Phlorin Screening in Various Citrus Species and Varieties

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Phlorin (3,5-dihydroxyphenyl β-D-glucopyranoside), an orange peel marker, has been searched in 45 species and varieties of *Citrus*. The phlorin content was determined by high-pressure liquid chromatography in juices and aqueous peel extracts of these different fruits. The phlorin content in *C. reticulata* peel extract varies from 0 to 1012 mg L^{-1} with a mean of 162 mg L^{-1}. On the contrary, phlorin was not found in mandarin and clementine juices except for mandarin "Commune" and "Beauty" (33 and 30 mg L^{-1}, respectively). In the 14 species of oranges and varieties, phlorin was detected in juices and peel extracts with a mean of 22 and 492 mg L^{-1}, respectively, while for grapefruits, means were 108 mg L^{-1} in juices and 982 mg L^{-1} for peel extracts. Tangors and tangelos which are hybrids (*C. reticulata* x *C. sinensis* and *C. reticulata* x *C. paradisi*, respectively) are characterized by the systematic presence of phlorin in peels (mean: 406 and 659 mg L^{-1}, respectively) while in juices its presence could be variable (0−73 mg L^{-1}). These heterogeneity and values may be explained by the genetic variability of these hybrids and the phlorin content of their parentage group.

Keywords: *Phlorin; peel marker; 3,5-dihydroxyphenyl-β-D-glucopyranoside (R.N. 28217-60-9); citrus fruit species and varieties; juice; HPLC*

INTRODUCTION

Continuing our researches on authentication of orange juices, we have investigated the content determination of 3,5-dihydroxyphenyl-β-D-glucopyranoside (phlorin) in various *Citrus* species and varieties. It has been already shown that phlorin is present in orange peel (Cancalon, 1995; Johnson et al., 1995; Hammond et al., 1996). This molecule is a natural peel marker highly concentrated in albedo and can be extracted with water (Louche et al., 1998). After this molecule isolation, we have determined its content in Valencia and Navel Late orange juices and peels. It has been shown that this molecule is present in oranges, and a few qualitative works have been done on other *Citrus* juices. Horowitz et al. (1961) have isolated phlorin from grapefruit (*C. paradisi*) and oranges (*C. sinensis*) and have quantitatively shown, by chromatographic methods, its presence in lemons (*C. limon*). As in food industries, mixtures of orange and other citrus juices can be found, and it is of importance to determine the phlorin content in various juices and peel extracts of *Citrus* fruits having industrial importance, for quality control. In this work, we researched the range of phlorin content in juices and peels for 45 species and varieties according to fruit maturity state.

MATERIALS AND METHODS

Materials. One to two kilograms of citrus fruit was collected on mature trees growing in the citrus germplasm of the Experimental Domain of San Giuliano, Agronomic Research Station (Corsica Island, France) during October 1998 and March and May 1999. All trees were healthy and grafted on different rootstocks depending to their genetic compatibility. The easy peelers citrus group and sweet oranges were grafted generally on *Poncirus trifoliata* (L.) Raf. Pomeroy or on citranges Carrizo and Troyer which are hybrids between *Poncirus trifoliata* (L.) Raf. and *Citrus sinensis* (L.) Osb. The acid citrus fruit group (lemon, lime, citron, and their hybrids) were grafted on *C. limonia* Osb. The 45 *Citrus* varieties are given in Table 1. The SRA number identifies the variety on the germplasm field and certifies the good controlled sanitary status of these trees.

Maturity Control of Fruits. The ratio *r*, Brix corrected from acidity divided by the percentage of acidity expressed versus anhydrous citric acid, was used to determine fruit maturity. Therefore, the sugar determination was achieved using the normative method given by the Fédération Internationale des Producteurs de Jus de Fruits (1991a). Each species and variety of fruit was cut in two pieces. Then, each part was hand-squeezed carefully with an electric juice squeezer avoiding contact with albedo without using strong pressure. The juice is therefore not completely extracted. The total soluble solid content as °Brix was measured with an RFM-91 refractometer (Bellingham and Stanley Ltd., England) for raw juices. Then, the titratable acidity at pH 8.1 was determined (Fédération Internationale des Producteurs de Jus de Fruits, 1991b). We attributed the letter I for immature fruit, M for mature, VM for very mature, and OM for overmature. The raw juices were immediately filtered through a sieve (1.25 mm, Prolabo, France) and analyzed by HPLC.

