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FATTY ACIDS OF TRIGLYCERIDES FROM *CITRUS* JUICE SACS

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Abstract—The fatty acid composition of trigylcerides from oranges grapefruit lemons and limes was determined by GLC Each species possessed its own intrinsic fatty acid pattern which might be used to differentiate it from the other species The five major acids in all species were palmitic, palmitoleic, oleic, linoleic and linolenic Collectively these acids comprised greater than 92% of the total acid content Lemons were distinguished from all other species by their higher 16/16 1 ratios while grapefruit showed the highest total percentage of 16 and 16 1 acids Lemons and limes contained higher percentages of branched-chain acids than oranges and grapefruit

INTRODUCTION

CHEMOTAXONOMY is being used with considerable success by taxonomists in determining the purity of citrus species Citrus species have been differentiated by their content of essential oils,[1-3] limonoids[4] and flavanones[5] Recently the authors[6] investigated the fatty acid composition of orange, grapefruit, mandarin, lemon and lime, and found that these species differed markedly in their total fatty acid profiles Cultivars of *C sinensis* (sweet orange) were shown to possess a similar total fatty acid pattern[7] Although total fatty acid patterns therefore cannot be utilized to distinguish cultivars within a species, fatty acid patterns associated with specific lipid subfractions can[8] In the present study the possibility that other citrus species also have specific patterns was investigated for orange, grapefruit, lemon and lime

RESULTS AND DISCUSSION

Fatty acid distribution patterns of triglycerides are shown in Tables 1–4 for the four citrus species Only fatty acids with relative percentages greater than 0 1% are recorded The authors have previously shown the complexity of citrus fatty acids by reporting values down to a level of 0 001%[6]

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Table 1 shows the triglyceride fatty acid distribution of the two midseason oranges, Homosassa and Queen, and the late season orange, Lue Gim Gong The five major acids are palmitic (16 0), palmitoleic (16 1) oleic (18 1) linoleic (18 2) and linolenic (18 3) Collectively these five acids comprise more than 94% of the total fatty acids The major acid in these oranges is either 18 1 (Homosassa) or 18 2 (Queen and Lue Gim Gong)

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

Carbon No	Homosassa	Queen	Lue Gim Gong	Carbon No	Homosassa	Queen	Lue Gim Gong
12	0 35 ± 0 03*	0 27 ± 0 04	0 20 ± 0 01	18	0 85 ± 0 06	0 64 ± 0 06	0 65 ± 0 04
14	0 46 ± 0 03	0 37 ± 0 04	0 38 ± 0 02	18 1	29 88 ± 0 61	29 92 ± 0 35	28 91 ± 0 22
15	0 24 ± 0 02	0 15 ± 0 03	0 2 ± 0 01	18 2	25 47 ± 0 76	35 ± 0 54	36 66 ± 0 31
15 1†	0 16 ± 0 01	0 12 ± 0 03	0 19 ± 0 01	18 3	21 9 ± 0 16	18 89 ± 0 50	16 62 ± 0 30
a16§	0 12 ± 0 01	Tr‡	0 10 ± 0 01	a19§	0 14 ± 0 01	0 26 ± 0 02	0 26 ± 0 02
16	10 07 ± 0 10	7 60 ± 0 14	7 30 ± 0 10	20 1	0 87 ± 0 08	0 88 ± 0 06	0 61 ± 0 04
16 1	6 72 ± 0 03	7 11 ± 0 25	6 22 ± 0 04	22	0 16 ± 0 01	Tr	Tr
a17§	0 10 ± 0 02	Tr	Tr	22 1	0 35 ± 0 03	0 2 ± 0 01	0 11 ± 0 01
17	0 80 ± 0 02	0 8 ± 0 04	0 54 ± 0 02	24	0 39 ± 0 03	0 26 ± 0 03	0 14 ± 0 01
17 1	0 47 ± 0 03	0 36 ± 0 02	0 36 ± 0 02	25	0 11 ± 0 01	0 13 ± 0 01	Tr
a18§	0 58 ± 0 02	0 45 ± 0 02	0 41 ± 0 04	26	0 28 ± 0 02	0 24 ± 0 01	0 11 ± 0 02

