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Further Knowledge on the Phenolic Profile of *Colocasia esculenta* (L.) Shott

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ABSTRACT: *Colocasia esculenta* (L.) Shott, commonly called taro, is an ancient species selected for its edible tuber. Its huge "elephant ear" like leaves are also consumed in sauces and stews or as soups. Forty-one phenolic metabolites (11 hydroxycinnamic acid derivatives and 30 glycosylated flavonoids) were identified by high-performance liquid chromatography−diode array detection−electrospray ionization/mass spectrometry (HPLC-DAD-ESI/MS^n) in the leaves of two *C. esculenta* varieties cultivated in Azores Islands. To our knowledge, 34 of the 41 phenolic compounds are being reported for the first time in this species. Phenolics quantification was achieved by an HPLC-DAD accurate and sensitive validated method. Although the qualitative profile of the two varieties is quite similar, quantitative differences were observed between them. "Giant white" and "red" varieties (local denomination) contain, respectively, ca. 14 and 21% of phenolic acids, 37 and 28% of flavones mono-*C*-glycosides, 42 and 43% of flavones di-*C*-glycosides, 3 and 4% of flavones mono-*C*-(*O*-glycosyl)glycosides, and both of them ca. 2% of flavones di-*C*-(*O*-glycosyl)glycosides and 2% of flavones-*O*-glycosides. Luteolin-6-*C*-hexoside was the compound present in higher amounts in both varieties. The established phenolic profile is an added value for the authenticity and quality control of *C. esculenta* and may be useful in the discrimination of its varieties.

KEYWORDS: *Colocasia esculenta (L.) Shott, phenolic compounds, HPLC-DAD-ESI/MS^n*

INTRODUCTION

Taro [*Colocasia esculenta* (L.) Schott], belonging to the Araceae family, is native to Southeast Asia and is among the first domesticated plants. Despite its adaptation to tropical conditions, it has long been cultivated in the Mediterranean and southern Europe.[1,2] Currently, it is a fundamental culture in tropical and subtropical regions, where it is cultivated for its edible energy-rich tuber, which mainly consists of starch, being a staple food for millions of people.[2] Besides the tuber, the leaves, flowers, and stems can be eaten either in sauces and stews or as soups.[3,4]

Taro leaves contain high levels of protein and are also an excellent source of β-carotene, potassium, calcium, phosphorus, iron, riboflavin, thiamine, niacin, vitamin A, vitamin C, and dietary fiber.[5] The fresh taro leaf lamina and petiole contain 80 and 94% moisture, respectively.[6] In addition to its nutritional characteristics, this plant has been known since ancient times for its therapeutic properties. It is used in traditional medicine in the treatment of various ailments, such as, asthma, arthritis, diarrhea, internal hemorrhage, and neurological and skin disorders.[7] These therapeutic properties may be due, in part, to the presence of secondary metabolites like phenolic compounds.[8]

Phenolics are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, or wine, and the interest in their analysis has increased as a result of their recognized physiological actions in humans. Numerous studies relate that the ingestion of polyphenols as part of a regular diet lowers the risk of cardiovascular diseases and development of cancers. Furthermore, they have long been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, and antiviral activities.[9,10]

Apart from their beneficial properties, which have granted them a relevant role in foods like nutraceuticals, polyphenols are chemotaxonomic markers, thus being useful in plants authentication.[9,11]

Rapid and reliable tools for the determination of phenolic compounds are important for the investigation of structure−activity relationships, taxonomy, and food quality control. Nowadays, the methods employed in the determination of these secondary metabolites are based on reversed-phase high-performance

