Lipids in Citrus Fruit Juice. 1. Lipid Content during Orange Ripening in Eastern Sicily

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Fatty acid composition of total lipids was determined during ripening and overripening in six widespread orange cultivars in eastern Sicily (Naveline, Tarocco, Moro, Sanguinello, Ovale, Valencia). Palmitic and palmitoleic acids decreased during maturation in the early ripening cultivars while palmitic acid increased in late ripening ones. Linoleic and linolenic acid levels were higher in the overripe than in the ripe stage. This was more common in the colored cultivars where the fruit juices presented unpleasant odors and taste.

Lipid content in citrus fruit influences the juice stability and markedly modifies the taste after pasteurization and storage (Galoppini, 1970; Galoppini and Carcea, 1983). The most common fatty acids present are palmitic, palmitoleic, oleic, linoleic, and linolenic acids, and together these acids form 92–97% of linear- and branched-chain fatty acids. The relationship between these five acids depends on their lipid class and citrus fruit species (Nordby and Nagy, 1971a). Very small amounts of fatty acids with a carbon number below 16 (C_{10}, C_{12}, C_{14}, C_{15}) and above 18 (C_{22}, C_{24}, C_{29}) are present (Nordby and Nagy, 1979). Stearic acid is less than 1%.

The significant difference in the lipid content of the various citrus fruits allows different species to be distinguished, and the fatty acid profile can even indicate the cultivar. For this reason the fatty acid profile has been proposed as the chemotaxonomic marker to determine parentage of hybrids (Nordby and Nagy, 1969, 1971b, 1974; Nagy and Nordby, 1974).

In most citrus juices linoleic is the major acid (21–39% of total content of fatty acids), followed by oleic (6–37%), palmitic (17–25%), linolenic (6–18%), and palmitoleic (1–7%) (Nordby and Nagy, 1969, 1971b).

Fatty acid formation during growth, maturation, and senescence of the fruit has revealed some variations: $C_{16:0}$, $C_{18:2}$, and $C_{18:3}$ decrease while $C_{16:1}$ and $C_{18:1}$ increase probably due to an enzymatic reduction of $C_{18:2}$ and $C_{18:3}$–$C_{18:1}$ during the growth period. At optimum maturation the alterations are slight ($C_{18:2}$ falling to the advantage of $C_{18:3}$); during the senescence the $C_{18:3}$ content varies, falling in some cultivars while rising in others (Nordby and Nagy, 1979). These authors suggest that oranges are capable of synthesizing linolenic acid throughout growth, causing a reduction of $C_{18:1}$ and $C_{18:2}$, as opposed to what occurs in climacteric fruit (Galliard, 1968). Moreover, it seems that during senescence the linolenic acid is converted into ethylene by a reaction catalyzed by lipoxygenase. Galliard et al. (1968) demonstrated that in apple fruit ethylene can be formed from linolenic acid by this mechanism. This conversion has been put forward for citrus fruits, even if Rasmussen (1975) saw an increased ethylene production in oranges during storage and Nagy et al. (1978) correlated it with a percentage decrease in polar lipids throughout the same period. During juice storage the increased production of fatty acids released from lipids by oxidative lysis causes formation of aldehydes, ketones, and acids with a low number of carbon atoms and having off-flavors and off-tastes (Hitchcock and Nichols, 1971; Galliard, 1975; Huskins et al., 1952; Huskins and Swift, 1953a,b). They generally develop during high-temperature storage (30 °C) of juices containing much suspended matter (Curl and Veldhuis, 1947; Swift, 1951).

The aim of this study was to evaluate the lipid variations of widespread oranges in eastern Sicily in relation to the cultivar and the maturation period. Special attention was paid to the senescence period since the processing industries often use fruit that has long passed optimum maturation.

EXPERIMENTAL SECTION

Sample Harvesting. Samples of Naveline, Tarocco nucellare, Moro, Sanguinello comune, Ovale, and Valencia late oranges were harvested from 12 chosen representative plants per cultivar in batches of 5 kg/sample, in farms of eastern Sicily. Samples were harvested every week in relationship to the biological cycle of each cultivar from the unripe to the ripe and overripe fruit.