Assessment of Phlorin Content in Peels of the Different *Citrus* Species and Varieties. To compare the phlorin content in peels of the different *Citrus* species and varieties, we systematically adopted the same protocol: the whole peels (flavedo + albedo without segment membranes or central core, 100−900 g) were cut in small pieces (0.5 × 0.5 cm) and extracted with exactly 2-fold weight water (200−1800 g, respectively) at 40 °C for 2 days. The aqueous parts were

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Table 1. List of Species and Varieties of *Citrus* Collected on the Experimental Domain of the Agronomic Research Station of Corsica

sample no.	cultivar names	SRA[a]	rootstock[b]	genetic origin	Tanaka[c]
			Mandarin Group		
1	Nova	158	Pomeroy		*C. reticulata* Blanco
2	Cravo	434	Pomeroy		*C. reticulata* Blanco
3	Oneco	429	Pomeroy		*C. reticulata* Blanco
4	Willow Leaf	113	Pomeroy		*C. deliciosa* Ten.
5	Natal Tightskin	481	Pomeroy		*C. deliciosa* Ten.
6	Beauty of Glen Retreat	261	Carrizo		*C. tangerina* Hort. ex Tan.
7	King of Siam	166	Carrizo		*C. nobilis* Lour.
8	Satsuma Okitsu	445	Troyer		*C. unshiu* (Mak.) Marc.
9	Sunki				*C. sunki* Hort. Ex Tan.
10	Cleopatre				*C. reshni* Hort. Ex Tan.
			Clementine Group (mandarin x sweet orange)		
11	Commune Corse	85	Pomeroy		*C. tangerina* Hort. ex Tan.
12	Nules	389	Carrizo		*C. tangerina* Hort. ex Tan.
			Tangor Group (Hybrid) (mandarin x sweet orange)		
13	Bergamota	164	Troyer	unknown parentage	*C. reticulata* Blanco x *C. sinensis* L. Osb
14	Ortanique	110	Pomeroy	unknown parentage	*C. reticulata* Blanco x *C. sinensis* L. Osb
15	Murcott	181	Troyer	unknown parentage	*C. reticulata* Blanco x *C. sinensis* L. Osb
16	Ellendale	592	Carrizo	unknown parentage	*C. reticulata* Blanco x *C. sinensis* L. Osb
			Tangelo Group (Hybrid) (mandarin x grapefruit)		
17	Orlando	46	Pomeroy	Dancy x Duncan	*C. tangerina Hort. ex Tan.* x *C. paradisi* Macf
18	Pearl	453	Pomeroy	Willow Leaf x Imperial	*C. deliciosa Ten.* x *C. paradisi* Macf
19	Pearl	454	Pomeroy	Willow Leaf x Imperial	*C. deliciosa Ten.* x *C. paradisi* Macf
20	Sampson	456	Pomeroy	Dancy x ?	*C. tangerina Hort. ex Tan.* x *C. paradisi* Macf
			Grapefruit Group		
21	Marsh	188	Troyer		C. paradisi Macf.
22	Star Ruby	293	Carrizo		C. paradisi Macf.
23	Oroblanco	603	Carrizo	(grapefruit x pummelo)	*C. paradisi* Macf.
			Orange Group		
24	Shamouti	299	Carrizo		*C. sinensis* (L.) Osb.
25	Navelate	308	Carrizo		*C. sinensis* (L.) Osb.
26	Navelina	306	Carrizo		*C. sinensis* (L.) Osb.
27	Hamlin	251	Pomeroy		*C. sinensis* (L.) Osb.
28	Maltaise Blonde	239	Pomeroy		*C. sinensis* (L.) Osb.
29	Washington Navel	203	Pomeroy		*C. sinensis* (L.) Osb.
30	Pera	399	Pomeroy		*C. sinensis* (L.) Osb.
31	Natal	398	Pomeroy		*C. sinensis* (L.) Osb.
34	Rosa	401	Pomeroy		*C. sinensis* (L.) Osb.
33	Salustiana	485	Carrizo		*C. sinensis* (L.) Osb.
34	Ruby	402	Pomeroy		*C. sinensis* (L.) Osb.
35	Baianinha	513	Pomeroy		*C. sinensis* (L.) Osb.
36	Itaborai	516	Pomeroy		*C. sinensis* (L.) Osb.
37	Yoshida navel	558	Carrizo		*C. sinensis* (L.) Osb.
			Lemon		
38	Frost Eureka	4	Volkamer		*C. limon* (L.) Burm.
			Pummelo		
39	Pink	322	Carrizo		*C. maxima* (Burm.) Merr.
			Lime		
40	IAC 1	617	Volkamer		*C. latifolia* Tan.
			Citron		
41	de Corse	613	Volkamer		*C. medica* L.
			Sour Orange		
42	Maroc		NG		*C. aurantium* L.
			Lemon Natural Hybrids		
43	Castagnaro Bergamot	612	Volkamer	(lemon x sour orange) ?c	*C. bergamia* Risso &Poit.
44	Rough lemon		NG	unknown parentage	*C. jambhiri* Lush
45	Rangpur lime		NG	unknown parentage	*C. limonia* Osb.

