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FATTY ACIDS OF MONOGALACTOSYL DIGLYCERIDES FROM *CITRUS*

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Key Word Index—*Citrus*; Rutaceae; orange; lemon; lime; grapefruit; tangelos; mandarin; satsuma; monogalactosyl diglycerides; fatty acid profiles; juice vesicles; leaves; chemotaxonomy.

Abstract—Fatty acids in vesicular and leaf monogalactosyl diglycerides (MGDG) of citrus were studied. Vesicular MGDG contained from 94.4 to 97.3% C_{16}, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$; whereas leaf MGDG contained *ca* 90% $C_{18:3}$, 3% C_{16} and 1.8 to 9.5% $C_{18:2}$. Species varied considerably in their percentages of vesicular $C_{18:2}$, $C_{18:3}$ and to a lesser degree, $C_{16:1}$ and $C_{18:1}$ fatty acids with lemons being the most distinctive. Branched fatty acids were present to the extent of 5.6% in vesicular and to only 0.1% in leaf MGDG.

INTRODUCTION

In previous publications we have shown that lipids are important chemotaxonomic markers for the differentiation of citrus fruit. These markers have included sterols, hydrocarbons and fatty acids [1–6]. One goal of our chemotaxonomic investigations has been to find a lipid which is easily isolated, found in all citrus plant tissue and shows enough heterogeneity to be useful for the differentiation of citrus species and cultivars (cvs). Monogalactosyl diglyceride (MGDG) , the most prevalent lipid in photosynthetic tissues of higher plants [7] has been analyzed for its fatty acid composition in numerous plant genera and species [8]. The major fatty acid found in all leaf MGDG examined to date has been linolenic acid, MGDG of runner bean leaf has one of the highest percentages of this acid (95.5%) [9]. In nonphotosynthetic tissue, no specific fatty acid appears to be unique to MGDG. For example, in rice [10] the major MGDG fatty acid is palmitic (51.4%) while linolenic acid is found in trace quantities. In contrast, wheat MGDG has 57.0% linoleic acid, 13.9% palmitic acid and only 2% linolenic acid [11]. Comparative studies of the composition of fatty acids in MGDGs from photosynthetic and nonphotosynthetic tissues of the same plant have apparently never been undertaken. In a preliminary investigation, we found differences in the fatty acid compositions of MGDG from orange and tangor juice vesicles [3]. These differences and the relatively high concentration of MGDG in citrus leaves (unpublished data) prompted us to compare the fatty acid profiles of MGDGs from leaves and juice vesicles of several citrus species for the purpose of enhancing our understanding of citrus chemotaxonomy.

RESULTS AND DISCUSSION

Table 1 shows the percentages of the five major fatty acids found in vesicular MGDGs from 29 citrus cvs. These acids comprised from 94.4 to 97.3% of the total MGDG fatty acids. Palmitic acid percentages in the 29 cvs were the most consistent of the percentages for the five major acids with a mean of 3.2 and a range 2.0–5.1. The palmitoleic acid in lemons was 1.0 while in all other citrus cvs, except Key lime (2.8%), the mean was 5.3 with a range of 4.1–7.0%. For oleic acid, lemons showed a mean of 5.7%, while mid-season oranges and grapefruit had a mean of 25.4%. Tangelos, mandarins and satsumas had a $C_{18:1}$ mean of 30.3%, while Key lime had 14.7%. Late season oranges—Pope Summer and Lue Gim Gong—differed from mid-season oranges by showing about 6% more oleic acid than the mid-season oranges. Late season oranges could be differentiated from all other citrus species examined by their high linoleic percentages (25.9% mean). Mid-season oranges and grapefruit were quite similar, with means of 13.0 and 13.8% for $C_{18:2}$, respectively. Eureka lemon with 14.6% and Key lime with 12.3% were also in the grapefruit-mid-season orange range while the other lemons (mean 7.2%) and Persian lime (6.0%) were nearer to the mean of mandarins, satsumas and tangelos (4.3%). The low percentages of $C_{18:2}$ in MGDGs of mandarin hybrids were similar to the percentage [3] in MGDGs of Temple tangor (6.9%). Linolenic acid was the most prevalent acid in the MGDGs of all citrus except Pope Summer orange. Pope Summer contained 6% more oleic than linolenic acid. Lue Gim Gong, the other late season orange, had about the same percentages of oleic acid and linolenic acid. The lemons, with a mean of 76.6% $C_{18:3}$, could be differentiated from late season oranges (30.2% mean) and all other citrus cvs (51.9% mean). Each citrus species could generally be distinguished from other species by their profiles for these five major acids, viz., lemons by their oleic and linoleic percentages, late-season oranges by their linoleic and linolenic contents, grapefruit by their linoleic and linolenic contents, tangelos (except K-Early and Orlando) by their oleic–linoleic contents, mandarins by a combination of all five fatty acids and satsumas (except Sugiyama and Silverhill) by linoleic and linolenic percentages.