* Mean ± s d of 2 GLC determinations from each of 4 6 separate fruit extracts
† Number of double bonds
‡ Trace, less than 0 1%
§ Value represents the combined total of both saturated and unsaturated branched fatty acids

In Queen and Lue Gim Gong these acids are found in the approximate ratio 1 1 4 5 2 while in Homosassa the ratio is 1 0 7 3 3 2, respectively The 16/16 1 ratio is between 1 and 1 5 This ratio has been shown previously to be important in distinguishing various lipid classes within a species[8] The major acid found above 18 is 20 1

TABLE 2 FATTY ACID COMPOSITION OF TRIGLYCERIDES FROM GRAPEFRUIT JUICE SACS (%)

Carbon No	Marsh	Ruby Red	Thompson	Foster
12	0 13 ± 0 01	0 18 ± 0 02	0 27 ± 0 02	0 14 ± 0 01
14	0 27 ± 0 01	0 37 ± 0 05	0 63 ± 0 04	0 30 ± 0 02
15	0 27 ± 0 02	0 35 ± 0 06	0 45 ± 0 05	0 23 ± 0 02
15 1	0 17 ± 0 01	0 20 ± 0 01	0 30 ± 0 01	0 17 ± 0 01
a16	0 15 ± 0 01	0 21 ± 0 06	0 27 ± 0 06	Tr
16	14 26 ± 0 20	15 85 ± 1 80	13 55 ± 1 24	14 51 ± 1 25
16 1	6 65 ± 0 27	6 50 ± 0 23	9 35 ± 0 21	8 83 ± 0 09
a17	Tr	Tr	Tr	Tr
17	0 51 ± 0 05	0 55 ± 0 07	0 55 ± 0 02	0 42 ± 0 02
17 1	0 57 ± 0 01	0 62 ± 0 01	0 62 ± 0 09	0 58 ± 0 09
a18	0 71 ± 0 01	0 72 ± 0 01	0 68 ± 0 02	0 43 ± 0 08
18	1 31 ± 0 14	1 52 ± 0 20	1 47 ± 0 10	0 98 ± 0 14
18 1	30 69 ± 0 54	28 97 ± 0 92	27 65 ± 0 40	30 28 ± 0 23
18 2	28 56 ± 0 27	27 76 ± 0 96	26 21 ± 0 46	25 95 ± 0 65
18 3	13 05 ± 0 86	13 64 ± 0 49	15 29 ± 0 38	14 69 ± 0 71
a19	0 20 ± 0 01	0 22 ± 0 01	0 24 ± 0 02	0 34 ± 0 06
20 1	1 04 ± 0 05	1 12 ± 0 09	1 10 ± 0 08	1 02 ± 0 10
22	0 23 ± 0 01	0 20 ± 0 03	0 12 ± 0 01	0 15 ± 0 01
22 1	0 35 ± 0 03	0 30 ± 0 03	0 27 ± 0 02	0 30 ± 0 02
24	0 39 ± 0 01	0 33 ± 0 02	0 37 ± 0 04	0 24 ± 0 01
25	0 22 ± 0 03	0 16 ± 0 01	0 29 ± 0 01	0 18 ± 0 01
26	0 27 ± 0 02	0 23 ± 0 04	0 32 ± 0 02	0 26 ± 0 03

Surprisingly, for orange triglycerides 20, 20 2 and 20 3 are always found at less than 0 1% Above 18 the only odd-numbered acid of any prominence is 25 The odd-numbered acids, 19 and 21, are always found in citrus but below 0 1%[6,7] Citrus synthesizes a multitude of saturated and unsaturated branched-chain fatty acids[6] Unsaturated branched acids are very difficult to determine by a single GLC run because they are overlapped by other major linear acids To obviate this difficulty, triglyceride samples were hydrogenated and the relative percentage of the total branched acid content (saturate and unsaturate) were recorded