In order to determine optimum maturation, parameters related to the fruit horticultural characteristics, their internal and external pigmentation, the amount of juice, and mainly the S/A ratio (degrees Brix/acid) (Spina, 1985) were used. Production areas, harvesting period, optimum maturation period, and the total number of samples for each cultivar are reported in Table I. The plant physiology determined the number of samples obtained. Climatic conditions caused an almost complete fall of the Ovale and Valencia late fruits and brought sample harvesting to a halt.

Methods. In each sample weighing approximately 5 kg the mean weight and amount of juice per fruit were calculated. In the juice the degrees Brix, pH, acidity, and S/A ratio were determined. The juice was then freeze-dried and stored at −25 °C.

Total lipid extraction according to Nagy and Nordby's method (1970) was performed on an aliquot of the freeze-dried product. Stearic acid was added as internal standard since it had already been seen that less than 0.6% was present. The sample of crude lipids obtained was purified on a Sephadex column as described by Wuthier (1966). Two different methods for acid esterification were tested: one using boron trifluoride (Nagy and Nordby, 1970) and the other with acetyl chloride–methanol (1:20, v/v) at 50 °C for 1 h (Lillington et al., 1981). Both methods gave the same recoveries; Lillington's method was preferred because BF_3 could cause damage to both glass and column-packing material (Lillington et al., 1981). GLC analyses were performed on a 2 m × 2 mm (i.d.) silanized glass column packed with 10% SP-2330 on Chromosorb WHP (100–120 mesh) by Varian, Model 3700. Working conditions were as follows: injector temperature, 250 °C; detector temperature, 250 °C; column temperature, 180 °C isothermal; carrier nitrogen, 30 mL/min; quantity injected, 1 μL. The gas chromatographic responses were directly integrated by Varian Model 4270, and fatty acids

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Table I. Production Areas, Harvesting Period, Optimum Maturation, and Total Number of Samples for Each Cultivar

cultivar	production area	start of harvesting	end of harvesting	optimum maturation period	no. samples
Naveline (blonde)	Lentini (Siracusa)	Oct 2, 1984	Jan 16, 1985	Oct 30/Nov 6	15
Tarocco nucellare (colored)	Misterbianco (Catania)	Oct 23, 1984	Feb 6, 1985	Nov 27/Dec 4	15
Moro (colored)	Lentini (Siracusa)	Oct 23, 1984	March 6, 1985	Dec 11/Dec 19	19
Sanguinello comune (colored)	Lentini (Siracusa)	Nov 20, 1984	March 13, 1985	Jan 23/Feb 6	16
Ovale (blonde)	Carlentini (Siracusa)	Feb 13, 1985	April 16, 1985	April 10/April 16	10
Valencia late (blonde)	Paternò (Catania)	Feb 13, 1985	May 22, 1985	May 15/May 22	15

Table II. Naveline: S/A Ratio, ppm and Percentage Content of Single Fatty Acids, and ppm of Total Fatty Acids[a]

