Phytochemistry, 1979, Vol. 18, pp. 825–827. Pergamon Press. Printed in England.

FATTY ACID PROFILES OF ESTERIFIED STEROL GLUCOSIDES FROM JUICE VESICLES OF CITRUS FRUITS

HAROLD E. NORDBY and STEVEN NAGY
U.S. Citrus and Subtropical Products Laboratory*, Winter Haven, FL 33880, U.S.A.

(*Received* 3 *October* 1978)

Key Word Index—*Citrus*; Rutaceae; juice sac; taxonomy; esterified sterol glucosides; fatty acids.

Abstract—Vesicular lipids of 24 citrus cultivars were extracted and purified, and the esterified sterol glucosides (ESG) separated from other vesicular lipids by chromatography. Fatty acids of the ESG were analysed by gas–liquid chromatography as their methyl esters. Statistical analysis showed that five out of six citrus species or biotypes could be differentiated from each other on the basis of profiles of the five major ESG fatty acids. Differences between mid- and late-season sweet orange cultivers were detected. Small differences were observed between white- and red-fleshed grapefruit cultivars. Profiles of the minor acids showed higher relative percentages of *iso*-branched fatty acids in lemons and limes, and higher relative percentages of 12:0 and 14:0 fatty acids in mandarin and mandarin hybrids than in other cultivars.

INTRODUCTION

Prior to 1970, limited amounts of data were available dealing with the chemotaxonomy of the genus *Citrus*. During this early period, citrus chemotaxonomy essentially involved only the essential oils [1] and flavanones [2]. Since 1970 not only have these markers been further explored [3–5] but polyphenol oxidase [6], peroxidase isoenzymes [7] and lipids have also been investigated. Lipids have included sterols, alkanes and fatty acids [8] from the juice sac as well as alkanes from other tissues [9, 10]. We undertook to determine whether closely related citrus species could be differentiated on the basis of the fatty acid compositions of their juice-sac lipids. We, therefore, separated and analysed four major classes of juice-sac lipids present in six orange and two tangor cultivars [11]. Differences among the fatty acid profiles prompted us to continue the research, using larger numbers both of species and of members within species. Thus far, we have reported in depth on the acid profiles for the triglycerides (TG) [12], sterol esters (SE) [13] and monogalactosyl diglycerides (MGDG) [14]. The fourth major class of juice-sac lipids in citrus is esterified steryl glucosides (ESG). To determine correlations between ESG fatty acid patterns and citrus taxonomic classification, we examined 24 citrus cultivars.

Table 1. Fatty acids of esterified sterol glucosides from citrus vesicular lipids of 24 cultivars (%)

Carbon No.	Percentage Mean	Range	Carbon No.	Percentage Mean	Range
12	0.2	tr*– 0.6	18	1.2	0.7– 1.9
13	0.2	tr– 0.7	18:1	33.3	10.6–45.8
14	0.5	0.2– 1.1	18:2	17.0	10.3–31.9
14:1	0.1	tr– 0.3	18:3	7.2	4.1–16.6
†15	0.5	0.2– 0.9	†19:2	0.4	tr– 0.9
15	0.6	0.3– 0.9	‡20	0.3	tr– 1.0
15:1	0.3	tr– 0.8	20	0.6	0.1– 2.2
‡16	0.3	0.1– 1.4	20:1	1.1	0.2– 1.9
16	23.6	13.7–32.3	21	0.4	tr– 1.0
16:1	6.2	2.3– 9.7	22	0.8	0.3 1.9
‡17	0.1	tr– 0.5	22:1	0.5	0.1– 1.1
†17	0.2	tr– 0.6	23	0.3	0.1– 0.4
†17:1	0.3	tr– 0.6	24	1.1	0.4– 1.9
17	0.4	2.0– 0.7	24:1	0.1	tr– 0.2
17:1	0.3	0.1– 0.6	25	0.4	tr– 0.8
‡18	0.4	0.1– 1.1	26	0.4	0.1– 0.6
‡18:1	0.7	0.1– 2.2			

* Trace, less than 0.1%.
† *Anteiso*-branched.
‡ *Iso*-branched.

RESULTS AND DISCUSSION

Table 1 shows the composite ESG fatty acid composition of 24 citrus cultivars. Thirty-three C_{12}–C_{36} fatty acids were detected, and their relative percentages ranged from a trace (less than 0.1%) to 46%. Generally, the fatty acid profiles follow patterns observed previously for other citrus lipids. The major five acids, namely, $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$, comprised a mean ESG fatty acid value of *ca* 87%. This mean value is between values reported for TG [11, 12], MGDG [11, 14] and SE [11, 13]. The differences among TG, MGDG, SE and ESG are apparently due to differences in the percentages of C_{20}–C_{26} acids. We have found that ESG and SE contain more of these acids than TG and MGDG, and that SE contains the most [13].