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Table 1. Major fatty acids of monogalactosyl diglycerides from citrus juice sacs (%)

Cultivar	16:0	16:1	18:1	18:2	18:3	Other
Lemons						
—Eureka	2.4	1.2	3.8	14.6	72.9	5.1
—Lisbon	3.1	1.0	7.8	8.7	75.3	4.0
—Malta	3.3	0.9	4.9	7.1	78.3	5.6
—Kusner	2.8	0.9	6.5	5.8	79.7	4.3
Avg. Lemons	2.9	1.0	5.7	9.1	76.6	4.7
Limes						
—Key	5.0	2.8	14.7	12.3	60.2	5.1
—Persian	3.2	5.2	27.9	6.0	53.8	4.0
Avg. Limes	4.1	4.0	21.3	9.1	57.0	4.6
Oranges						
—Pope Summer (L)*	5.1	4.5	33.2	27.2	27.3	2.7
—Lue Gim Gong (L)	4.2	4.8	30.5	24.7	33.0	2.9
—Jaffa (M)*	3.5	4.8	25.5	12.7	49.2	4.4
—Queen (M)	3.1	6.8	27.6	12.1	45.7	4.6
—Abers (Granita) (M)	3.2	4.5	24.6	14.3	50.7	2.7
Avg. Oranges (L)	4.7	4.6	31.9	25.9	30.2	2.8
Avg. Oranges (M)	3.3	5.4	25.9	13.0	48.5	3.9
Grapefruit						
—Foster	3.8	5.0	24.1	15.8	47.7	3.6
—Ruby Red	3.7	5.7	22.3	12.7	51.8	3.9
—Thompson	4.3	6.9	26.2	13.3	45.7	3.5
—Marsh	4.0	5.9	28.7	11.9	45.8	3.7
—Duncan	3.0	4.9	24.1	15.0	49.9	3.1
Avg. Grapefruit	3.7	5.7	25.1	13.8	48.2	3.6
Tangelos						
—Seminole	2.7	4.9	29.8	3.2	55.9	3.5
—Minneola	1.9	7.0	32.8	5.9	48.1	4.3
—K-Early	2.8	4.5	27.5	5.0	56.5	3.7
—Orlando	2.3	5.1	27.5	5.2	56.8	3.1
Avg. Tangelos	2.4	5.4	29.4	4.8	54.3	3.7
Mandarins						
—Dancy	3.1	5.0	31.4	4.8	51.5	4.2
—Clementine	3.4	6.2	33.4	2.3	47.5	7.1
—Mediterranean	4.5	4.9	30.8	4.9	51.3	3.6
—King	2.5	4.7	30.1	2.7	55.3	5.0
—Kinnow	3.2	5.3	28.2	5.7	53.8	3.7
Avg. Mandarin	3.3	5.2	30.8	4.1	51.9	4.7
Satsumas						
—Sugiyama	2.1	4.7	35.1	2.6	52.2	3.2
—Owari	3.0	6.1	29.1	6.6	50.6	4.5
—Silverhill	2.5	5.1	34.9	2.4	51.6	3.6
—Nobilis	2.2	4.1	23.8	4.9	61.3	3.8
Avg. Satsumas	2.5	5.0	30.7	4.1	53.9	3.8

* L (late-) and M (mid-) season cultivars.