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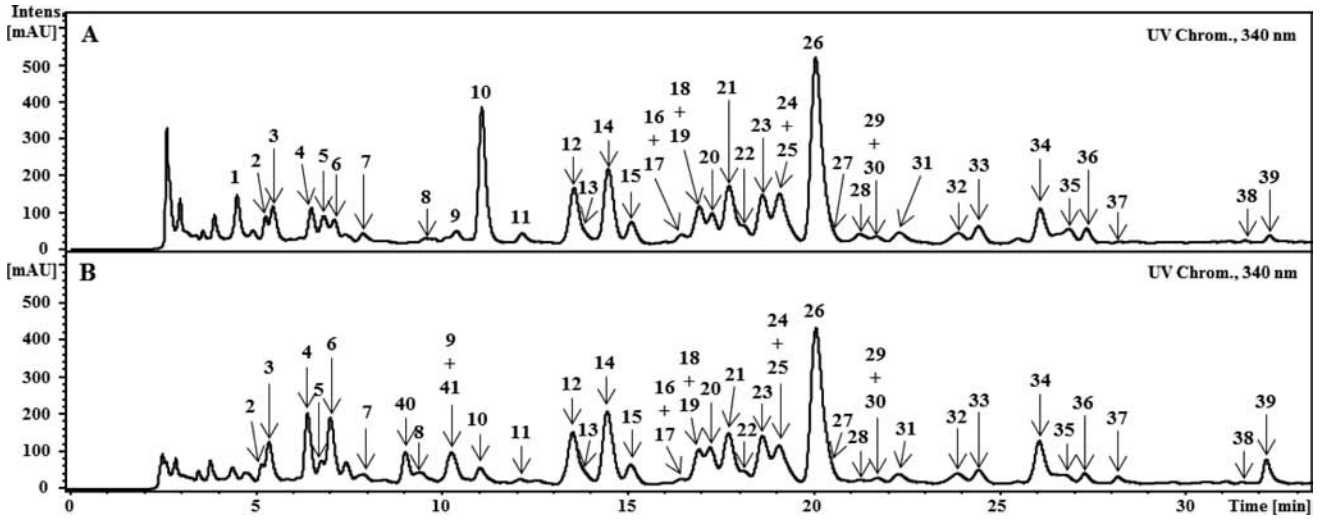

Figure 1. HPLC-DAD (340 nm) phenolic profile of aqueous extract from leaves of *C. esculenta*. (A) "Giant white" and (B) "red" varieties. Peaks: 1−6, caffeic acid derivatives; 7, sinapoyl hexoside; 8, apigenin-6-*C*-pentoside-8-*C*-hexoside-7-*O*-hexoside; 9, luteolin-6,8-di-*C*-hexoside; 10, caffeic acid; 11, luteolin-6-*C*-(6-*O*-hexosyl)hexoside; 12, luteolin-6-*C*-hexoside-8-*C*-pentoside; 13, apigenin-6,8-di-*C*-hexoside; 14, luteolin-6-*C*-hexoside-8-*C*-pentoside; 15, luteolin-6-*C*-pentoside-8-*C*-hexoside; 16, apigenin-6-*C*-(6-*O*-hexosyl)hexoside; 17, apigenin-6-*C*-pentoside-8-*C*-hexoside; 18, apigenin-6-*C*-pentoside-8-*C*-hexoside; 19, luteolin-6-*C*-(3-*O*-hexosyl)hexoside-8-*C*-pentoside; 20, *p*-coumaric acid; 21, apigenin-6-*C*-pentoside-8-*C*-hexoside; 22, luteolin-6-*C*-pentoside-8-*C*-hexoside; 23, luteolin-8-*C*-hexoside; 24, apigenin-6-*C*-hexoside-8-*C*-pentoside; 25, chrysoeriol-6-*C*-hexoside-8-*C*-pentoside; 26, luteolin-6-*C*-hexoside; 27, luteolin-6-*C*-(2-*O*-pentosyl)hexoside; 28, apigenin-6-*C*-pentoside-8-*C*-(2-*O*-hexosyl)hexoside; 29, diosmetin-6-*C*-hexoside-8-*C*-pentoside; 30, apigenin-6-*C*-(2-*O*-hexosyl)hexoside-8-*C*-pentoside; 31, apigenin-8-*C*-hexoside; 32, apigenin-8-*C*-(2-*O*-pentosyl)hexoside; 33, apigenin-6-*C*-hexoside-8-*C*-pentoside; 34, apigenin-6-*C*-hexoside; 35, chrysoeriol-8-*C*-hexoside; 36, chrysoeriol-6-*C*-hexoside; 37, luteolin-7-*O*-rhamnosyl(1→2)hexoside; 38, chrysoeriol-7-*O*-hexoside; 39, chrysoeriol-7-*O*-rhamnosyl(1→6)hexoside; and 40 and 41, dihydrocaffeoylquinic acid derivatives.