As compared with SE [13], ESG contained a lower percentage of total linear acids but higher percentages of $C_{20:1}$, $C_{22:1}$ and $C_{24:1}$. Branched C_{20}–C_{26} acids appeared to be absent in ESG. An acid which does not follow a specific pattern but which was found in relatively high percentages as a minor acid in this study as well as other

* U.S. Department of Agriculture, Science and Education Administration, Federal Research.

Table 2. Major fatty acids of esterified sterol glucosides from citrus juice sacs (%)

Biotype/cultivar	16:0	16:1	18:1	18:2	18:3
Mandarin					
—Dancy	20.6	6.6	40.0	12.1	5.6
—Clementine	19.9	7.4	45.8	10.3	5.0
—Ponkan	22.2	9.7	35.5	14.3	5.4
King	21.5	6.9	43.9	11.0	5.0
Mean*	21.1[a]	7.7[a]	41.3[a]	12.0[a]	5.3[a]
Tangor					
—Murcott	23.8	5.5	31.4	18.2	5.3
Tangelo					
—Orlando	20.9	6.4	36.9	14.8	6.8
—Minneola	19.2	8.0	39.2	13.1	5.7
—Seminole	21.7	7.2	35.8	17.2	5.7
—Page†	21.6	7.4	40.3	13.6	5.9
Mean*	20.9[a]	7.3[a]	38.1[a, b]	14.7[a, b]	6.0[a]
Sweet orange					
—Homosassa	27.3	5.7	35.3	13.8	5.9
—Queen	24.6	5.9	38.0	16.2	6.8
—Jaffa	29.0	4.7	33.1	14.7	7.4
—Lue Gim Gong	32.3	5.7	31.2	16.1	5.1
Mean*	28.3[b]	5.5[b]	34.4[b]	15.2[a, b]	6.3[a]
Grapefruit					
—Marsh	24.0	6.9	34.8	18.6	5.5
—Duncan	23.9	6.7	32.6	19.3	5.6
—Foster	24.5	6.8	33.2	18.0	6.2
—Thompson	26.7	6.9	34.5	16.7	5.4
Ruby Red	27.5	6.6	32.5	17.5	5.7
Mean*	25.3[b]	6.8[a]	33.5[b]	18.0[b, c]	5.7[a]
Lemons					
—Lisbon	25.9	2.8	15.1	23.1	16.6
—Eureka	25.0	2.3	10.6	31.9	16.5
Mean*	25.5[b]	2.6[c]	12.9[c]	27.5[d]	16.6[b]
Sour limes					
—Key	26.4	5.5	21.6	20.7	9.7
—Persian	23.8	4.8	20.5	22.1	13.2
Mean*	25.1[b]	5.2[b]	21.1[d]	21.4[c]	11.5[c]
Sweet lime					
—Columbia	19.2	6.9	41.2	13.9	4.1
Mandarin hybrid					
—Rangpur	13.7	5.5	36.7	21.0	8.2

* a, b, c, d—Means in each column with different letters are significantly different at the 5% level.

† Tangelo hybrid (Minneola tangelo × Clementine mandarin).

studies [8, 12, 13] is *anteiso* $C_{19:2}$. Linear $C_{21:0}$ has not been found above 0.1% in other citrus lipids but was present in ESG at percentages about equal to those of $C_{13:0}$, $C_{15:0}$, $C_{17:0}$, $C_{23:0}$ and $C_{25:0}$. *Iso* $C_{16:0}$, $C_{18:0}$ and $C_{20:0}$ are at about equal percentages. *Iso* $C_{18:1}$ appeared to be the only *iso*, monounsaturated acid in ESG. As observed from Table 1, the 33 ranges of values were large. The ranges were greatest for the five major acids (C_{16}, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$).

Table 2 lists the relative percentages of the five major ESG acids for the 24 citrus cultivars. The biotype sequence (mandarin, tangor, tangelo, etc.) in which fatty acid patterns are discussed follows a sequence used previously for peel [9] and leaf [10] alkanes. The sequence also is one of generally decreasing mean oleic acid content, as reported earlier for the total lipids [8], TG [12], SE [13] and MGDG [14].

The four mandarins in the first biotype are morphologically alike and, thus, were grouped together. The percentage values of $C_{16:0}$ and $C_{18:3}$ for these four cultivars were not different at the 1% level of significance as determined by ANOVA. The low $C_{18:1}$ and high $C_{18:2}$ contents of Ponkan suggest that this cultivar might be a mandarin hybrid. In support of this suggestion is our observation [10] that the leaf-alkane profile of Empress, a chance seedling of Emperor (identical to Ponkan), was closer to that of a tangor than of a mandarin biotype.