		$C_{16:0}$		$C_{16:1}$		$C_{18:1}$		$C_{18:2}$		$C_{18:3}$		
sample harvesting	S/A	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%	total, ppm
Oct 2, 1984	5.3	58.3	23.3	14.5	5.8	55.2	22.1	100.5	40.2	21.3	8.5	249.8
Oct 10	5.7	61.0	22.9	15.8	5.9	67.3	25.3	98.4	37.0	23.5	8.9	266.0
Oct 17	7.4	59.9	21.9	15.4	5.6	69.1	25.3	105.6	38.7	22.9	8.4	272.9
Oct 23	7.3	59.3	22.2	15.6	5.8	70.0	26.2	98.7	37.0	23.5	8.8	267.1
Oct 30	8.2	58.1	21.5	15.3	5.7	70.2	26.0	95.2	35.2	31.4	11.6	270.2
Nov 6	9.7	58.0	21.4	15.0	5.5	72.1	26.6	94.3	34.8	31.6	11.7	271.0
Nov 14	9.9	58.1	21.2	14.5	5.3	73.8	26.9	94.9	34.6	32.8	12.0	274.1
Nov 20	10.1	58.2	20.4	14.6	5.1	77.5	27.2	97.2	34.1	37.4	13.1	284.9
Nov 27	13.0	55.1	20.5	13.7	5.1	73.4	27.3	85.2	31.7	41.4	15.4	268.8
Dec 4	12.2	53.7	20.3	11.9	4.5	72.4	27.4	83.4	31.5	43.0	16.3	264.4
Dec 11	16.0	51.8	21.3	11.7	4.8	69.2	28.5	68.1	28.0	42.2	17.4	243.0
Dec 19	16.0	51.3	21.0	11.5	4.7	67.7	27.7	69.3	28.4	44.5	18.2	244.3
Jan 2, 1985	15.3	51.0	21.1	11.4	4.7	65.0	26.9	68.2	28.2	46.2	19.1	241.8
Jan 9	15.0	51.2	21.2	10.3	4.3	64.2	26.6	68.6	28.4	47.2	19.5	241.5
Jan 16	13.8	51.0	21.5	10.4	4.4	60.1	25.3	66.7	28.1	49.2	20.7	237.4

[a] Optimum maturation period: Oct 30–Nov 6.

Table III. Tarocco Nucellare: S/A Ratio, ppm and Percentage Content of Single Fatty Acids, and ppm of Total Fatty Acids[a]

		$C_{16:0}$		$C_{16:1}$		$C_{18:1}$		$C_{18:2}$		$C_{18:3}$		
sample harvesting	S/A	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%	total, ppm
Oct 23, 1984	5.1	62.6	23.1	15.7	5.8	70.1	25.9	101.9	37.6	20.6	7.6	270.9
Oct 30	6.0	61.2	22.7	15.1	5.6	70.0	26.0	103.1	38.3	19.8	7.4	269.2
Nov 6	6.5	59.3	21.8	15.2	5.6	72.9	26.8	106.5	39.1	18.2	6.7	272.1
Nov 14	6.8	60.9	22.3	15.1	5.5	71.3	26.1	107.0	39.1	19.0	7.0	273.3
Nov 20	7.2	66.0	22.2	15.4	5.2	78.4	26.4	115.7	39.0	21.6	7.2	297.1
Nov 27	8.6	64.8	21.7	15.5	5.2	78.3	26.2	117.9	39.4	22.5	7.5	299.0
Dec 4	9.5	67.3	21.2	15.9	5.0	86.9	27.4	121.0	38.1	26.3	8.3	317.4
Dec 11	10.9	65.4	19.0	15.8	4.6	93.2	27.1	140.4	40.8	29.2	8.5	344.0
Dec 19	9.7	70.2	19.9	16.9	4.8	98.3	27.9	135.8	38.5	31.4	8.9	352.6
Jan 2, 1985	11.8	72.3	20.8	16.3	4.7	96.2	27.7	130.6	37.6	32.0	9.2	347.4
Jan 9	10.9	74.5	19.6	17.1	4.5	103.8	27.3	149.0	39.2	35.8	9.4	380.2
Jan 16	12.2	77.8	20.0	16.7	4.3	106.9	27.5	152.0	39.1	35.4	98.1	388.8
Jan 23	13.4	74.3	19.4	16.1	4.2	107.7	28.1	149.4	39.0	35.6	9.3	383.1
Jan 30	11.7	72.9	18.5	15.4	3.9	108.8	27.6	159.3	40.4	37.9	9.6	394.3
Feb 6	11.5	73.3	18.0	17.9	4.4	105.9	26.0	170.8	41.9	39.5	9.7	407.4

[a] Optimum maturation period: Nov 27–Dec 4.

were calculated by the internal standard method. The recovery of each fatty acid analyzed was 95 ± 2%, and detector response was 1.

RESULTS AND DISCUSSION

Tables II–VII report the S/A ratios, ppm and percentage contents of single fatty acids, and their totals expressed in ppm.

