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Phloroglucinol from Phlorin Hydrolysis for Testing Quality of Commercial Orange Juices and Beverages

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The European Association of Juices and Nectars Producers (AIJN) is evaluating the opportunity to introduce the content of phlorin (3,5-dihydroxyphenyl β-D-glucopyranoside), a peel marker for oranges, as a parameter for testing the quality of orange juices. Because of the lack of a commercial standard of phlorin and its laborious isolation procedures, in this contribution is developed a simple and reliable method for measuring the phlorin level as the corresponding aglycon phloroglucinol, obtained after a total enzymatic hydrolysis of the sample. The method was applied to the quantification of phloroglucinol in several industrial and commercial blond and pigmented orange juices and beverages based on 12% orange juice. Under the same extraction procedure, the phloroglucinol content in the pigmented juices was higher than in the blond ones. No significant difference was obtained between not from concentrate juices and reconstituted from concentrate juices. The marker amount increases in the highly processed orange fruits and in the byproducts of citrus processing due to the contact of the juice with the albedo, which is the major source of phlorin. In orange-based beverages the phloroglucinol content revealed a large heterogeneity and a poor quality of the raw juices used.

KEYWORDS: 3,5-Dihydroxyphenyl β-D-glucopyranoside; orange juice; orange-based beverages; orange peel marker; phloroglucinol; phlorin; pigmented orange juice; quality control

INTRODUCTION

The quality and possible adulteration of food and beverages are long-standing problems. Authentication of food products is of primary importance for both consumer and industries, at all levels of the production process, from the raw materials throughout the products. Because pure fruit juices and fruit juice beverages are an important and fast-growing sector of the food industry, they have always been prime targets for adulteration (*1*). Orange is the most popular juice flavor in both pure juices and beverages: the increasing market demand for these products, containing different percentages of orange juice, has enhanced adulteration opportunities. They may be simple dilution with water and addition of sugar, other citrus juices, or fruit byproducts such as second-pressure extracts or other cheaper alternatives (*2*). Peel wash, pulp wash, and juice sacs are second extracts obtained by washing the separated peel, pulp, and membrane material, respectively, with water after the first pressing of oranges. Their chemical composition is similar to that of the juice, although they have lower soluble solids, different color, odor, and taste, and higher levels of pectins and bitter compounds. In accordance with U.S. federal regulations, pulp wash produced during extraction (in-line pulp wash) can be added back, up to 10%, to the juice before concentration; in-line addition of pulp wash to fresh and pasteurized juices and off-line addition to any categories of orange juice are not permitted. Peel wash, pulp wash, and juice sacs addition to every kind of orange juice at the present time is forbidden in the European Union (EU). Many components are present in citrus juices and could be determined to assess product quality. Among them, the phenolic compounds provide an excellent fingerprint for orange juices (*3*) and are simple to quantify with widespread techniques (*2*). Several methods, based on specific markers (*4*−*8*) or on the distribution of different phenolic compounds in pulp or peel with respect to the juice (*9*, *10*), have been proposed to prove either adulteration or authenticity of the orange juices. In 1995 Johnson et al. proposed phlorin (3,5-dihydroxyphenyl β-D-glucopyranoside) as a marker for orange peel extract (*11*); it is a secondary metabolite originating from acetyl-CoA by the polyketide pathway (*12*). In 1996 Hammond et al. used this phenolic compound to detect the addition of pulp wash to orange juice (*13*). The results of the studies about the concentration of phlorin in the different parts of the orange (*14*−*16*) have shown that the albedo is the major source of this compound, which is quite absent in the juice. The small amount of phlorin found in the flavedo may come from contamination with albedo; in fact, a large amount of this marker is present in peel extract (*14*). Regardless of the interest in using phlorin as a juice authentication marker (*17*), commercial standards are not available and pure phlorin has to be isolated from aqueous albedo extracts of oranges using C_{18} reversed phase column chromatography (*15*) or by purifying a clarified grapefruit albedo extract with an adsorbing resin (*17*). The above proposed procedures are difficult and time-consuming, producing a limited quantity of