[a] SRA: Agronomical Research Station number. [b] Varieties are grafted on different rootstocks; NG: nongrafted; trifoliate orange Pomeroy, Carrizo citrange, Troyer citrange, Volkamer lemon. [c] According to *Citrus of the World. A Citrus Directory*, 1997. [d] According to Luro et al., 1998.

separated from raw material by centrifugation (1500 rpm) and the surnatant filtered on a 0.45 μm micropore (Sartorius) before HPLC analyses for phlorin contents.

High-Performance Liquid Chromatography Determinations. A Beckman system Gold liquid chromatograph equipped with a diode array detector (512 diodes) was used with a supelcosil LC-ABZ analytical column (Supelco; 250 × 4.6 mm, 5 μm). The eluting solvent was a 25 mmol L^{-1} potassium dihydrogen phosphate (Sigma) solution adjusted at pH 2 with 85% orthophosphoric acid (Carlo Erba). A flow rate of 1 mL min^{-1} at 25 °C was used; the UV detector was scanned at 214 nm and the diode array was scanned from 190 to 310 nm. Ascorbic acid (vitamin C, Fluka), citric acid (Fluka), phlorin (isolated according to Louche et al., 1998), and phloroglucinol (1,3,5-trihydroxybenzene, Fluka) have a retention time of 4.1, 6.3, 10.1, and 14.6 min, respectively. Phlorin was used as standard; ascorbic and citric acids and phloroglucinol were used as internal standards for peak position only. Before analysis, samples were filtered on 0.45 μm micropore (Sartorius) and injected into the column with a 10 μL loop for phlorin determination.

Statistical Study. A statistical study on one orange variety ("Rosa") was investigated to follow the reproducibility and the influence of the cardinal point position on the phlorin determination. Six fruits for each cardinal point were collected in October 1998 and analyzed.

Table 2. Statistical Study on the Phlorin Content in Orange Rosa Juices and Peel Extracts

	juice				peel			
cardinal point	min[a] mg L^{-1}	max[a] mg L^{-1}	mean[b] mg L^{-1}	c.v.	min[a] mg L^{-1}	max[a] mg L^{-1}	mean[b] mg L^{-1}	c.v.
South	8	17	11	3	406	807	553	159
West	6	14	11	4	406	689	562	98
East	6	13	10	2	408	722	561	128
North	6	13	9	3	464	728	632	77
General	6	17	10	3	406	807	577	116

[a] HPLC 214 nm. [b] Mean of six determinations from six different fruits collected on the same tree.

RESULTS AND DISCUSSION

Citrus Species and Varieties Investigated. The list of the 45 samples collected on the Experimental Domain of Corsica island (EDC) is presented in Table 1. According to the taxonomical position and the Citrus phylogenetic relationships, these samples have been grouped in three essential categories: (1) mandarin, sweet orange, clementine, sour orange, and tangor which is a hybrid between mandarin and sweet orange; (2) grapefruit, pummelo; (3) lemon, lime, citron, and lemon hybrids. The tangelo group, which is hybrid between mandarin and grapefruit, has an intermediate position and the fruit characters are close to either mandarin fruit or grapefruit fruit, depending on the tangor considered. The botanical Latin known name of cultivars has been given using the Tanaka classification (Cottin,1997; Saunt, 1990). These species and varieties were chosen among the 700 species and varieties of the EDC according to their industrial food importance and