Table 2 lists the minor fatty acids found present in citrus MGDGs. Lemons differed markedly from other citrus and from each other in these minor acids. They resembled Key lime having 1.5–2.8% iso $C_{18:1}$; a range observed previously for lemon and lime total lipids [1] triglycerides [4] and sterol esters [5]. The mean stearic acid content for Lisbon, Malta and Kusner lemons, and Key lime was 0.7% while the mean for the other cvs, except Eureka lemon, was 0.20%. Eureka lemon with 0.1% stearic acid could be differentiated from all other (0.2%) citrus cvs examined. Eureka and Malta with 1.3% anteiso C_{19} could be characterized as different from the other lemons, and from all the other cvs. Previously we reported that the triglycerides of these two lemon cvs and Persian lime contained from 1.1 to 1.9% anteiso C_{19} [4]. The lemons were also characterized by the lack of fatty acids greater than anteiso C_{19}. Key lime having a low C_{17} combined with a high C_{20} content was set apart from other cvs. In contrast to previous reports for triglycerides [4, 6] and sterol esters [5, 6], Persian lime MGDG had no difinitive minor acid percentages which characterized it as being a lime. As observed for citrus triglycerides [4, 6] and for sterol esters [5, 6], citrus MGDGs always had more anteiso C_{19}s than linear C_{19}s.

All cvs examined except those of lemons contained 0.3–0.7% of two fatty acids (A and B) of unknown structure. Their methyl esters migrated with those of normal fatty acids on TLC; hence, they were not hydroxy fatty acids. When the methyl esters were hydrogenated, these unknowns became methyl stearate. These two acids were found in the monoenoic fraction when the methyl esters were subjected to $AgNO_3$–TLC. Their equivalent chain lengths (ECL), determined by GLC with SP-1000, were 19.5 and 19.8, respectively. These data indicated that the unknowns may have been monoynoic or isomeric monoenoic C-18 fatty acids. Monoynoic fatty acids although unreported in food tissues, have been found in seeds [12]. It was observed in the GLC profiles of lemons that a doublet occurred in the $C_{18:1}$ area instead of the usual sharp peak for oleate. This doublet, like fatty acids A and B, was reduced to methyl stearate upon hydrogenation. Thus, it appears that lemons contain different monoenic fatty acids from those present in other citrus examined.

Table 3 lists the major fatty acids found in citrus leaf MGDGs. Linolenic acid accounted for *ca* 90% of the fatty acids in the MGDGs of this tissue. This was in agreement with the MGDG composition of leaves from higher plants [7]. Grapefruit appeared to have a lower (86.1%) and mandarins a higher (92.5) percentage of $C_{18:3}$ than the mean $C_{18:3}$ content of citrus leaf MGDG.

Palmitoleic, stearic and oleic acids were present in MGDGs of citrus leaves at percentages which were not significantly different (means of 0.2, 0.6 and 0.9%, respectively) for differentation of the species and cvs examined. The percentages of linoleic acid, however, appeared to fall into five ranges according to citrus species; grapefruit 7.0%, mid-season oranges 5.7%, lemon–limes 3.7%, tangelos 2.9% and mandarin–satsumas 1.9%. The other four cvs listed were not fully characterized because of insufficient data. Because of their hybrid nature, these four cvs yielded leaf-fatty acid profiles slightly different from those of the other cvs.

Percentages of minor acids in total fatty acids from MGDGs were between 2.7 and 5.6% for vesicles and only 0.9 for leaves (Table 2). This difference was essentially due to the higher percentage of branched-chain fatty acids in vesicular MGDG. Malta lemon and late season oranges contained 4.3 and 0.4% branched-chain acids, respectively, in their vesicular MGDGs. In contrast to these values, the total amount of branched acids in citrus leaf MGDG was less than 0.1%. Previously we reported that for Duncan grapefruit, the percentages of branched long-chain hydrocarbons were 55.6% of the total saturated hydrocarbons in vesicular lipids and 1.2% of the saturated hydrocarbons in the leaf wax [13]. This difference in tissue composition correlates with the vesicular-leaf MGDG differences in fatty acids since long-chain hydrocarbons are synthesized from fatty acids [14]. The two, unknown C_{18} acids (A and B) found pres-

Table 2. Minor fatty acids of monogalactosyl diglycerides from citrus juice vesicles and leaves (%)