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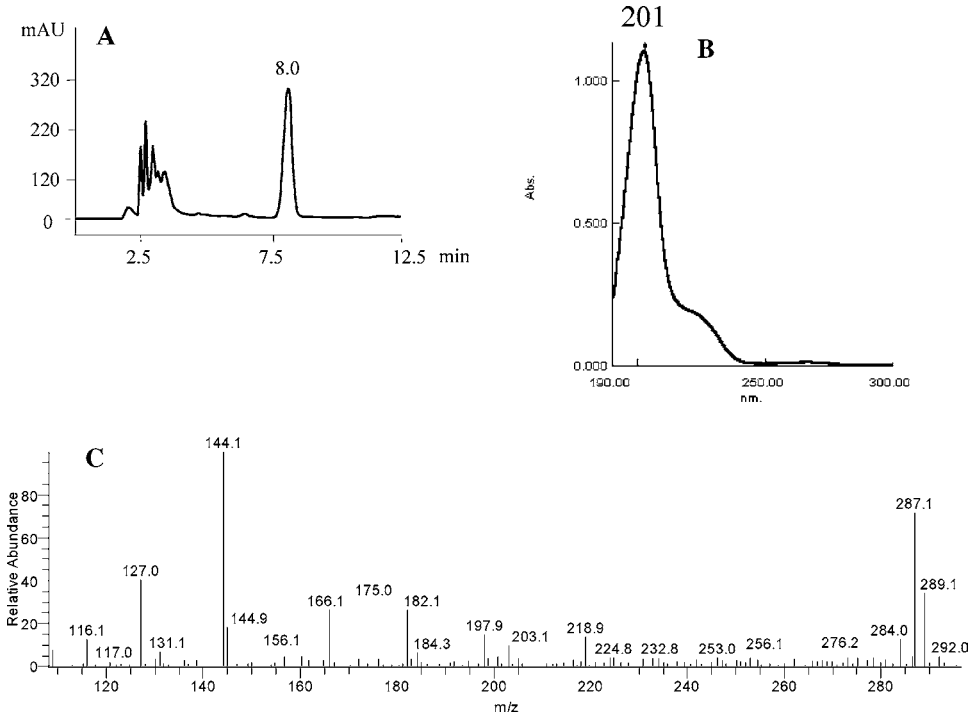

Figure 1. Phlorin: (**A**) HPLC chromatogram of the purified extract; (**B**) UV spectrum; (**C**) mass spectrum.

a hygroscopic compound, which is hard to weigh accurately and susceptible to gradual oxidation and browning (*17*).

The aim of this work is to propose a reliable and routinely suitable method to quantify the content of phlorin in juices and beverages as the corresponding aglycon phloroglucinol after total enzymatic hydrolysis of the sample. We also applied this quantitative determination on different industrial products and byproducts of blond and pigmented orange processing and in commercial orange juices and beverages. No data are available in the literature about commercial 12% beverages and pigmented orange juices, although there is a positive trend in the blood orange juice consumption in EU during the past decade. Furthermore, the aim of this contribution is also to obtain the reference guideline of the phlorin content in blond and pigmented orange juices obtained with the FMC technology to assess the quality of commercial juices and beverages. In fact, the European Association of Juices and Nectars Producers (AIJN, Bruxelles, Belgium) is evaluating the opportunity to introduce, among other parameters, the level of phlorin as an element for evaluating juice authenticity.

MATERIALS AND METHODS

Samples. Phlorin quantification as phloroglucinol aglycon has been carried out on the following products and byproducts of the blond and pigmented orange processing: (1) single-strength juices processed using FMC extractor and FMC finisher; (2) concentrated juices obtained with FMC technology and TASTE evaporator; (3) hand-squeezed juices, prepared in the laboratory from carefully squeezed fruits avoiding albedo contact; (4) laboratory-made juices obtained by putting a weighed amount of wet pulp from the FMC finishers into a citric acid/potassium citrate buffer solution (Sigma Aldrich) at pH 3.5; (5) byproducts from orange processing such as enzymatically treated peel wash, pulp wash, and juice sacs; (6) commercial 100% juices not from concentrate (NFC); (7) commercial juices reconstituted from concentrate (RFC) and beverages containing different percentages of orange juice; (8) commercial 12% orange-based beverages.