Table 3. Phlorin Content in Various Species and Varieties in Fruit Juices

	period								
	October			March			May		
sample name	state of mat[a,b]	°Brix[c]	phlorin[d] (mg L^{-1})	state of mat[a,b]	°Brix[c]	phlorin[d] (mg L^{-1})	state of mat[a,b]	°Brix[c]	phlorin[d] (mg L^{-1})
mandarin group									
Nova	I	10.2	traces	VM	13.0	n.d.[e]	n.f.[f]	n.f.[f]	n.f.[f]
Cravo	I	7.9	n.d.[e]	VM	10.5	n.d.[e]	OM	9.0	n.d.[e]
Oneco	I	7.5	n.d.[e]	M	10.3	n.d.[e]	OM	11.0	n.d.[e]
Willow Leaf	I	10.0	18	VM	13.4	33	OM	10.5	111
Natal Tightskin	I	8.2	n.d.[e]	M	10.2	n.d.[e]	n.f.[f]	n.f.[f]	n.f.[f]
Beauty of Glen Retreat	I	9.0	7	VM	10.9	30	n.f.[f]	n.f.[f]	n.f.[f]
King of Siam	I	8.6	n.d.[e]	M	12.1	n.d.[e]	VM	10.6	n.d.[e]
Satsuma Okitsu	M	8.0	n.d.[e]	OM	11.1	n.d.[e]	n.f.[f]	n.f.[f]	n.f.[f]
Sunki	I	8.1	traces	M	8.5	n.d.[e]	VM	7.8	n.d.[e]
Cleopatre	I	10.1	n.d.[e]	M	10.5	n.d.[e]	OM	n.j.[g]	n.j.[g]
clementine group									
Commune Corse	M	9.1	n.d.[e]	OM	13.6	n.d.[e]	n.f.[f]	n.f.[f]	n.f.[f]
Nules	M	10.6	n.d.[e]	OM	13.3	n.d.[e]	n.f.[f]	n.f.[f]	n.f.[f]
tangor group									
Bergamota	I	9.2	n.d.[e]	M	10.8	15	OM	n.j.[g]	n.j.[g]
Ortanique	I	8.5	11	M	11.7	19	OM	11.9	15
Murcott	I	8.6	87	I	13.3	63	M	14.5	24
Ellendale	I	9.1	traces	M	16.1	n.d.[e]	VM	13.3	n.d.[e]
tangelo group									
Orlando	I	9.2	n.d.[e]	VM	11.1	n.d.[e]	OM	9.7	n.d.[e]
Pearl (SRA 453)	I	9.8	12	M	12.5	19	OM	12.4	15
Pearl (SRA 454)	I	9.8	50	M	11.5	73	OM	11.9	65
Sampson	I	8.9	70	M	12.7	40	VM	12.6	35
grapefruit group									
Marsh	I	9.9	49	I	10.3	54	M	10.5	34
Star Ruby	I	10.2	60	I	11.5	50	M	10.8	30
Oroblanco	I	11.2	166	M	12.3	108	VM	11.1	75
orange group									
Shamouti	I	9.5	12	M	10.3	16	VM	10.6	30
Navelate	I	9.6	24	M	12.2	19	VM	13.2	13
Navelina	I	9.2	23	VM	12.9	28	n.f.[f]	n.f.[f]	n.f.[f]
Hamlin	I	8.1	27	M	10.7	13	VM	11.0	22
Maltaise Blonde	I	9.0	14	M	9.7	34	VM	9.7	19
Washington Navel	I	9.2	9	VM	11.2	14	OM	11.3	17
Pera	I	8.8	10	I	9.3	35	M	9.1	30
Natal	I	8.8	n.d.[e]	I	10.6	17	M	11.0	11
Rosa	I	9.7	8	M	11.0	37	VM	11.0	23
Salustiana	I	9.3	27	VM	11.8	19	OM	13.0	12
Ruby	I	9.5	29	M	12.2	18	VM	12.2	21
Baianinha	I	9.6	20	M	12.0	17	OM	12.1	21
Itaborai	I	8.6	23	M	10.5	28	VM	9.6	25
Yoshida navel	I	8.4	27	VM	9.7	27	OM	11.5	18
lemon									
Frost Eureka	I	7.3	73	M	7.3	43	VM	6.4	25
pummelo									
Pink	I	9.2	235	I	11.2	175	M	8.0	91
lime									
IAC 1	n.c.[h]	8.1	26	n.c.[h]	7.4	28	n.c.[h]	6.7	29
lemon hybrids									
Castagnaro Bergamot	I	7.2	114	M	6.8	52	VM	6.3	54
Rough lemon	I	8.8	30	M	9.0	26	VM	9.2	18
Rangpur lime	I	8.8	16	M	8.5	34	VM	7.3	21
sour orange									
Maroc	I	11.4	105	M	10.5	213	VM	9.3	146