A careful study of these tables shows the following:

Naveline. Very early ripening blonde fruit (Hodgson, 1967; Baldini and Scaramuzzi, 1980). Commercial maturation occurred between Oct 30 and Nov 6, 1984, when the S/A ratio was between 8 and 9. In later samples this ratio rose to a maximum value of 16, which fell as the overripening took place.

Percentage contents of fatty acids $C_{16:0}$ and $C_{16:1}$ were 21.5% and 5.7% at maturation, respectively. These levels fell slightly later. $C_{18:1}$ (26% at maturation) increased up to optimum maturation and then presented small variations. At maturation, percentage content of $C_{18:2}$ was 35%, which decreased gradually and was proportionally inverse to $C_{18:3}$ content. Linolenic values in unripe fruit was 8.5%, rising to Ca. 12% at maturation and over 20% at the end of the study. This behavior was also seen in $C_{18:2}$ and $C_{18:3}$ values (expressed in ppm) where the respective levels of 95 and 31 ppm reached 67 and 49 ppm. The total fatty acid ppm increased until immediately after maturation, before falling off gradually up to overripening when the levels were slightly lower than those observed at the start of the study (Table II).

Tarocco Nucellare. Midseason ripening cultivar with blood pigmentation quite widespread (Spina, 1985). Optimum maturation was between Nov 27 and Dec 4, 1984, when the S/A ratio was 8–9. This value rose to 13.4 before falling off.

Palmitic and palmitoleic acids values were 21% and 5%, respectively, at maturation, and then they sloped off throughout the study. Only small variations of oleic acid were observed. However, linoleic and linolenic acids values (at maturation 39% and 7.5%, respectively) increased gradually from start to finish of the study, reaching 42% and 9.7%, respectively; $C_{18:2}$ and $C_{18:3}$ ppm values and total

Table IV. Moro: S/A Ratio, ppm and Percentage Content of Single Fatty Acids, and ppm of Total Fatty Acids[a]

		$C_{16:0}$		$C_{16:1}$		$C_{18:1}$		$C_{18:2}$		$C_{18:3}$		
sample harvesting	S/A	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%	total, ppm
Oct 23, 1984	4.6	53.8	20.9	12.6	4.9	56.3	21.9	112.9	43.9	21.6	8.4	257.2
Oct 30	4.7	55.7	21.4	12.2	4.7	60.1	23.1	110.9	42.6	21.3	8.2	260.2
Nov 6	5.3	54.3	19.9	12.6	4.6	63.0	23.1	119.5	43.8	23.5	8.6	272.9
Nov 14	5.6	54.5	19.3	12.4	4.4	66.7	23.6	122.6	43.4	26.1	9.2	282.3
Nov 20	5.0	54.6	19.9	11.5	4.2	62.8	22.9	118.4	43.2	26.9	9.8	274.2
Nov 27	6.4	57.7	21.2	10.6	3.9	61.0	22.4	114.4	42.0	28.6	10.5	272.4
Dec 4	6.1	57.1	21.2	10.5	3.9	64.4	23.9	107.9	40.1	29.4	10.9	269.3
Dec 11	6.6	60.4	20.3	11.9	4.0	67.0	22.5	121.2	40.7	37.2	12.5	297.7
Dec 19	6.6	58.6	20.1	11.7	4.0	68.2	23.4	114.6	39.3	38.5	13.2	291.6
Jan 2, 1985	6.3	56.3	19.7	10.6	3.7	64.0	22.4	116.9	40.9	38.0	13.3	285.8
Jan 9	6.8	55.5	19.1	11.0	3.8	65.4	22.5	121.0	41.6	37.8	13.0	290.7
Jan 16	7.3	59.3	20.4	11.9	4.1	64.5	22.2	119.0	40.9	35.9	12.4	290.6
Jan 23	7.6	63.3	19.1	12.3	3.7	69.3	20.9	143.7	43.3	43.1	13.0	331.7
Jan 30	6.0	64.1	18.3	11.9	3.4	76.3	21.8	152.6	43.6	45.3	12.9	350.2
Feb 6	7.5	67.1	17.8	12.8	3.4	86.4	22.9	158.1	41.9	52.8	14.0	377.2
Feb 13	7.3	65.0	17.7	12.1	3.3	88.9	24.2	147.2	40.1	54.0	14.7	367.2
Feb 20	7.8	60.8	17.4	12.0	3.4	85.3	24.4	136.3	39.0	55.2	15.8	349.6
Feb 27	7.7	62.7	17.9	11.5	3.3	82.6	23.6	140.0	40.0	53.2	15.2	350.0
March 6	8.4	72.0	18.7	12.3	3.2	85.8	22.3	152.8	39.7	61.9	16.1	384.8