Samples 1, 2, and 5 and pulp for preparing sample 4 were kindly supplied by RUBY Compagnia Internazionale Prodotti Agricoli s.r.l. (Catania, Italy) and, together with the laboratory-made juices, were characterized by the measurements of the soluble solids content and percent acidity. The soluble solids content was expressed as °Brix by direct reading on a Zeiss refractometer, and the acidity, expressed as percent of anhydrous citric acid, was determined by neutralizing 5 mL of juice with 0.1 N NaOH (Fixanal Riedel-de Haën) up to pH 8.1. Different brands of samples 6−8 were collected on the market, and three different lots of each brand were analyzed.

Phlorin Identification. To confirm the phlorin structure HPLC-PDA and LC-ESI-MS analyses were carried out on an albedo extract prepared by overnight maceration of 150 g of cv. Moro orange albedo in 250 mL of hot water (50 °C). The sample was centrifuged at 9000 rpm for 20 min at 4 °C (Braun Biotech GmbH DR 15, Melsungen, Germany), the supernatant was filtered through a Whatman no. 1 filter paper by suction using a Büchner funnel and then concentrated under reduced pressure to a final volume of 25 mL. An aliquot of 6 mL was loaded on a Sep-Pak C18 cartridge (Supelco), to purify the phlorin from interfering compounds. The HPLC-PDA analysis of the extract was performed as specified under HPLC Determination (**Figure 1A**). For the LC-ESI-MS analysis, 100 μL of filtrate was diluted with 50:50 (v/v) methanol/water up to 500 μL and injected (loop = 100 μL) in a Thermoquest Spectra series P200 HPLC [column, LiChrosphere 100 C_{18} 5 μm, 250 × 4 mm i.d., Merck; flow, 1 mL/min of 5:95 (v/v) methanol/water for 12 min] coupled with a Finnigan LC QDUO mass spectrometer (positive mode; capillary temperature, 200 °C; mass range, *m*/*z* 100−300; scan mode, MS/MS). The UV spectrum of the peak at a retention time of 8 min (**Figure 1B**) was superimposable with that reported by Louche et al. (*15*). The ESI mass spectrum confirmed the proposed structure (**Figure 1C**). The more informative peaks were the following [*m*/*z* (relative abundance), assignation]: 289.1 (35%), M + H; 288.1 (10%), M; 287.1 (70%), M − H; 144.1 (100%), phloroglucinol moiety + H_2O; 127 (40%), phloroglucinol moiety + H.

Sample Preparation for Phlorin Determination. All samples were analyzed without any pretreatment to maintain the natural cloud. To perform hydrolysis of phlorin, the samples were treated with Rapidase PW (DMS Food Specialties, Seclin, France), a commercial pectinase preparation used in the citrus industry to reduce viscosity of second-extract juices. Sample aliquots (5 mL) were put in contact with Rapidase PW in a bath (35 °C) under constant stirring (300 rpm) until phlorin was completely hydrolyzed. The enzyme quantity and the treatment time were dependent on the sample type. An enzyme amount of 0.2−0.3 mL and a contact time of 30 min were used for the natural juices and beverages, whereas the enzyme amount was increased 5 times for concentrated industrial samples. For byproducts the contact time of 1.5−2 mL of the enzymatic preparation was 45 min. Control samples