[a] State of mat: state of maturity. [b] Maturity state: I for immature; M for mature; VM for very mature; OM for overmature (dry fruits). [c] See Materials and Methods for sugar determination. [d] HPLC at 214 nm. [e] Not detected. [f] No fruit. [g] No juice. [h] No criteria because fruits became from different seasons of flowering time.

use. Forty varieties were considered to be important in citrus fruit production and the five others were used only as rootstock in different part of the world (Maroc sour orange, Rangpur lime, Rough lemon, Cleopatre mandarin, and Sunki mandarin).

Fruits were harvested during the entire season to follow the effect of maturity on phlorin content. Fruit maturity was determined using three factors: fruit color, size, and sugar ratio *r* value. So, the first collect began in October when the most important parts of the fruits were immature, then in March when almost all species and varieties were mature, and finally in May for the latest ones, like oranges Natal and Pera, Murcott tangor, and Frost Eureka lemon. The state of maturity is given in Table 3. For the October collect, 42/45 samples were immature (Satsuma mandarin and the two clones of clementines have given mature fruits in October); then for the March collect all fruits were mature or just mature while for the last collect, all fruits were mature, very mature, or overmature.

Vitamin C, Citric Acid, and Phloroglucinol Detections. In our preceding paper (Louche et al., 1998), vitamin C and a compound noted unknown (now clearly identified as citric acid) were presented on chromatograms. They were used as internal standard for peak position only in our HPLC analyses. Since phlorin hydrolysis during maceration or maturity may be considered, phloroglucinol (1,3,5-trihydroxybenzene) detection was investigated. As previously observed, this compound was not detected.

Statistical Study. The reproducibility and the influence of the cardinal point of collection of fruits was investigated to follow the range of phlorin content in juices and peel extracts. Results are given in Table 2 for the orange Rosa variety. As shown in this table, no influence of the cardinal point was observed either in juice (10 ± 3 mg L^{-1}) or in peels (577 ± 116 mg L^{-1}).

Phlorin Content in Fruit Juices. The content of 3,5-dihydroxyphenyl-β-D-glucopyranoside of different species and varieties in juices is presented in Table 3. For the mandarin group, we can see that only Willow Leaf mandarin and Beauty of Glen Retreat mandarin, present a peak of phlorin (see Figure 1) during the period collect. The increased value detected in May for Willow Leaf mandarin can be explained by the particularly low percentage of juice in the fruit at this time and consequently an increase of the metabolic concentrations. For the other mandarin varieties, rootstocks, and cultivars, no phlorin was detected. For tangor and tangelo groups, the behavior of phlorin is different from one variety to another. In Ortanique tangor and Pearl tangelo (SRA 453) varieties, the phlorin content is almost similar. For Bergamota tangor and Orlando tangelo, no phlorin was detected. For Murcott tangor and Sampson tangelo, the phlorin content decreases during increase of maturity. Only Pearl tangelo (SRA 454) presents a higher content in March which correspond to the high level of maturity. The last period correspond to fruit senescence. In the *C. paradisi* group, Star Ruby and Oroblanco grapefruit show a decreased content of phlorin during increasing maturity while Marsh grapefruit has a maximum content for the second period, in agreement with the optimum maturity.