[a] Optimum maturation period: Dec 11–19.

Table V. Sanguinello Comune: S/A Ratio, ppm and Percentage Content of Single Fatty Acids, and ppm of Total Fatty Acids[a]

		$C_{16:0}$		$C_{16:1}$		$C_{18:1}$		$C_{18:2}$		$C_{18:3}$		
sample harvesting	S/A	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%	total, ppm
Nov 20, 1984	5.3	58.7	27.4	12.0	5.6	51.2	23.9	74.4	34.7	18.0	8.4	214.3
Nov 27	6.5	56.5	25.9	11.3	5.2	52.6	24.1	80.5	36.9	17.2	7.9	218.1
Dec 4	6.1	62.4	19.6	11.8	3.7	75.7	23.8	137.2	43.1	31.2	9.8	318.3
Dec 11	5.9	65.2	20.9	10.9	3.5	72.0	23.1	133.9	42.9	30.0	9.6	312.0
Dec 19	6.7	62.7	19.3	11.4	3.5	74.5	22.9	139.8	43.0	36.7	11.3	325.1
Jan 2, 1985	7.3	67.3	18.9	11.8	3.3	82.7	23.2	153.2	43.0	41.3	11.6	356.3
Jan 9	5.7	71.6	18.5	11.6	3.0	90.2	23.3	166.8	43.1	46.8	12.1	387.0
Jan 16	7.8	70.2	18.8	11.9	3.2	82.9	22.2	163.1	43.7	45.2	12.1	373.3
Jan 23	7.9	68.8	19.1	11.6	3.2	74.9	20.8	160.6	44.6	44.3	12.3	360.2
Jan 30	7.1	68.0	18.7	10.6	2.9	85.1	23.4	155.7	42.8	44.3	12.2	363.7
Feb 6	8.9	63.5	18.1	10.2	2.9	82.8	23.6	147.7	42.1	46.7	13.3	350.9
Feb 13	7.5	64.9	17.8	11.3	3.1	85.3	23.4	147.6	40.5	55.4	15.2	364.5
Feb 20	8.4	59.5	17.6	10.2	3.0	81.4	24.1	138.5	41.0	48.3	14.3	337.9
Feb 27	7.6	58.2	17.9	10.4	3.2	80.1	24.6	131.1	40.3	45.6	14.0	325.4
March 6	8.6	63.7	18.7	11.9	3.5	75.2	22.1	132.4	38.9	57.2	16.8	340.4
March 13	8.7	58.1	21.2	9.6	3.5	57.0	20.8	103.1	37.6	46.4	16.9	274.2

[a] Optimum maturation period: Jan 23–Feb 6.

Table VI. Ovale: S/A Ratio, ppm and Percentage Content of Single Fatty Acids, and ppm of Total Fatty Acids[a]