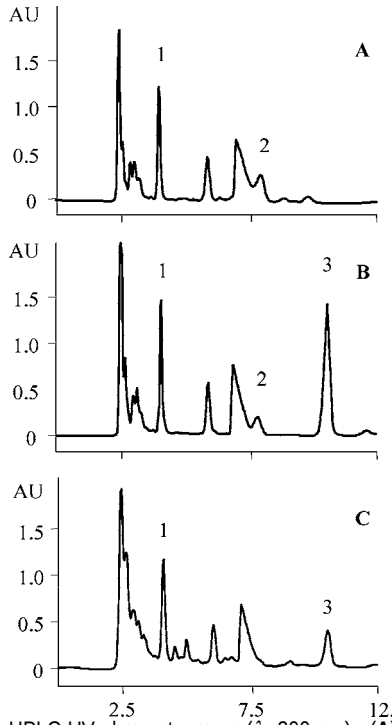

Figure 2. HPLC-UV chromatograms (λ, 200 nm): (**A**) orange juice; (**B**) orange byproduct; (**C**) orange juice after enzymatic treatment. Peaks: 1, ascorbic acid; 2, phlorin; 3, phloroglucinol.

were also run without enzyme addition. The samples were filtered through 0.45 μm PTFE filters and analyzed by HPLC. All experiments were carried out in duplicate.

HPLC-PDA Determinations. Phlorin and phloroglucinol were analyzed before and after the enzymatic treatment of the sample. A Varian 9012Q liquid chromatograph equipped with a 20 μL loop, a LiChrosphere 100 C_{18} 5 μm column (250 × 4 mm i.d., Merck), and a photodiode array detector (Varian Prostar 330), scanning from 190 to 300 nm, were used. According to Louche et al. (*15*), the eluent was a 25 mmol L^{-1} potassium dihydrogen phosphate (Sigma Aldrich) solution adjusted at pH 2.0 with 85% orthophosphoric acid (Sigma Aldrich). Flow rate was 1 mL min^{-1} at 25 °C for 12.5 min and detection wavelength, 200 nm (λ_{max} of both phlorin and phloroglucinol). In these conditions, phlorin and phloroglucinol have retention times of about 8.0 and 10.5 min, respectively; the peak at 3.8 min was assigned to ascorbic acid after comparison with the retention time and the UV spectrum of an ascorbic acid standard solution (Carlo Erba). After the complete enzymatic hydrolysis of the juice, the quantitative determinations were carried out using external standard solution of phloroglucinol (Fluka). The calibration curve showed R^2 values of 0.997 ± 0.002. The corresponding amount of phlorin (milligrams per liter) can be calculated by multiplying the milligrams per liter of phloroglucinol by the ratio between the molecular weights of phlorin and phloroglucinol (2.29).

RESULTS AND DISCUSSION

Simple and reliable Method for Determining Phlorin as Phloroglucinol. Preliminary HPLC runs have evidenced the presence of phlorin in orange juices, whereas in the orange-processing byproducts both phlorin and phloroglucinol were observed (**Figure 2A**,**B**). Byproducts of citrus processing, which are in high demand by the soft drink industry, are an attractive resource for developing natural beverage clouds due to the low cost and high sugar content (*18*). Because pectic enzymes can be used in byproducts processing to improve the extraction of soluble solids, therefore resulting in a higher yield with a lower viscosity (*19*), the hydrolysis of phlorin into the corresponding aglycon phloroglucinol may be expected, due to the β-glucosidase activity of commercial pectinase (**Figure 3**). Although pectinases are used within producers' recommended dosages,

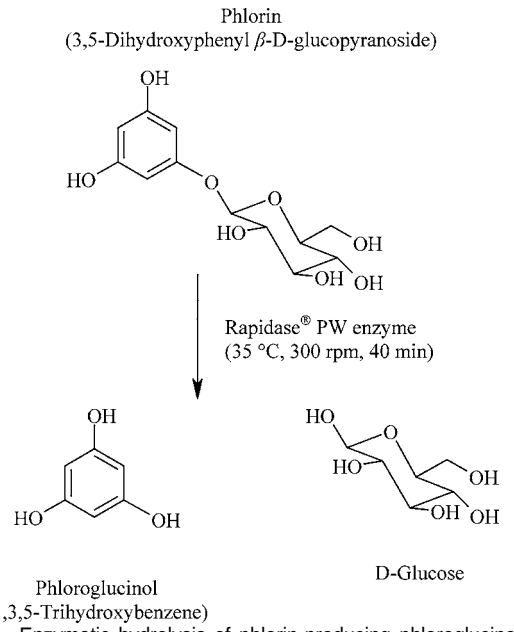

Figure 3. Enzymatic hydrolysis of phlorin-producing phloroglucinol and D-glucose.

