The profiles of Seminole tangelo were different from those of both parents and of its sister hybrids.

INTRODUCTION

CHEMICAL profiles of lipid constituents are reliable indicators for distinguishing citrus fruit. The profiles include total juice sac fatty acids,[1,2] lipid subfraction fatty acids,[3] sterols[4,5] and hydrocarbons.[6–11] In two recent publications emphasis was placed on the fatty acid profiles associated with triglycerides[12] and sterol esters.[13] In both studies, each citrus species appeared to possess an intrinsic fatty acid profile which distinguished it from other species.

One of the long-range goals of our chemotaxonomic studies has been to determine parental influence in citrus hybrids. The present study is concerned with the fatty acid profiles of three tangelo hybrids resulting from a cross of Dancy mandarin with Duncan grapefruit.

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TABLE 1. FATTY ACID COMPOSITION OF TRIGLYCERIDES FROM CITRUS JUICE SACS (%)

Cultivar	Major fatty acids 16	16:1*	18:1	18:2	18:3	Total major fatty acids	Ratio of major fatty acids to 16:1 16	16:1	18:1	18:2	18:3
Duncan grapefruit	15·31	8·31	30·14	30·08	11·76	95·60	1·84	1·00	3·63	3·62	1·42
Seminole tangelo	11·13	9·26	35·24	22·41	15·29	93·33	1·20	1·00	3·81	2·42	1·65
Orlando tangelo	13·94	8·26	40·29	18·02	13·12	93·63	1·69	1·00	4·88	2·18	1·59
Minneola tangelo	13·15	8·46	41·10	17·75	13·01	93·47	1·55	1·00	4·86	2·10	1·54
Dancy mandarin	12·99	7·10	42·74	16·32	12·90	92·05	1·83	1·00	6·02	2·30	1·82

Cultivar	Minor fatty acids 12	14	15	15:1	17	17:1	18	18:1	18	20	20:1	22	22:1	24	Other
Duncan grapefruit	0·19	0·37	0·27	0·16	T†	0·45	0·22	0·44	0·61	0·21	0·69	0·11	0·27	0·21	0·20
Seminole tangelo	0·78	1·51	0·30	0·31	0·12	0·68	0·18	0·22	0·21	0·12	0·78	T	0·27	0·14	1·05
Orlando tangelo	0·19	0·59	0·41	0·37	0·25	0·75	0·36	0·47	1·16	0·15	0·55	0·13	0·20	0·17	0·32
Minneola tangelo	0·31	0·62	0·39	0·29	0·23	0·76	0·43	0·56	1·10	0·11	0·78	T	0·24	0·16	0·55
Dancy mandarin	0·63	0·97	0·70	0·42	0·19	0·99	0·10	0·16	0·66	0·25	1·26	0·23	0·71	0·20	0·48

* Number of double bonds.
† Value in trace amount (0·01–0·1%).
I—*iso*-branched acid.

RESULTS AND DISCUSSION

Table 1 lists the triglyceride fatty acids above the 0·1% level found in the five citrus cultivars. Duncan grapefruit has a triglyceride fatty acid profile characteristic of grapefruit[12] with over 95% of the acids comprising palmitic (C_{16}), palmitoleic ($C_{16:1}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and linolenic ($C_{18:3}$). As for other grapefuit, the ratio of palmitic to palmitoleic acid is in the range 1·5–2·4. The triglycerides of Dancy mandarin have a $C_{16}/C_{16:1}$ ratio of 1·83, nearly identical to that of Duncan (1·84). Dancy however, has relatively less C_{16} and $C_{16:1}$ than Duncan. Comparison of the C_{18} triglyceride fatty acids for Duncan and Dancy show marked differences. In Duncan essentially equal amounts (30%) of oleic and linoleic acids are present, whilst Dancy shows 43% oleic and 16% linoleic acid. In the three tangelo hybrid triglycerides, the five major acids comprise over 93% of the total. The 16/16:1 ratio for these hybrids is 1·20–1·69, noticeably lower than those for either of their parents. The relative percentages of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ in Orlando and Minneola are closer to those found in the Dancy parent than those in the Duncan. The Seminole tangelo shows noticeably different profiles from those of either of its parents or sister hybrids. Table 1 also shows the ratio of the five major acids; the ratio found for Duncan triglycerides is typical of grapefruit.[12] Whether the ratio shown for Dancy is typical of mandarins is uncertain as an insufficient number of mandarins have been analyzed. Orlando and Minneola tangelos have similar ratios which although different from either of their parents tend to resemble the Dancy more than Duncan parent. The percentages of C_{16} and $C_{18:3}$ are nearly the same in Dancy, Orlando and Minneola. The ratio of the five major acids in Seminole tangelo is different from those of either of its parents or its sister hybrids. The noticeably higher percentages of the unsaturated fatty acids, $C_{18:2}$ and $C_{18:3}$, in Seminole contrast sharply with the percentages observed for Orlando, Minneola and Dancy.