		$C_{16:0}$		$C_{16:1}$		$C_{18:1}$		$C_{18:2}$		$C_{18:3}$		
sample harvesting	S/A	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%	total, ppm
Feb 13, 1985	7.4	66.8	18.6	15.4	4.3	76.2	21.2	148.0	41.2	52.8	14.7	359.2
Feb 20	6.6	78.4	19.0	15.7	3.8	82.5	20.0	181.1	43.9	54.9	13.3	412.6
Feb 27	6.8	77.8	19.3	15.4	3.8	78.2	19.4	179.4	44.5	52.4	13.0	403.2
March 6	6.5	78.0	19.2	15.9	3.9	79.2	19.5	179.6	44.2	53.7	13.2	406.4
March 13	7.4	84.6	19.4	16.1	3.7	81.9	18.8	200.9	46.1	52.3	12.0	435.8
March 20	8.4	83.9	20.5	15.2	3.7	77.0	18.8	184.4	45.0	49.2	12.0	409.7
March 27	7.6	81.9	20.8	14.6	3.7	73.6	18.7	174.5	44.3	49.2	12.5	393.8
April 3	9.2	78.2	19.9	14.1	3.6	73.5	18.7	174.5	44.4	52.7	13.4	393.0
April 10	10.0	76.3	19.9	14.6	3.8	70.9	18.5	168.7	44.0	52.9	13.8	383.4
April 16	10.2	62.6	22.1	10.8	3.8	54.9	19.4	120.4	42.5	34.6	12.2	283.3

[a] Optimum maturation period: April 10–16.

fatty acids rose constantly (Table III).

Moro. Midseason ripening cultivar deeply pigmented very widespread in Sicily, especially in the provinces of Catania and Siracusa (Hodgson, 1967; Baldini and Scaramuzzi, 1980). It reached commercial maturation at the beginning of December with an S/A ratio of about 6, which increased up to the last sample. The samples were harvested up to March 1985 when the fruit was decidedly overripe and contained bad-smelling juice.

$C_{16:0}$ and $C_{18:1}$ values were almost stationary, whereas $C_{16:1}$ presented percentage decreases. There was also a slight fall in $C_{18:2}$, especially during the last weeks of the study, while $C_{18:3}$ increased constantly. The latter rose from initial values of 8–12% at maturation and 16% later. $C_{18:2}$ and $C_{18:3}$ ppm values rose throughout the study especially during the overripening period. Total fatty acids increased uniformly during the whole study (Table IV).

Sanguinello Comune. Midseason–late ripening cultivar, depending on growing area. It is the latest ripening variety of the colored cultivars (Baldini and Scaramuzzi, 1980). Commercial ripening occurred between Jan 23 and Feb 6, 1985 with an S/A ratio of about 8, which rose constantly throughout the study.

Palmitic and palmitoleic percentages decreased gradu-

Table VII. Valencia Late: S/A Ratio, ppm and Percentage Content of Single Fatty Acids, and ppm of Total Fatty Acids[a]

		$C_{16:0}$		$C_{16:1}$		$C_{18:1}$		$C_{18:2}$		$C_{18:3}$		
sample harvesting	S/A	ppm	%	ppm	%	ppm	%	ppm	%	ppm	%	total, ppm
Feb 13, 1985	4.9	70.0	20.0	16.8	4.8	68.2	19.5	158.8	45.4	36.0	10.3	349.8
Feb 20	6.0	67.8	20.6	14.5	4.4	65.5	19.9	146.5	44.5	35.0	10.6	329.3
Feb 27	5.3	66.0	21.0	14.7	4.7	61.6	19.6	141.1	44.9	30.8	9.8	314.2
March 6	6.3	68.6	21.0	15.0	4.6	63.3	19.4	142.4	43.6	37.2	11.4	326.5
March 13	6.2	66.8	21.0	14.6	4.6	63.0	19.8	139.0	43.7	34.7	10.9	318.1
March 20	6.4	63.6	21.1	13.6	4.5	54.6	18.1	136.1	45.1	33.8	11.2	301.7
March 27	7.4	62.7	22.4	12.9	4.6	53.8	19.2	120.5	43.0	30.3	10.8	280.2
April 3	7.6	57.3	22.0	11.7	4.5	49.4	19.0	113.5	43.6	28.4	10.9	260.3
April 10	8.4	59.2	21.2	12.9	4.6	53.7	19.2	120.7	43.2	33.0	11.8	279.5
April 16	8.8	54.3	21.2	12.5	4.9	54.5	21.3	101.8	39.8	32.8	12.8	255.9
April 23	8.8	51.7	21.5	11.5	4.8	51.0	21.2	94.7	39.4	31.5	13.1	240.4
May 2	9.6	45.7	21.4	10.2	4.8	46.0	21.5	82.7	38.7	29.1	13.6	213.7
May 8	9.7	43.9	21.7	9.9	4.9	42.6	21.1	79.6	39.4	26.1	12.9	202.1
May 15	10.1	46.6	21.9	10.2	4.8	46.4	21.8	79.6	37.4	30.1	14.1	212.9
May 22	11.9	49.3	23.4	10.8	5.1	43.7	20.7	76.8	36.4	30.4	14.4	211.0