a loss of anthocyanins and other phenolic compounds correlated with β-glucosidase activity was in fact detected in cranberry (*20*, *21*) and strawberry fruits (*22*). Moreover, phlorin hydrolysis in mature fruits can occur due to the activity of endogenous β-glucosidase, even if relatively low levels of β-glucosidase activity were observed in the sweet orange fruit vesicles (*23*). The partial hydrolysis of phlorin, which could occur in byproducts, resulting in an underestimation of the marker content, suggested to us that we develop a quantitative method for its measurement as phloroglucinol after complete enzymatic hydrolysis of the phlorin. In fact, the addition to the sample of a higher dosage of Rapidase PW recommended for viscosity reduction outside citrus juices brings about the total conversion of phlorin to phloroglucinol (**Figure 2C**), which can be easily quantified by comparison with an external standard. Phloroglucinol is available as an HPLC pure standard (>99%) at low cost. Moreover, the position of the phloroglucinol peak in the HPLC chromatogram allows a correct integration. The choice of Rapidase, commonly used by citrus processors, was made also because it is cheaper than more appropriate reagent kits based on β-glucosidases.

Phloroglucinol Content of Blond and Pigmented Orange Products and Byproducts. The phlorin content was examined in some industrial juices, obtained from blond and pigmented oranges, and in some citrus-processing byproducts as well. In **Table 1** the characteristics and phlorin content (as phloroglucinol) of 22 juices are reported; the amount of the concentrated samples was referred to 12.5 °Brix. The results show that the phloroglucinol content in orange juices is quite low. However, a considerable difference exists between blond and pigmented orange juices: the phloroglucinol amount in pigmented samples (16.2 ± 3.3 mg/L) is higher than in the blond ones (5.1 ± 1.8 mg/L). Because the orange juices were obtained with the same FMC technology, the difference in the marker content may be ascribed only to the varieties. This result is in accordance with those of a previous study (*16*) that found a great heterogeneity in the phlorin content among several *Citrus* species and varieties. On the contrary, the marker content for both types of orange juice does not change during the different production periods, even when organic and conventional fruits and pasteurized and not pasteurized juices were considered. A comparison of these results with those of laboratory-made samples obtained with

Table 1. Phloroglucinol Content of FMC Processed Orange Juices after Enzymatic Hydrolysis of the Samples

juice typology	batch date	°Brix	% acidity	ratio	phloroglucinol[a] (mg/L)	mean	SD[b]
blond							
not pasteurized	May 22, 2003	12.7	1.50	8.46	3.92		
	June 3, 2003	13.7	1.32	10.38	3.26	3.59	0.47
not pasteurized (organic)	May 23, 2003	13.5	1.50	9.00	6.12		
	July 4, 2003	13.4	1.29	10.38	2.74	4.43	2.39
pasteurized	May 30, 2003	12.6	1.30	9.69	5.22		
	June 13, 2003	13.1	1.42	9.22	3.81	4.50	1.00
concentrated	May 26, 2003	60.0	6.45	9.30	8.15[c]		
	Jan 14, 2004	50.0	4.37	11.44	6.01[c]		
	Feb 13, 2004	58.9	5.75	10.24	6.95[c]	7.15	1.25
pigmented							
not pasteurized	Jan 31, 2003	12.0	1.29	9.30	11.48		
	Feb 21, 2003	12.9	1.44	8.95	11.39		
	April 23, 2003	14.1	1.25	11.28	19.33	14.07	4.56
not pasteurized (organic)	Feb 22, 2003	12.9	1.43	9.02	18.33		
	March 17, 2003	13.1	1.27	10.3	17.82		
	April 24, 2003	13.3	1.50	8.80	15.34	17.16	1.60
pasteurized	Feb 21, 2003	13.1	1.42	9.22	16.51		
	March 19, 2003	13.3	1.22	10.9	15.40		
	April 24, 2003	13.3	1.20	10.3	16.95	16.29	0.80
concentrated	Jan 20, 2003	52.8	5.60	9.30	11.83[c]		
	Feb 21, 2003	52.0	5.60	9.28	16.62[c]		
	March 19, 2003	50.2	5.50	9.10	18.65[c]		
	April 24, 2003	50.8	6.18	8.22	23.59[c]	17.67	4.87