Carbon no.	12	14	15	16:2	17+ 17:1	◂18	◂18:1	18	‖19+ ‖19:1	19 + 19:1	Unknown A	Unknown B	20 20:1 20:2	21
Cultivars														
Lemons														
—Eureka	0.1	0.1	0.1	tr*	0.5	0.1	2.2	0.1	1.2	0.1	0.2	–†	—	—
—Lisbon	0.1	0.1	0.1	tr	0.6	0.2	2.0	0.5	0.4	tr	—	—	—	—
—Malta	0.1	0.1	0.1	tr	0.4	0.3	2.8	0.6	1.3	tr	—	—	—	—
—Kusner	0.2	0.1	tr	tr	0.7	0.2	1.6	1.0	0.4	tr	—	—	—	—
Limes														
—Key	0.2	0.6	0.2	0.1	0.2	0.3	1.5	0.7	0.6	tr	0.1	—	0.7	—
—Persian	0.1	0.2	0.2	0.1	0.5	0.1	0.2	0.3	0.4	tr	0.6	0.5	0.7	0.3
Oranges— Late season-mean‡	0.1	0.1	0.2	tr	0.8	0.1	0.3	0.4	0.1	0.1	0.4	0.3	0.2	tr
Oranges— Mid-season-mean‡	0.6	0.5	0.2	0.2	0.4	0.1	0.3	0.2	0.3	tr	0.5	0.7	0.5	0.2
Oranges— Sour Abers	0.1	0.1	0.1	tr	0.5	0.1	0.4	0.1	0.1	tr	0.4	0.2	0.4	0.1
Grapefruit-mean‡	0.2	0.2	0.2	0.1	0.4	0.1	0.4	0.3	0.2	tr	0.4	0.4	0.6	0.1
Tangelos-mean‡	tr	0.1	0.1	0.1	0.6	0.1	0.5	0.2	0.2	tr	0.6	0.7	0.5	tr
Mandarins-mean‡	0.2	0.4	0.2	0.1	0.6	0.1	0.2	0.4	0.3	tr	0.7	0.6	0.8	0.3
Satsumas-mean‡	0.1	0.1	0.1	0.1	0.6	0.1	0.1	0.1	0.4	tr	0.6	0.7	0.6	0.1
Citrus leaves—mean§	0.1	0.1	0.1	tr	0.3	0.1	0.1	0.6	—	0.1	—	—	0.1	—

* Trace, less than 0.01%. † Not detected at 0.001% level. ‡ Mean of 2 to 5 cultivars, differences between cultivars not significant. § Mean of 19 cvs, differences between species not significant. ◂ Iso-branched. ‖ Anteiso-branched.

Table 3. Fatty acids of monogalactosyl diglycerides from citrus leaves (%)

	16	16:1	18	18:1	18:2	18:3	Total minor
Lemons							
—Lisbon	2.5	0.3	0.4	0.7	3.0	92.3	0.9
—Kusner	3.2	0.2	0.5	1.4	4.3	89.5	0.9
Limes							
—Key	2.8	0.4	0.6	1.1	4.4	89.6	1.1
—Persian	1.9	0.1	0.4	0.4	3.2	93.7	0.3
Oranges							
—Lue Gim Gong	2.5	0.3	0.6	0.9	3.0	91.8	0.8
—Jaffa	1.8	0.2	0.4	1.0	5.4	90.4	0.9
—Pineapple	3.1	0.2	0.5	1.0	6.0	89.4	0.7
Grapefruit							
—Marsh	3.0	0.2	0.5	1.5	7.2	86.8	0.8
—Duncan	4.4	0.2	0.9	1.3	6.8	85.4	1.0
Tangelos							
—Seminole	3.6	0.3	0.9	0.5	2.2	91.4	1.1
—Orlando	3.1	0.2	0.7	1.1	3.7	90.5	0.9
Mandarin							
—Dancy	3.6	0.2	0.7	0.5	1.5	92.3	1.3
—Mediterranean	3.3	0.2	0.7	0.5	2.0	92.6	0.8
Satsumas							
—Sugiyama	3.2	0.4	1.0	1.0	1.8	91.5	1.1
—Owari	4.2	0.3	0.6	0.7	2.3	91.0	1.0
Hybrid Citrus							
"Page Orange"	4.5	0.2	0.7	1.5	5.5	87.9	0.7
"Rangpur lime"	2.7	0.1	0.5	1.0	4.1	90.9	0.7
Murcott	3.5	0.2	0.7	0.4	3.1	91.5	0.6
Calamondin	1.5	0.2	0.5	0.4	9.5	87.2	0.7
Mean of all citrus	3.1	0.2	0.6	0.9	4.1	90.3	0.9

ent in vesicular MGDG were not detected in leaf MGDG. Fatty acids greater than C_{21} were only detected in trace amounts in both vesicular and leaf MGDGs.