[a] Optimum maturation period: May 15–22.

ally but then augmented slightly in the last samples. Oleic acid remained almost stationary with only small fluctuations. Linoleic acid increased up to a maximum about 45% and then fell before stabilizing itself above initial values. Linolenic acid followed its normal behavior and presented double percentage values and almost tripled ppm values at the end of the study. In the first 3 weeks of the trial there was a marked increase in the total fatty acids. In the overripening period these values reduced gradually and then abruptly (Table V).

Ovale. Late-ripening blonde cultivar known as "Calabrese", which is very widespread in southern Italy (Baldini and Scaramuzzi, 1980; Spina, 1985). Sampling went on until April 16, 1985, this being its normal maturation period with an S/A ratio of around 10. Owing to the complete fall of fruits, we were not able to observe the overripening period. During maturation we saw a slight increase of palmitic acid and a fall in palmitoleic and oleic acid values. Linoleic acid did not present any significant variations. There was a slight fall in percentage and absolute values of linolenic acid. After an initial increase, the total fatty acids decreased constantly (Table VI).

Valencia Late. Very late ripening blonde cultivar. Today it is the most widespread in the world and will likely oust the Ovale in Sicily (Baldini and Scaramuzzi, 1980). It reached its commercial maturation in the middle of May 1985 with an S/A ratio above 10. For complete fruit fall we were unable to study its overripening period. Palmitic acid percentage values increased slightly to maturation, while the palmitoleic and oleic percentage values were stationary. Linoleic acid levels decreased constantly whereas linolenic acid increased from 10 to 14%. The down-sloping linoleic acid curve was also evident in the ppm values, whereas the modest rise in percentage linolenic acid values was not mirrored in the absolute values, which presented a slight decrease. Total fatty acids fell constantly from an initial value of 350 to approximately 200 ppm (Table VII).

Concluding Remarks. Our results showed that there was a slight progressive reduction in percentage values of fatty acids $C_{16:0}$ and $C_{16:1}$ in early-ripening and midripening but not in late-ripening cultivars. We also observed that palmitic acid tended to increase in late-ripening cultivar. The quantity of oleic acid varied very little throughout maturation in all the cultivars studied.

The variations between $C_{18:2}$ and $C_{18:3}$ in Naveline were also observed in the other blonde cultivars even if the differences were not so evident. However in the colored oranges there was small reduction in $C_{18:2}$ percentage values. In Sanguinello comune there was a very slight fall in ppm values of this acid while it rose markedly in Tarocco nucellare and Moro cultivars. Linolenic acid increased about 40% in the overripening period of almost all the cultivars studied, being generally higher in colored ones. In the Ovale there was a slight reduction in percentage and absolute values of linoleic and linolenic acids during ripening. This behavior was also reported by Nordby and Nagy, (1979) in Hamlin and Pineapple oranges.

The total fatty acid content rose during maturation and overripening in the Tarocco and Moro, increased up to maturation, and then fell off in the Sanguinello and Naveline while it fell constantly in the two late-ripening blonde cultivars Ovale and Valencia late.

The results obtained from this study indicate that the reduced quantity of linoleic and linolenic acids and the low content of total fatty acids in the blonde cultivars make them more suitable for juice extraction purposes as compared with colored cultivars. Moreover, the reduced amount of total fatty acids in Sicilian oranges is a favorable indication for their use in industrial process.

Today modern technology used to stabilize the juice extracted (low-temperature storage and enzymatic activity control) slows down the fatty acid degeneration processes that cause the formation of the described lipid fragments.