[a] Mean value of duplicates; the corresponding amount of phlorin (mg/L) can be calculated by multiplying by 2.29. [b] Standard deviation. [c] Referred to 12.5 °Brix.

Table 2. Phloroglucinol Content in Hand-Squeezed Samples of Orange Juices from Different Cultivars after Enzymatic Hydrolysis of the Samples

juice typology	batch date	°Brix	% acidity	ratio	phloroglucinol[a] (mg/L)	mean	SD[b]
blond							
navel	Jan 16, 2004	12.8	1.53	8.36	0.59		
	Jan 20, 2004	13.2	1.27	10.39	0.35		
	Jan 30, 2004	12.9	1.35	9.56	0.66	0.53	0.17
Valencia	Feb 10, 2004	10.8	1.20	10.69	0.84		
	Feb 15, 2004	11.4	1.01	9.50	0.82		
	Feb 23, 2004	11.2	1.00	11.20	0.85	0.84	0.02
pigmented							
Moro	Jan 9, 2004	11.8	1.22	9.70	2.76		
	Feb 10, 2004	11.8	1.30	9.08	2.52	2.64	0.17
Tarocco	Jan 7, 2004	13.0	1.15	11.30	1.94		
	Jan 13, 2004	12.5	1.30	9.62	1.91		
	Feb 21, 2004	13.0	1.10	11.82	2.20		
	Feb 23, 2004	11.2	1.03	10.87	3.39		
	Feb 24, 2004	11.8	1.02	11.57	3.35	2.56	0.75
Sanguinello	Jan 10, 2004	11.2	1.20	9.33	2.60		
	Feb 2, 2004	12.2	1.7	11.40	4.58		
	Feb 9, 2004	11.8	1.00	11.80	4.99	4.05	1.28

[a] Mean value of duplicates; the corresponding amount of phlorin (mg/L) can be calculated by multiplying by 2.29. [b] Standard deviation.

gentler conditions of pressing pointed out a decrease of the content to <1 mg/L and to ~3 mg/L in blond and pigmented orange juices, respectively (**Table 2**). The screening of different blood cultivars (Moro, Tarocco, and Sanguinello) has confirmed that the phloroglucinol amount is ~3 times higher than in the blond ones (Navel and Valencia), even if no considerable difference exists among the blood cultivars. Although the phlorin values reported by Louche et al. (*16*) for blond hand-squeezed orange juices were in the range of 11−37 mg/L, our values are in agreement with those of Johnson et al. (*12*), who found 3−5 ppm of phlorin, which corresponds to ~1−2 mg/L of phloroglucinol. No comparison is possible for pigmented samples due to the lack of data in the literature. The strong influence on the juice composition due to the mechanical treatment of fruits was already considered by Cancalon (*14*) and Braddock et al. (*17*); due to the higher extraction pressure and the presence of membrane material, the phlorin content of FMC technology juices was significantly higher than in domestic gently hand-squeezed juices. For the same reason, the phlorin content in orange-processing byproducts is higher with respect to the mean values detected in the juices; the marker amount increases from about 30 to 150 mg/L depending on the specific byproduct (**Table 3**). The analyzed samples of pulp wash showed mean values of 32.4 mg/L for the blond and 33.7 mg/L for the