TABLE 3 FATTY ACID COMPOSITION OF TRIGLYCERIDES FROM LEMON JUICE SACS (%)

Carbon No	Kusner	Lisbon	Malta	Eureka
12	0 22 ± 0 03	0 24 ± 0 01	0 22 ± 0 02	0 16 ± 0 05
14	0 47 ± 0 04	0 52 ± 0 02	0 53 ± 0 04	0 40 ± 0 12
15	0 12 ± 0 01	0 18 ± 0 01	0 33 ± 0 03	0 10 ± 0 01
15 1	0 23 ± 0 02	0 30 ± 0 02	0 41 ± 0 03	0 18 ± 0 01
i16	0 13 ± 0 02	0 20 ± 0 03	0 25 ± 0 02	Tr
16	6 20 ± 0 10	5 45 ± 0 21	8 56 ± 0 38	7 43 ± 0 47
16 1	1 99 ± 0 08	1 35 ± 0 05	0 65 ± 0 02	1 02 ± 0 12
a17	0 16 ± 0 01	0 24 ± 0 01	0 45 ± 0 05	0 25 ± 0 02
17	0 28 ± 0 01	0 25 ± 0 03	0 32 ± 0 09	0 20 ± 0 02
17 1	0 36 ± 0 02	0 41 ± 0 01	0 46 ± 0 05	0 27 ± 0 02
17 2	0 54 ± 0 03	0 60 ± 0 03	0 69 ± 0 03	0 55 ± 0 02
i18	1 17 ± 0 02	1 33 ± 0 04	2 76 ± 0 24	2 05 ± 0 15
18	0 50 ± 0 06	0 48 ± 0 03	1 17 ± 0 10	0 52 ± 0 09
18 1	10 95 ± 0 32	15 14 ± 0 28	8 70 ± 0 62	8 52 ± 0 62
18 2	32 37 ± 0 31	29 29 ± 0 34	39 83 ± 0 51	39 62 ± 0 69
18 3	42 97 ± 0 07	42 40 ± 0 25	32 26 ± 0 47	36 51 ± 0 94
a19	0 49 ± 0 04	0 78 ± 0 07	1 94 ± 0 09	1 64 ± 0 02
20 1	0 45 ± 0 03	0 54 ± 0 02	0 19 ± 0 01	0 21 ± 0 01
20 2	0 17 ± 0 01	0 16 ± 0 01	0 12 ± 0 01	0 17 ± 0 01
20 3	0 23 ± 0 01	0 14 ± 0 01	0 16 ± 0 01	0 20 ± 0 01

Table 2 shows the triglyceride fatty acid distribution of the four grapefruit cultivars As with oranges, 16, 16 1, 18 1, 18 2 and 18 3, are the major acids and comprise greater than 92% of all acids In all four grapefruit cultivars, these acids are found in the approximate ratio 2 1 4 4 2, respectively The major acid in all grapefruit is 18 1 and the 16/16 1 ratio ranges between 1 5 and 2 4 The major branched acid is *iso* 18 followed by *anteiso* 19, *iso* 16 and *anteiso* 17

The fatty acid distribution of the four lemons is shown in Table 3 In Kusner and Lisbon, the five major acids are found in the approximate ratio 1 0 3 2 5 7 while Malta and Eureka have the ratios 1 0 1 1 5 4 and 1 0 2 1 5 5, respectively Lemons, in comparison with oranges and grapefruit, show a high relative percentage of 18 3, and the major acid is either 18 2 or 18 3 The 16/16 1 ratio of 3–13 is noticeably higher for lemons than for oranges and grapefruit Lemons also show higher relative percentages for the branched acids The branched-acid percentage order is *iso* 18 > *anteiso* 19 > *anteiso* 17 > *iso* 16 Fatty acids with carbon lengths greater than 20 are found in lemons but are never detected at percentages above 0 1 In contrast to observations in oranges and grapefruit, 20 2 and 20 3 are detected in lemons above the trace level, i e 0 1%