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Quantitative Survey of Narirutin, Naringin, Hesperidin, and Neohesperidin in *Citrus*

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Narirutin, naringin, hesperidin, and neohesperidin concentrations in juices from 52 citrus cultivars were determined using HPLC. Neither naringin nor neohesperidin was detected in sweet orange (*Citrus sinensis*), tangerine (*Citrus reticulata*), tangerine-like hybrids, or most tangelos. Grapefruit (*Citrus paradisi*), sour orange (*Citrus aurantium*), and K-Early (a grapefruit-like tangelo) juices all contain similar amounts of naringin and widely varying amounts of neohesperidin. Therefore, naringin cannot be used as the sole indicator for the presence of grapefruit juice in orange juice. However, neohesperidin concentrations can be used to differentiate grapefruit, sour orange, and K-Early juices. Naringin concentrations in these cultivars ranged from 100 to 800, 150 to 350, and 150 to 200 ppm, respectively, whereas, neohesperidin concentrations ranged from 4 to 10, 100 to 200, and 600 to 950 ppm, respectively.

Although flavonoids are ubiquitous in the plant kingdom, there are several flavanone glycosides unique to *Citrus* and specific citrus cultivars. Swingle (1943) suggested that these glycosidic compounds might be useful taxonomic markers. In an early citrus chemotaxonomic study, Albach and Redman (1969) studied the flavanone glycoside content in whole fruit of 41 citrus cultivars representing 18 recognized citrus species and 49 hybrids. Subsequent investigators (Tatum et al., 1974; Kamiya et al., 1979; Anis and Aminuddin, 1981) used flavonoid patterns in leaves and fruit, as determined by TLC, to study citrus taxonomy. There is general agreement as to the major flavanones in most citrus cultivars, but perhaps due to the subjective estimation of the intensity of TLC spots, there is considerable disagreement as to the presence of minor flavanones in several citrus cultivars. Little, if any, flavanone chemotaxonomic work has been reported on citrus juices.

Historically, orange juice (*Citrus sinensis*) has commanded a higher price than grapefruit juice (*Citrus paradisi*). There is the temptation for those who supply and market citrus juices to add the lower priced juice, i.e., grapefruit juice, to the more expensive juice for financial gain. However, this would violate orange juice standards of identity in most countries. To guard against the addition of grapefruit juice to orange juice, Greiner and Wallrauch (1984) proposed using the presence of naringin to indicate the addition of grapefruit juice. Using HPLC, they analyzed over 50 orange juice samples, three Murcott juices (an orange–tangerine hybrid), and eight tangerine juices without finding naringin (detection limit was 3 ppm). This confirmed the TLC work of Horowitz (1961) and Albach and Redman (1969) but conflicted with the work of Drawert et al. (1980) who reported between 30 and 40 ppm naringin in commercial orange juice.

The purpose of this study was to use the sensitivity and resolving power of HPLC to determine the concentrations of the major citrus flavanone glycosides, narirutin, naringin, hesperidin, and neohesperidin, in a systematic study of the major citrus cultivars produced in Florida. From such a survey, it would be possible to determine whether naringin is a natural component in any commercially significant orange cultivar. The survey will also determine whether cultivars added into orange juice under U.S. FDA Standards of Identity (USFDA, 1984) contain naringin, or other neohesperidosides. A final goal of the survey is to quantify the flavanone glycoside concentration levels in order to establish a data base from which it might be possible to differentiate various naringin-containing juices based upon these concentration profiles.

MATERIALS AND METHODS

Equipment. The high-performance liquid chromatographic system consisted of a Waters (Milford, MA) Model M-6000A pump, a Model 710B WISP autosampler, and a Model 440 fixed-wavelength UV–vis detector. The column effluent was monitored at 280 nm. Chromatographic peaks were integrated by a Spectra-Physics (San Jose, CA) Model 4000 recording integrator.

Chromatography. A Du Pont (Wilmington, DE) Zorbax ODS (C-18) column 25 cm × 4.6 mm (i.d.) was used with a Brownlee (Santa Clara, CA) spheri-5 C-18 preco-

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