The tangelo biotype consisted of three commercial cultivars—Orlando, Minneola and Seminole. The other tangelo, Page, is a cross between the Minneola tangelo and the Clementine mandarin. Fatty acid profiles of the seed parent, Minneola, and sibling, Page, were similar. Both, however, differed from Clementine and the other tangelos in their $C_{18:1}$ and $C_{18:2}$ relative percentages.

Differences in fatty acid profiles among early-, mid-, and late-season fruit have been observed for citrus lipid classes [11] and for MGDG's [14]. Of the four sweet oranges used in this study, three—Homosassa, Queen and Jaffa—are mid-season maturing while the fourth—Lue Gim Gong—is late maturing. In a preliminary study [11], we examined oranges maturing in the early (4 cvs), middle (1 cv) and late (1 cv) seasons. When the profiles of those six cultivars were re-examined with the four profiles obtained in this study, definite trends were observed, and the trends reflected the season of maturity. The mean $C_{16:0}$ values tended to increase with lateness of maturing season, i.e. $C_{16:0}$ for early < mid < late. Conversely, $C_{16:1}$ and $C_{18:1}$ decreased in the order of early > mid > late. Differences in $C_{18:2}$ and $C_{18:3}$ with respect to the three seasons were less noticeable.

The grapefruit cultivars examined included the Marsh seedless and Duncan, and the pink-fleshed Foster, Thompson and Ruby Red. ANOVA (1% level of significance) showed the two flesh types to differ in their $C_{16:0}$ and $C_{18:2}$ values. Whether this difference is due to a mutant influence or to some other factor, such as maturity (as observed for sweet oranges), was not determined.

Previous studies showed lemons and limes to differ appreciably from other biotypes in fatty acid profiles for MGDG [14], TG [12] and SE [13]. Table 2 reveals that lemons could readily be differentiated by their $C_{16:1}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ contents. Sour limes, on the other hand, could be differentiated by their $C_{18:1}$ and $C_{18:3}$ contents. The high percentages of $C_{18:3}$ in total lipids of lemons and sour limes have been observed previously [8]. Columbia sweet lime profile was quite different from that of sour limes; it showed twice the $C_{18:1}$ level and less than half the $C_{18:3}$ level found in sour limes. These biotypes also differed widely in $C_{18:1}$ and $C_{18:3}$ of their TG [12] and SE [13]. Columbia's profile was similar to the mean profile for the mandarins; thus, the cultivar might have some mandarin background. Rangpur differed from the other biotypes in its very low $C_{16:0}$. Rangpur might be a hybrid of mandarin and sour orange parentage [17].

Statistical analysis of the six biotypes revealed some differences in one or more of the five major fatty acids. Mandarins and tangelos could not be differentiated from each other based on their major fatty acid patterns, but both could be differentiated from the other biotypes. Both mandarins and tangelos had significantly lower percentages of $C_{16:0}$. Previous studies have shown sweet oranges and grapefruit to have similar fatty acid [8] and alkane

[10] profiles. Except for $C_{16:1}$, the ESG fatty acid percentages of oranges and grapefruit were statistically nonsignificant and, therefore, could not be used to adequately differentiate these two biotypes.

Minor fatty acid values may be used to help distinguish the various citrus biotypes. Biotypes which contain some mandarin parentage have relatively more $C_{12:0}$ and $C_{14:0}$ than the other biotypes. The three *iso* acids $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, singly and collectively, were present in higher relative percentages in lemons and limes than in the other cultivars. This has been shown in previous citrus taxonomic studies [8, 12–14] as well.

The fatty acid profiles for esterified sterol glucosides in juice sacs of 24 citrus cultivars were analysed, and most could be grouped according to citrus biotype. These biotypes could, in turn, be arranged sequencially, according to a general decrease or increase in the level of each of the five major acids. This sequence is similar to that used previously for citrus peel [9] and leaf [10] alkanes. The similarity in trends supports the postulate that both fatty acids and long-chain hydrocarbons are synthesized and deposited in citrus in a systematic and highly reproducible manner. Also, some idea of a citrus cultivar's background might be obtained if its fatty acid profile is compared with profiles for citrus fruit of known parentage.

In recent studies conducted at our laboratory [18], we have shown fatty acid profiles of citrus fruit to change during the maturation of the fruit. In this study, all samples were collected when mature from Florida groves (Eureka lemon from California) over a period of 1 year. At present we do not know whether the same profiles would be obtained from these cultivars grown in different years or at other locations around the world, where maturation times differ from those in Florida.