Table 4 shows the fatty acid distribution of lime juice sac triglycerides In comparison with oranges, grapefruit and lemons, limes appear to be rather diverse and show no specific distinguishing patterns Key and Persian limes are regarded as small-fruited and large-fruited sour limes, respectively While Key lime is regarded as a pure species, Persian lime is thought to be of hybrid origin with acid lime and citron parentage [9] The hybrid nature of Persian may be one reason for the contrasting pattern when compared to Key Columbia, which belongs to the second major lime group, viz sweet limes, is a pure lime species but its fatty acid pattern is noticeably different from that of sour limes The two sour limes show 18 2 and 18 3 as being the two most prominent acids while the sweet lime Columbia shows 18 1 and 18 2

TABLE 4 FATTY ACID COMPOSITION OF TRIGLYCERIDES FROM LIME JUICE SACS (%)

Carbon No	Key	Persian	Columbia	Carbon No	Key	Persian	Columbia
12	0 10 ± 0 06	0 15 ± 0 02	0 16 ± 0 04	17 2	0 21 ± 0 05	0 48 ± 0 02	0 21 ± 0 07
14	0 42 ± 0 14	0 31 ± 0 04	0 25 ± 0 01	18	1 62 ± 0 06	1 80 ± 0 06	2 40 ± 0 22
15	0 15 ± 0 06	0 15 ± 0 03	0 18 ± 0 01	18	0 68 ± 0 05	0 28 ± 0 03	0 34 ± 0 02
15 1	0 13 ± 0 02	0 13 ± 0 02	0 14 ± 0 01	18 1	18 57 ± 0 07	11 54 ± 0 55	26 35 ± 0 42
i16	0 27 ± 0 06	0 26 ± 0 03	0 16 ± 0 03	18 2	42 36 ± 0 49	38 81 ± 0 61	35 12 ± 0 15
16	7 67 ± 0 15	3 90 ± 0 10	9 45 ± 0 25	18 3	23 91 ± 0 15	35 72 ± 0 24	13 97 ± 0 42
16 1	4 58 ± 0 27	3 94 ± 0 15	8 91 ± 0 20	a19	0 61 ± 0 03	1 14 ± 0 02	0 84 ± 0 05
a17	0 31 ± 0 01	0 30 ± 0 01	0 12 ± 0 02	20 1	0 47 ± 0 02	0 33 0 01	0 56 ± 0 05
17	0 28 ± 0 05	0 23 ± 0 03	0 40 ± 0 05	20 2	0 14 ± 0 01	0 20 0 01	Tr
17 1	0 37 ± 0 04	0 25 ± 0 02	0 48 ± 0 05	20 3	0 11 ± 0 01	0 26 0 01	Tr

Table 5 is a compilation of factors that the authors believe might be useful in distinguishing triglycerides of the four species Oranges appear to differ from grapefruit by their higher total percentage of 18 acids and lower total percentage of 16 acids The 16/16 1 ratio in oranges is lower than that found in grapefruit While the total 18 acid content of oranges has a range similar to those of lemons and limes, the 16 content is noticeably higher Also the percentages of branched acids and in particular *iso* 18, are consistently lower when compared to lemons or limes

TABLE 5 FACTORS WHICH MIGHT BE USEFUL IN DISTINGUISHING TRIGLYCERIDES OF *CITRUS* SPECIES

Species	Total % of all linear 18 (18 18 1 18 2 18 3)	Total % of all linear 16 (16 16 1)	16 16 1	% *iso* 18
Orange	78-83	13 17	11 15	0 31–0 45
Grapefruit	70 74	20 24	15-24	0 45–0 72
Lemon	81-88	7–10	31-132	1 17–2 76
Lime				
Sour	82–87	7 13	10 17	1 62–1 80
Sweet	76	17	11	2 40