The five major sterol ester fatty acids comprise 86% (Dancy) to 91% (Orlando) of the total (Table 2). This range is similar to that reported for sterol esters of oranges and grapefuit.[13] The 16/16:1 ratio (1·65) for Duncan is characteristic of grapefuit sterol esters. The low 16/16:1 ratios for the three tangelo hybrids are not characteristic of any citrus species.[3,13] Unlike the percentage differences observed among the three tangelo triglycerides, the percentage differences observed in the C_{18} sterol ester fatty acids were negligible. The

major fatty acid percentages in Orlando and Minneola appear to resemble the percentages of the Dancy parent more than the Duncan parent. The ratio of the five major acids in Duncan is typical of grapefruit sterol esters.[13] The ratios of the five acids in the three tangelos are different from either of their parents but appear to be closer to those of Dancy than those of Duncan.

Minor differences are found among the five cultivars when the minor fatty acids of triglycerides are examined (Table 1). More fatty acids, present at levels greater than 0·1%, are observed for Dancy than for the other four cultivars. The range of total amounts of these minor acids in these cultivars is from 4·4% (Duncan) to 8·1% (Dancy). Monoenes of chain lengths between 17 and 22 are present in greater percentages than are their respective saturate homologs. Total branched-chain acids range from 0·26% (Dancy) to 1·54% (Minneola).

TABLE 2. FATTY ACID COMPOSITION OF STEROL ESTERS FROM CITRUS JUICE SACS (%)

Cultivar	Major fatty acids 16	16:1	18:1	18:2	18:3	Total major fatty acids	Ratio of major fatty acids to 16:1 16	16:1	18:1	18:2	18:3
Duncan grapefruit	4·22	2·55	9·28	58·38	13·75	88·18	1·65	1·00	3·64	22·89	5·39
Seminole tangelo	3·16	3·74	18·24	47·18	16·17	88·49	0·84	1·00	4·88	12·61	4·32
Orlando tangelo	3·55	3·39	21·02	46·63	16·01	90·60	1·05	1·00	6·20	13·76	4·72
Minneola tangelo	2·36	4·73	20·01	45·34	16·27	88·71	0·50	1·00	4·23	9·59	3·44
Dancy mandarin	3·97	3·87	22·71	38·93	16·64	86·12	1·03	1·00	5·87	10·06	4·30

	Minor fatty acids 12	14	15	15:1	17	17:1	AI I18:1	18	AI 19:2	22	23	124	24	25	126	26	Other
Duncan grapefruit	0·17	1·34	0·13	0·20	0·18	0·25	0·79	0·41	0·69	0·35	0·33	0·51	2·48	1·13	0·73	1·18	0·95
Seminole tangelo	0·28	0·99	0·10	0·17	0·22	0·39	0·50	0·25	0·52	0·52	0·44	0·33	2·89	1·15	0·33	1·28	1·15
Orlando tangelo	0·36	1·05	0·11	0·15	T	0·46	0·69	0·20	0·77	0·50	0·32	0·39	2·20	0·66	0·30	0·60	0·64
Minneola tangelo	0·32	0·95	0·12	0·15	0·20	0·41	0·98	T	0·59	0·44	0·50	0·57	2·90	0·96	0·58	1·10	0·52
Dancy mandarin	0·67	1·38	0·11	0·20	0·19	0·59	0·28	0·66	0·35	1·04	0·31	0·51	3·14	1·33	0·26	1·71	1·15

AI = *anteiso*-branched acid.

The sterol ester minor acids range from 11·8% (Duncan) to 13·9% (Dancy) (Table 2). As previously observed,[3,13] citrus sterol esters contain greater amounts of long-chain fatty acids, C_{20}–C_{28}, than the other citrus lipid classes. As found in the minor acids of the triglycerides, no distinct differences are found between the cultivars in the minor acids of the sterol esters. Total branched-chain acids range from 1·5% (Dancy) to 2·9% (Minneola) of the total. The two major branched-chain acids in all cultivars are *iso* $C_{18:1}$ and *anteiso* $C_{19:2}$. The four most dominant acids of the minor acids are C_{14} (0·9–1·4%), C_{24} (2·2–3·1%), C_{25} (0·7–1·3%) and C_{26} (0·6–1·7%). Except for these four acids, all other minor acids are found at percentages less than 1. As with the triglyceride monoenes, sterol ester monoenes in the region C_{15} to *iso* C_{18} are always found at percentages greater than their respective saturated homologs.