Comparison of the major acids in the MGDGs of vesicles and leaves showed that palmitic acid occurred in both tissues at *ca* the 3% level with a variation spread of 2% for all cvs. Stearic acid was present in both tissues at about the same level (Tables 2 and 3). Vesicular MGDG showed a rather consistent ratio of 5 to 6/1 for the oleic/palmitoleic acids. In leaf MGDG this ratio varied considerably, 2 to 8/1, and showed a mean of 4.4/1. Mandarins contained the least amount of linoleic acid in both tissues.

EXPERIMENTAL

Isolation of vesicular and leaf lipids. 24 of 29 citrus cvs studied were from Whitmore Foundation Farm (U.S. Horticultural Research Laboratory, Orlando, Fla.), Thompson grapefruit from Adams Citrus Nursery, Haines City, FL., Key lime from a USDA grove in Homestead FL, and Persian lime from a USDA grove in Ft. Pierce, FL. The K-Early tangelo was from a grove in Leesburg, FL and the Eureka lemon was from a local market. All samples were harvested at their peak maturities. The juice sacs were removed from the fruit, extracted for lipids and the lipid extracts purified [15]. Each cv was run in quadruplicate. Leaves from 17 of 19 cvs were from Whitmore Experimental Farm. Leaves from Persian lime were from Budwood Register, Winter Haven, FL and those of Key lime were from the Winter Haven area. The leaves were collected from various areas of the tree; they were free of decay and uniform in maturity, size and greenness. 30 leaves of each cv were blended with 150 ml $CHCl_3$–MeOH (2:1) for 5 min. The extract was decanted, and the leaves were re-extracted with an additional 150 ml of the solvent mixture. Combined lipid extracts were separated from the leaf residue

by filtration and reduced to near dryness. Concentrated leaf lipids were extracted into 50 ml $CHCl_3$ and washed (2 × 50 ml) with H_2O. After evaporation of the $CHCl_3$ the leaf lipid extract was stored in $CHCl_3$–MeOH (4:1) at −27° until fractionated.

Separation of MGDGs and preparation of fatty acid methyl esters. Purified vesicular and leaf lipids were separated into neutral, glyco- and polar lipids by Si gel column chromatography [3] using 200 ml Me_2CO to elute the glycolipids. This fraction was streaked on 0.5 mm Si gel G plates and developed in $CHCl_3$–MeOH (17:3) [3]. MGDG areas were purified from esterified sterol glucoside (ESG), free fatty acids, and minor polar lipids by TLC for 2 hr in a continuous development chamber [6] with $CHCl_2$–MeOH (23:2). Representative samples of vesicular and leaf MGDGs were transmethylated with HCl in MeOH and the sugar moieties chromatographed with standards [3]. Purified MGDG areas were transesterified directly without removal of the Si gel [3] and the esters purified by TLC with hexane–Et_2O (9:1). One-half of each purified ester sample was hydrogenated in a Parr apparatus with 10 mg PtO in hexane (3 kg cm^2) at 20° for 1 hr. Saturated esters were eluted from the catalyst by washing the sample on sintered glass with MeOH (2 × 0.5 ml) followed by Me_2CO (2 × 0.5 ml). For each citrus species a representative sample of purified Me esters was subjected to $AgNO_3$–TLC [1].

Quantitation. Fatty acid Me esters were injected onto a 3 m 3% SP-1000 GLC column and the peaks integrated with an electronic integrator as reported previously [6]. 4 to 8 analyses were run on each cv and the analyses were averaged. Coefficient of variation (CV) determined for several mean ranges (MR) were: MR 0.01–0.10; CV 10–35%, MR 0.1–1.0; CV 5–10%; MR 1.05–5.0; CV 3–5%v, and MR above 5.0; CV less than 2%.

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