Table 3. Phloroglucinol Content in Byproducts of Orange Processing after Enzymatic Hydrolysis of the Samples

byproduct	batch date	°Brix	% acidity	ratio	phloroglucinol[a] (mg/L)	mean	SD[b]
pulp wash	Feb 13, 2003	36.4	2.89	12.60	36.68[c]		
	May 15, 2003	60.0	5.05	11.88	30.81[c]		
	June 24, 2003	39.3	2.40	16.97	32.35	33.28	3.04
juice sacs		60.0			110.76		
peel wash	June 3, 2003	55.0	2.83	19.43	149.72		
pigmented pulp							
5% w/v	March 19, 2003				1.22		
	April 24, 2003				1.25	1.24	0.02
10% w/v	March 19, 2003				2.40		
	April 24, 2003				2.47	2.44	0.05

[a] Mean value of duplicates expressed for juices at 12.5 °Brix; the corresponding amount of phlorin (mg/L) can be calculated by multiplying by 2.29. [b] Standard deviation. [c] Pigmented sample.

pigmented oranges. These marker amounts are higher with respect to pure orange juice values, even if these byproducts are obtained by water-washing finisher pulp, which may have a low phlorin concentration (*14*, *15*). The results of the laboratory-made samples obtained by the addition of a fixed amount of pigmented pulp into an aqueous phase at pH 3.5 showed that the phloroglucinol content was ~1.2 mg/L for every pulp addition of 5% (**Table 3**). In accordance with Braddock et al. (*17*), this suggests that the presence of phloroglucinol in commercial pulpwash samples is an indication of the content of peel and/or core extracts. The large amount of phloroglucinol detected in the juice sacs (111 mg/L) and in the peel wash (150 mg/L), which were obtained from the treatment of membranes and peel respectively, confirms the usefulness of this phlorin to monitor juice blending with albedo extracts and segmented wall membranes.

Phloroglucinol Screening in Commercial Orange Juices and Orange Beverages. Different brands of juices and beverages were collected from the market to investigate the quality of commercial samples. **Table 4** reports the phloroglucinol content for these samples, together with the commercial types and the percentage of juice reported on the label. In addition, **Figure 4** shows the mean values of phloroglucinol (adjusted for the percentage of juice) of the NFC and RFC blond and pigmented orange juices: they confirm that no substantial difference exists between NFC and RFC orange juices, but that it is possible to distinguish between blond and pigmented orange juice due to the higher phloroglucinol content of the latter. The phlorin content in the commercial juices is approximately the same as for the analyzed industrial samples, pointing out that the above proposed mean of the phloroglucinol values can be used to evaluate the quality of the juice extraction procedure. The results of the commercial NFC and RFC juices of different brands show comparable mean values for the blond and also for the pigmented juices. On the contrary, the phloroglucinol content of the 12% orange-based beverages (**Table 4**) revesls a large heterogeneity among the collected samples even if the three analyzed lots of each brand have proved a good reproducibility of the data. With the exception of two samples that show lower amounts, the phloroglucinol contents of the 12% orange beverages (adjusted for the percentage of juice) show mean values >50 mg/L. Only one sample has a marker amount comparable with the pure juice (8.9 mg/L). These results point out the very low quality of most raw materials used for these beverages and also the significant differences among the various brands. Moreover, the possible addition of byproducts and the possible blend of blond and pigmented juices to produce red beverages do not permit the blond and pigmented orange beverages to be distinguished.

Table 4. Phloroglucinol Content in Commercial Samples of Orange Juices and Beverages after Enzymatic Hydrolysis of the Samples