In studies on the chemotaxonomy of citrus species and hybrids, it is relevant to relate the biochemistry of the fruit to its morphology, physiology and flavor properties. In 1905 Webber and Swingle designated hybrids resulting from the cross of a mandarin with a grapefruit as tangelos. With regard to morphology and flavor, tangelos are a highly diverse group of hybrids showing characteristics that are both typical of their parents and intermediate between them.[14] The flavor of Orlando and Minneola tangelos resemble that of

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Dancy mandarin in being sweet, juicy and rich. The Seminole tangelo, on the other hand, is acid and extremely tart. This property is more characteristic of grapefruit than of mandarins and would indicate that Seminole tangelo shows flavor properties more akin to its grapefruit parent. The results obtained in the present investigation indicate that there may be some correlation between fatty acid profiles and inherited flavor properties. The profiles suggest that the Orlando and Minneola tangelos are more closely related to Dancy mandarin than Duncan grapefruit. Likewise, Seminole tangelo, while not showing profiles typical of grapefruit does not show profiles typical of the mandarin. Morphologically, Seminole is oblate (typical of Dancy mandarin) but highly seedy (typical of Duncan grapefruit). Further studies on tangelo hybrids should prove the feasibility of correlating lipid chemistry with the nutritionally important properties of the fruit.

EXPERIMENTAL

Duncan grapefruit, Dancy mandarin and the three tangelos Orlando, Minneola and Seminole were obtained from Whitmore Foundation Farm (U.S. Horticultural Research Laboratory, USDA, Orlando, Florida). Samples of the five citrus fruits were cut in half and the intact 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 juice sac powder by a method previously described.[2] Quadruplicate extractions were run on a single batch of fruit from each cultivar.

Separation of triglycerides and sterol esters and preparation of fatty acid methyl esters. The purified juice sac lipid (*ca* 200 mg) was dissolved in $CHCl_3$ and percolated onto an 0·9 × 30 cm column containing 9 g 60–200 mesh silica gel. The neutral lipids were eluted with 200 ml $CHCl_3$, evaporated, taken up in hexane, streaked on silica gel G plates and developed with hexane–Et_2O (9:1). The triglyceride and sterol ester bands were visualized with Rhodamine 6G and eluted from the adsorbent with Et_2O. Methyl esters of the triglycerides were prepared by transesterification with BF_3–MeOH.[3] Sterol esters were separated from the colored carotenoid esters by a second TLC development for 2 hr in a continuous developing chamber using hexane–Et_2O (97:3). The purified sterol esters were evaporated to dryness under N_2 and hydrolyzed with 3 ml 6% KOH in 95% EtOH in sealed tubes for 1 hr at 75°. After neutralization the products were extracted into Et_2O, dried, evaporated to dryness and the fatty acids separated from the sterols by TLC using $CHCl_3$. The acid band was removed and after elution methylated with BF_3-MeOH.[3] Representative fatty acid methyl ester samples of both triglycerides and sterol esters from each species were hydrogenated at 3·4 kg/cm² at 20° for 1 hr with 10 mg of 10% Pd–C catalyst using a Parr apparatus.

GLC. Fatty acid methyl esters were analyzed on a FID instrument using a glass column 3·05 m × mm i.d. packed with 3% SP-1000 on 100/120 mesh Gas Chrom Q with a He flow rate of 55 ml/min. The optimum temperature program was 180–204° at 2°/min, then 204–210° at 1°/min, then 210–230° at 20°/min and finally a hold at 230° until the C_{28} methyl esters had eluted from column. The fatty acids were identified by comparing their carbon numbers with linear and branched chain fatty acid standards as previously described. The percentages were calculated with the aid of a computing integrator. The values represent the mean of 2 GLC determinations on each of 4 separate tissue juice sac extractions (8 values total). Coefficient of variation (CV) determined for several mean ranges (MR) showed the following: MR 0·1–1·0: CV 5–10%; MR 1·0–5·0: CV 3–5%, and MR above 5·0: CV less than 2%.