EXPERIMENTAL

Isolation of vesicular lipids. The majority of citrus cultivars studied (20 of 24 cvs) was obtained from Whitmore Foundation Farm (U.S. Horticultural Research Laboratory, Orlando, Fla.). Thompson grapefruit was from Adams Citrus Nursery, Haines City, Fla., while Key and Persian limes were from USDA groves at Homestead, Fla., and Ft. Pierce, Fla., respectively. Eureka lemon was from a local market. All grove samples were collected at the time of their respective peak maturities. Fruits of the 24 cvs were cut in half and the intact juice sacs (vesicles) were carefully separated from core, peel, seeds and carpellary membrane with the aid of a citrus spoon. The samples were freeze-dried to powders and the lipids extracted from these powders as previously described [11]. Each cv sample was run in quadruplicate.

Chromatography and fatty acid methyl ester preparation. Purified vesicular lipids were separated into neutral lipids, glycolipids and polar lipids by silica gel column chromatography [11]. The glycolipid fraction was eluted from the column with 200 ml Me_2CO; then it was streaked onto 0.5 mm thin layers of silica gel G and developed with $CHCl_3$–MeOH (17:3) [11]. The ESG band, visualized with Rhodamine 6G under UV light, was scraped from the plate, eluted with $CHCl_3$–MeOH (1:1), concd to dryness and transmethylated [15] by reaction in a sealed acetylation tube with 4 ml 0.5 N dry HCl in MeOH for 22 hr at 75° [11]. Methyl esters were purified on 0.25 mm layers of silica gel with C_6H_6–hexane (7:4) [11].

Quantitation. Fatty acid methyl esters were analysed on an FID instrument equipped with a glass column, 3.05 m × 0.4 mm, packed with 3% SP-1000 on 100/120 mesh Gas Chrom Q with He at 55 ml/min. The optimum temp. program was 180–204° at 2°/min, then 204–210° at 1°/min, then 210–230° at 20°/min and, finally isothermal at 230° until $C_{26:0}$ was eluted from the column. The fatty acids were identified by their retention times, as compared with those of standard linear and branched-chain fatty acids [11]. The percentages were calculated with the aid of an Autolab System IV computing integrator. The values represent the mean of one or more GLC determinations on each of four separate tissue juice sac extractions. 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%. Analysis of variance (ANOVA) and a Duncan's multiple range test, adapted for unequal sample sizes [16] were run on each CV within a specific biotype and between biotypes.

REFERENCES

1. Scora, R. W., England, A. B. and Bitters, W. P. (1966) *Phytochemistry* **5**, 1139.
2. Albach, R. F. and Redman, G. H. (1969) *Phytochemistry* **8**, 127.
3. Scora, R. W., Esen, A. and Kumamoto, J. (1976) *Euphytica* **25**, 201.
4. Tatum, J. H. and Berry, R. E. (1974) *Proc. Fla. State Hortic. Soc.* **87**, 70.
5. Tatum, J. H., Berry, R. E. and Hearn, C. J. (1977) *Proc. Int. Soc. Citriculture* **2**, 614.
6. Esen, A. and Soost, R. K. (1977) *Proc. Int. Soc. Citriculture* **2**, 616.
7. Button, J., Vardi, A., and Spiegel-Roy, P. (1976) *Theor. Appl. Genet.* **47**, 119.
8. Nordby, H. E. and Nagy, S. (1969) *Phytochemistry* **8**, 2027.
9. Nordby, H. E. and Nagy, S. (1977) *Phytochemistry* **16**, 1393.
10. Nordby, H. E., Nagy, S. and Smoot, J. M. (1979) *J. Am. Soc. Hortic. Soc.* **104**, 3.
11. Nordby, H. E. and Nagy, S. (1971) *Lipids* **6**, 556.
12. Nagy, S. and Nordby, H. E. (1974) *Phytochemistry* **13**, 153.
13. Nordby, H. E. and Nagy, S. (1974) *Phytochemistry* **13**, 443.
14. Nordby, H. E., Nagy, S. and Hall, N. T. (1976) *Phytochemistry* **15**, 957.
15. Sweeley, C. C. and Walker, B. (1964) *Analyt. Chem.* **36**, 1461.
16. Bancroft, T. A. (1968) *Topics in Intermediate Statistical Methods.* Iowa State University Press, Ames.
17. Barrett, H. C. and Rhodes, A. M. (1976) *Syst. Bot.* **1**, 105.
18. Nordby, H. E. and Nagy, S. (1979). *J. Agric. Food Chem.* **27**, 15.