Grapefruit are distinguished from oranges, lemons and limes by their lower total percentage of 18 acids and higher 16 acid content Grapefruit, like oranges, differ from lemons and limes by having lower percentages of branched acids

[9] HODGSON R W (1967) in *The Citrus Industry* (REUTHER W WEBBER H J and BATCHELOR L D eds) Vol 1 p 577 University of California Press

The most noticeable feature of lemons useful in differentiating this species from the other three species is the very high 16/16 1 ratio The sour and sweet limes, when compared as a whole, show some features useful in distinguishing this citrus group from orange and grapefruit Limes, like lemons, contain relatively high percentages of branched acids The two sour limes, Key and Persian, differ from oranges and grapefruit by showing a lower total percentage of 16 acids

EXPERIMENTAL

Citrus sources Oranges Citrus sinensis, cultivars Queen, Homosassa and Lue Gim Gong were obtained at a mature stage from Whitmore Experimental Farm (Plant Science Research Division, U S D A, Orlando, Florida) *Grapefruit C paradisi*, cultivars Marsh seedless, Redblush and Foster were from Whitmore Experimental Farm and Thompson was from Adams Citrus Nursery, Haines City, Florida *Lemons C limon* cultivars Lisbon Malta and Kusner were from Whitmore Experimental Farm and Eureka was from a local market *Limes C aurantifolia* Swingle (sour lime) cv Key and *C latifolia* Tanaka cv Persian and *C limettioides* Tanaka (sweet lime) cv Columbia were from U S D A groves in Homestead, Ft Pierce and Leesburg, Florida, respectively *Juice sac lipids* were prepared and purified by a method previously described[10] 4–6 separate extractions were made from tissue from each cultivar

Separation of triglyceride and preparation of fatty acid methyl esters The purified lipid (*ca* 200 mg) was dissolved in $CHCl_3$ and applied to a (column 0 9 × 30 cm) containing 9 g 60–200 mesh silica gel (J T Baker Chemical Company, Phillipsburg, New Jersey) The neutral lipids, which contained the triglycerides, were eluted with 200 ml $CHCl_3$ This fraction was concentrated, taken up in hexane and streaked on precoated silica gel G plates (20 × 20 cm, 500 μ, Analtech, Inc, Wilmington, Delaware) The TLC plate was placed in a chamber lined with filter paper and developed with hexane–ethyl ether (9 1) containing 0 1% ditert-butyl-cresol The triglyceride band was visualized with Rhodamine 6G and eluted with ethyl ether Methyl esters were prepared from TLC-separated triglycerides by the transesterification BF_3–MeOH method[6] Representative fatty acid methyl ester samples from each species were hydrogenated under 4 kg/cm^2 at room temp for 1 hr with 10 mg of 10% Pd–C catalyst in a Parr apparatus

GLC The fatty acid Me esters were determined with an F & M Model 5750 gas chromatograph equipped with FIDs Me esters were analyzed on two glass columns column 1 was 3 05 m long and 4 mm i d and column 2 was 2 44 m long and 2 mm i d Both columns were packed with 10% SP-1000 (Supelco, Inc, Bellefonte, Pennsylvania) coated on 100–120 mesh Gas Chrom Q (Applied Science, State College, Pennsylvania) The injection port and detector were at 245° for both columns For analysis of Me esters from 12 0 to 20 3, column 1 was used The conditions for column 1 were on-column injection, He flow rate 80 ml/min, isothermal temp 210° The conditions for column 2 were on-column injection, He flow rate 55 ml/min, isothermal temp 225° Two columns were necessary because although column 1 gave excellent resolution between 12 0 nd 20 3, considerable peak broadening was manifest in the region 20 3 to 26 0 With column 2, resolution was excellent in the 20 3 to 26 0 region without peak broadening Results were quantitated by measuring peak areas with the aid of a disc integrator and also with a planimeter

[10] NAGY, S and NORDBY, H E (1970) *J Agric Food Chem* **18,** 593