In the *C. sinensis* group, varieties like the oranges Navelina, Hamlin, Baianinha, Itaboraï, and Yoshida navel present an almost stable content of phlorin. For Rosa, Pera, and Maltaise Blonde varieties, a higher

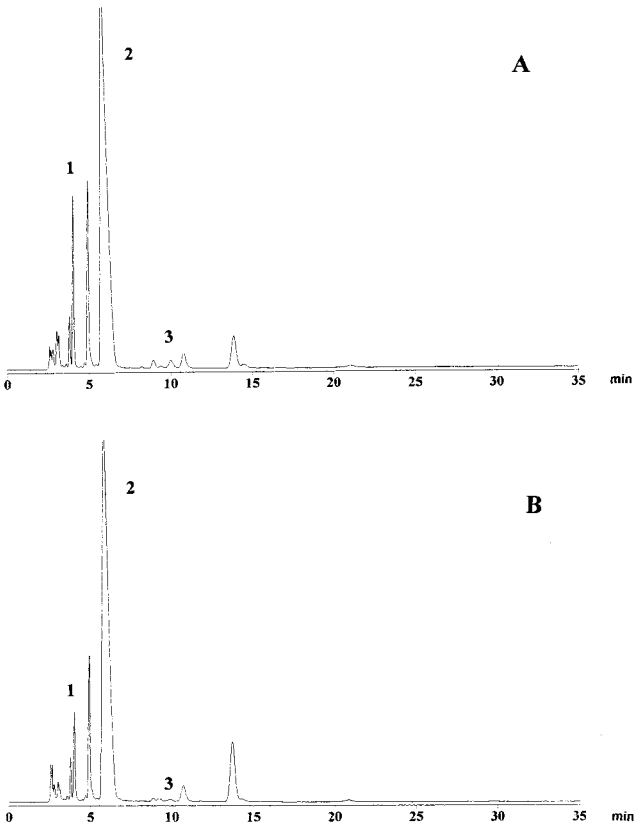

Figure 1. Chromatographic profiles of (A) Willow Leaf mandarin and of (B) Beauty of Glen Retreat mandarin juices. For chromatographic conditions, see Materials and Methods.

content (34−37 mg L^{-1}) in phlorin was observed in the second collect period. About Shamouti and Washington navel varieties, the phlorin content increased (from 12 to 30 and from 9 to 17 mg L^{-1} respectively) while for Navelate its decreases (from 24 to 13 mg L^{-1}) with fruit maturity. For the IAC 1 lime, the content is quite similar whatever the collect period (26, 28, and 29 mg.L^{-1}).

For Pink pummelo a higher content in phlorin compared to the other *Citrus* varieties (235 mg L^{-1}) was observed whatever the period of collect.

Content of 3,5-Dihydroxyphenyl-β-D-Glucopyranoside in Peel Extracts. Previous works showed that the peels contained a major quantity of phlorin compared with the fruit juices (Cancalon, 1995; Johnson et al., 1995). In our preceding paper (Louche et al., 1998), we studied the extraction conditions of phlorin in peels of two orange varieties: orange Valencia and Navel Late. The results showed that the concentration of phlorin in the maceration waters varied little at the end of 48 h using 2-fold water weight than peels weight. Therefore, a similar protocol was applied to the various fruits investigated. Phlorin was detected in all *Citrus* species and variety peels in higher amount than in juices, excluding only Nules and Commune clementines, in which this compound was characterized neither in juice nor in peel (Table 4). In the mandarin group, in Beauty of Glen Retreat and Cravo an increasing maturity peak of phlorin was observed. For Nova and Satsuma Okitsu varieties, the content of peel marker was stable in immature and mature fruits. In Willow Leaf and Natal Tightskin varieties, the higher content of phlorin was observed for immature fruits (1301 and 532 mg L^{-1}, respectively). For the tangor group, the content of