sample				phloroglucinol[a] (mg/L)	
no.[b]	color	type	% of juice	mean value[c]	mean value adj % juice
1	blond	NFC	100	8.68 (0.33)	8.68
2	blond	NFC	100	5.25 (0.80)	5.25
3	blond	NFC	100	3.77 (0.57)	3.77
4	blond	RFC	100	11.43 (0.61)	11.43
5	blond	RFC	100	8.38 (0.11)	8.38
6	blond	RFC	100	7.87 (0.20)	7.87
7	blond	RFC	50	5.02 (0.17)	10.04
8	blond	beverage	12	7.87 (0.03)	65.60
9	blond	beverage	12	8.03 (0.36)	66.90
10	blond	beverage	12	3.38 (1.92)	28.10
11	blond	beverage	12	6.38 (0.20)	53.20
12	blond	beverage	12	6.10 (0.41)	50.80
13	blood	NFC	100	15.72 (0.92)	15.72
14	blood	NFC	100	14.61 (0.52)	14.61
15	blood	NFC	100	12.10 (0.23)	12.10
16	blood	RFC	83	12.37 (0.28)	14.90
17	blood	RFC	50	8.10 (0.03)	16.20
18	blood	RFC	35	6.90 (2.00)	19.7
19	blood	RFC	30	3.57 (0.62)	11.88
20	blood	RFC	30	5.27 (0.11)	17.55
21	blood	RFC	25	4.99 (0.04)	19.94
22	blood	RFC	25	3.29 (0.12)	13.14
23	blood	beverage	12	10.77 (1.00)	89.70
24	blood	beverage	12	7.03 (0.78)	58.50
25	blood	beverage	12	1.08 (0.25)	8.90

[a] Mean value of duplicates of three different lots of the same brand; the corresponding amount of phlorin (mg/L) can be calculated by multiplying by 2.29. [b] Each number is referred to a brand. [c] Standard deviations in parentheses.

Conclusions. The phlorin content of orange juices may provide a useful marker for authenticity investigations; however, the use of commercial pectinases in the treatment of citrus-processing byproducts may cause an underestimation of the phlorin content, limiting its utility as an index of the addition of pulp/peel water extracts to orange juice. We conclude that it can be difficult to prove juice adulteration on the basis of only on a phlorin analysis, without considering its possible hydrolysis to the aglycon phloroglucinol. Due to the total absence of phloroglucinol in fresh and untreated oranges, its presence is exclusively correlated with phlorin hydrolysis because of enzymatic treatments and/or endogenous β-glycosidase enzymes. An analytical procedure quantifying the phlorin content in orange juices as phloroglucinol after the addition of Rapidase

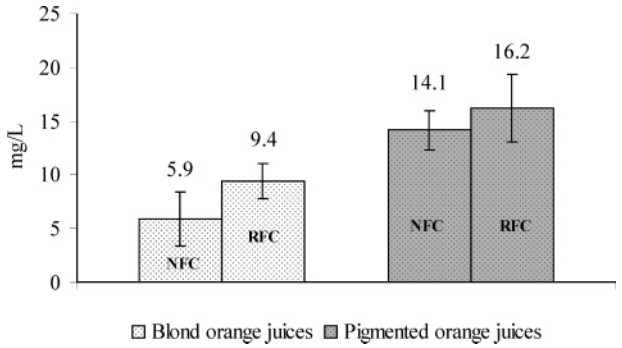

Figure 4. Mean values of phloroglucinol in milligrams per liter (adjusted for juice percentage) in commercial blond and pigmented orange juices after enzymatic hydrolysis of the samples. NFC, not from concentrate; RFC, reconstituted from concentrate.

PW for complete hydrolysis of the phlorin improves the quality control procedure due to its simplicity and reliability.

The quantification of the phlorin content in industrial and commercial orange juices has revealed that the marker amount is strictly correlated with the types of juices considered. Blond and pigmented orange juices by FMC processing have a mean phlorin content (as phloroglucinol) from 5−8 to ∼16 mg/L, respectively. The possible choice of phlorin as an authentication marker has to take into consideration this inherent difference between these two types of juice. On the contrary, no significant difference exists in the phlorin level between NFC and RFC, even if its amount is lower in the former. It was demonstrated that the phlorin content in the juice depends on the extraction technology and increases in highly processed juices. The analysis of the marker amount in 12% commercial orange-based beverages has shown, in contrast to the good homogeneity of commercial NFC and RFC juices, a wide phlorin heterogeneity of the samples present on the market. Despite the world oversupply of orange juice, this kind of commercial beverage is still made with poor raw materials.

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