Table 4. Phlorin Content in Various Species and Varieties in Aqueous Maceration Peel Fruits

	period					
	October		March		May	
sample name	°Brix[a]	phlorin (mg L^{-1})[b,c]	°Brix[a]	phlorin (mg L^{-1})[b,c]	°Brix[a]	phlorin (mg L^{-1})[b,c]
mandarin group						
Nova	3.9	32	6.3	31	n.f.[e]	n.f.[e]
Cravo	2.8	39	4.6	43	3.5	54
Oneco	2.7	n.d.[d]	4.4	traces	4.0	traces
Willow Leaf	3.5	1301	3.6	475	3.1	443
Natal Tightskin	3.6	532	2.8	traces	n.f.[e]	n.f.[e]
Beauty of Glen Retreat	4.7	219	3.3	1012	n.f.[e]	n.f.[e]
King of Siam	3.1	n.d.[d]	4.3	traces	3,5	traces
Satsuma Okitsu	3.2	59	4.8	53	n.f.[e]	n.f.[e]
Sunki	3.2	traces	3.2	n.d.[d]	2.4	traces
Cleopatre	4.3	traces	3.6	traces	3.5	traces
clementine group						
Commune Corse	2.9	traces	4.9	traces	n.f.[e]	n.f.[e]
Nules	2.9	n.d.[d]	6.2	n.d.[d]	n.f.[e]	n.f.[e]
tangor group						
Bergamota	2.5	854	4.5	372	3.9	375
Ortanique	3.0	1595	4.1	877	4.2	593
Murcott	3.7	611	4.4	371	5.5	364
Ellendale	2.8	traces	6.4	12	4.6	traces
tangelo group						
Orlando	2.7	n.d.[d]	3.8	traces	4.1	traces
Pearl (SRA 453)	3.2	880	4.6	869	4.1	426
Pearl (SRA 454)	3.9	1338	3.9	1189	4.5	883
Sampson	4.4	1180	4.4	578	5.2	952
grapefruit group						
Marsh	3.2	861	3.7	1035	4.1	779
Star Ruby	4.5	1270	4.6	923	4.3	1008
Oroblanco	3.8	1687	3.8	1158	3.3	888
orange group						
Shamouti	2.4	573	3.5	427	3.5	401
Navelate	2.6	1203	3.9	876	4.4	552
Navelina	4.2	643	5.2	157	n.f.[e]	n.f.[e]
Hamlin	2.7	696	4.4	359	4.0	242
Maltaise Blonde	3.7	554	3.8	803	3.1	391
Washington Navel	3.5	480	4.1	417	4.2	263
Pera	3.1	250	4.4	534	3.0	489
Natal	3.0	611	4.0	483	3.7	440
Rosa	3.0	577	3.3	424	4.0	366
Salustiana	3.5	494	3.8	373	4.6	298
Ruby	2.8	989	4.7	184	4.5	342
Baianinha	2.9	449	4.4	514	4.4	393
Itaborai	3.5	896	3.8	680	3.4	573
Yoshida navel	3.4	496	4.0	841	n.f.[e]	n.f.[e]
lemon						
Frost Eureka	4.3	677	2.5	548	2.4	383
pummelo						
Pink	3.2	1403	3.5	1684	3.0	299
lime						
IAC 1	3.2	758	3.1	818	2.5	1018
sour orange						
Maroc	3.8	2063	4.1	1304	4.1	1188
citron						
de Corse	3.9	33	3.6	80	2.9	79
lemon hybrids (natural)						
Castagnaro Bergamot	3.6	1268	3.7	599	2.7	585
Rough lemon	2.5	538	3.9	329	3.7	173
Rangpur lime	3.2	417	3.1	237	3.1	177

[a] See Materials and Methods for sugar determination. [b] HPLC at 214 nm. [c] See Materials and Methods for aqueous maceration at 40 °C. [d] Not detected. [e] No fruit.

phlorin in Ortanique and Murcott varieties decreases during maturity (from 1595 to 593 mg L^{-1} and from 611 to 364 mg L^{-1}, respectively). For these varieties and for Bergamota (854 mg L^{-1}), the higher content was observed in immature fruits as in Pearl tangelos SRA 453 (880 mg L^{-1}) and SRA 454 (1338 mg L^{-1}) and Sampson (604 mg L^{-1}). In Orlando tangelo, we noted just traces. In the *C. paradisi* group, the concentration of peel marker in the Marsh grapefruit is higher for the second period (1035 mg L^{-1}). In the Star Ruby and Oroblanco varieties, the phlorin content decreases with increasing maturity (from 1270 to 1008 mg L^{-1} and from 1687 to 888 mg L^{-1}, respectively). In *C. sinensis*, the phlorin content increases with maturity only for orange Yoshida navel. The phlorin content decreases for Shamouti, Navelate, Navelina, Hamlin, Washington Navel, Natal, Rosa, Salustiana, and Itaboraï with maturity. In Maltaise Blonde, Pera, and Baianinha varieties, the higher content is observed for the second period. For the Ruby variety, the high content is observed for the immature fruits and then for the very mature fruits (989 and 342 mg L^{-1}, respectively). For lemon Frost Eureka and its hybrids, the phlorin content decreases with maturity, while for the lime IAC 1, the phlorin content increases with maturity. The higher concentrations of peel marker were detected for the Maroc sour orange and Pink pummelo.

Concerning the de Corse citron which is characterized by a hard and thick peel and almost no juice, although

Table 5. Phlorin Content in Juices and Peel Extracts from Mature Citrus Fruits

		juice					peel[a]				
variety	no. of samples	presence (%)	min mg L^{-1}	max mg L^{-1}	mean mg L^{-1}	c.v.	presence (%)	min mg L^{-1}	max mg L^{-1}	mean mg L^{-1}	c.v.
mandarin	10	20	0	33	6	13	100	traces	1012	162	332
clementine	2	0			0		50	0	traces	traces	
tangor	4	75	0	24	15	10	100	12	877	406	356
tangelo	4	75	0	73	33	31	100	traces	1189	659	505
grapefruit	3	100	30	108	71	32	100	779	1158	982	191
orange	14	100	11	37	22	9	100	157	876	492	229
lemon	1				43					548	

[a] Phlorin content is determined using aqueous maceration peel, see Materials and Methods.

the entire fruits were taken for maceration, the phlorin content was very low (33−80 mg L^{-1}).

Phlorin Content in Mature *Citrus* Juices and Peel Extracts. In Table 5 are presented the minimum, maximum, mean, and c.v. of phlorin content in mature fruits. For the mandarin group, only two varieties of 10 samples present a peak of phlorin in juices. In mandarin peel extracts, phlorin was detected in all samples with a mean of 162 mg L^{-1}. For the clementine group, no phlorin was detected in juices and just as traces in one peel extract. For the tangor and tangelo groups, 75% of samples present a phlorin peak in juices with a mean of 15 and 33 mg L^{-1}, respectively, while in peel extracts, phlorin was detected in all samples with a mean of 406 and 659 mg L^{-1}, respectively. For the grapefruit group, phlorin was present in all samples in juices as well as in peel extracts (mean of 71 and 982 mg L^{-1}, respectively). A similar result was observed for the 14 samples of the orange group (mean of 22 and 492 mg L^{-1}). We can suggest that the phlorin content characterize the different taxonomic groups of *Citrus*. The mandarin group which is considered as a true ancestral species (Swingle et al., 1967) has the lower values of phlorin content both in juices and in the peel. The pummelo group (including grapefruits varieties), the other true ancestral *Citrus* species, was extremely differentiated by a very high concentration of phlorin in juices and also in peel extracts. The third ancestral important group is the combination of lime and citron varieties. In this last important phylogenetic group, the content of phlorin was quite different with a low content in lime juices and high concentration in peel extracts; the citron peel has lower phlorin content. The sweet oranges, which were genetically related to mandarin group and suspected to be at the origin of the citrus history a hybrid between mandarin and probably a pummelo, have intermediate mean values of phlorin between these two ancestral genitor groups. Considering that all sweet orange varieties became probably from somatically natural mutations selected by the growers, the content of phlorin is quite similar for all varieties. In contrast, the recently created hybrids like tangelos and tangors were characterized by wide variations of phlorin contents in juices and peel extracts related to the high genetic variability of these hybrids. Furthermore, the mean values of these two kinds of hybrids were also intermediate to their parentage groups. Consequently, and if the mean values observed in our Citrus population were confirmed for a wider representation, particularly for the "citrus acid" species and pummelo group, the quantitative concentration of phlorin would be used as a taxonomic marker.

CONCLUSION

The wide screening of phlorin in 45 *Citrus* species and varieties allows to certify that phlorin is absent from some fruit varieties. When its presence is observed, the concentration is higher in peel than in juice. The content is very heterogeneous in *Citrus* genera but the species or groups investigated present specific mean values. The phlorin content is in accordance with genetic relationships of each citrus taxonomic group and we have not observed a relationship between phlorin concentration and fruit maturity state.

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