liquid chromatography (RP-HPLC) coupled to diode array detection (DAD) and/or mass spectrometry (MS) or tandem MS with atmospheric pressure ionization techniques, that is, electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). Although liquid chromatography coupled to mass spectrometry (LC/MS) has become the best alternative for the analysis of polyphenols, DAD is an indispensable tool for the provisional identification of the main phenolic structures present in plants, since they show characteristic UV−vis absorbance.[9,12]

Few studies have previously addressed the phenolic composition of *C. esculenta*, anthocyanins (cyanidin and pelargonidin derivatives) and flavones (apigenin and luteolin derivatives) being described in different varieties.[4,13−15] As far as we know, there is no report on the leaves of "giant white" and "red" varieties of *C. esculenta*. The purpose of this study was to assess the variability of the phenolic composition of the leaves of these two varieties growing in Azores (Portugal), by using HPLC-DAD-ESI/MS^n, thus contributing to a further knowledge of the species.

MATERIALS AND METHODS

Plant Material. *C. esculenta* leaves from "giant white" and "red" varieties in the same stage of development were collected in Furnas (São Miguel Island, Azores), in June, 2010. The plant material was immediately transferred to the laboratory and dried in an oven at 30 °C for 2 weeks. The dried material was powdered (mean particle size lower than 910 μm) and stored in a desiccator until future use. The analyzed samples correspond to a mixture of the leaves of three different individuals from the same variety. Voucher specimens were deposited at the Laboratory of Pharmacognosy of the Faculty of Pharmacy of Porto University, under the identification CEb-L-062010 and CEv-L-062010 (leaves from "giant white" and "red" varieties, respectively).

Standards and Reagents. Standards of caffeic, 5-*O*-caffeoylquinic, and *p*-coumaric acids, luteolin-3′,7-di-*O*-glucoside, apigenin-8-*C*-glucoside, apigenin-6-*C*-glucoside, luteolin-8-*C*-glucoside, luteolin-6-*C*-glucoside, apigenin-6-*C*-glucoside-7-*O*-glucoside, and chrysoeriol were purchased from Extrasynthese (Genay, France). HPLC-grade methanol and acetic acid were obtained from Merck (Darmstadt, Germany). Water was deionized using a Milli-Q water purification system (Millipore, Bedford, MA).

Extraction. For phenolics identification, 2 mL of water was added to 0.2 g of dried leaves of each sample. The mixture was sonicated, centrifuged (12000 rpm, 5 min), and filtered through a 0.20 μm size pore membrane. For quantification purposes, phenolic compounds were extracted by mixing dried leaves of each sample with water (0.2 g/50 mL) as follows: 0.5 h of sonication followed by 1 h of stirring (200 rpm) at room temperature. The extract obtained was filtered under vacuum and concentrated to dryness, under reduced pressure (40 °C).

HPLC-DAD-ESI/MS^n Qualitative Analyses. Chromatographic analyses were carried out in an Agilent HPLC 1100 series equipped with a diode array detector and mass detector in series (Agilent Technologies, Waldbronn, Germany). The elution was performed on a Luna C18 column (250 mm × 4.6 mm, 5 μm particle size; Phenomenex, Macclesfield, United Kingdom). The mobile phase consisted of two solvents: water−acetic acid (1%) (A) and methanol (B), starting with 20% B and using a gradient to obtain 35% B at 20 min, 50% B at 30 min, and 90% B at 32 min. The flow rate was 1 mL/min, and the injection volume was 40 μL. Spectral data from all peaks were accumulated in the range of 240−400 nm, and chromatograms were recorded at 340 nm. The HPLC system was controlled by ChemStation software (Agilent, v. 08.03). The mass detector was an ion trap spectrometer equipped with an ESI interface and was controlled by LCMSD software (Agilent, v. 4.1). The ionization conditions were adjusted to 350 °C and 4 kV for capillary temperature and voltage, respectively. The nebulizer pressure and flow rate of nitrogen were 65.0 psi and 11 L/min, respectively. The full scan mass covered the range from m/z 100 up to m/z 1500. Collision-induced fragmentation experiments were performed using helium as

Table 1. R_t, UV, and MS: $[M - H]^-$ and $MS^2[M - H]^-$ Data of Flavonoids from the Leaves of *C. esculenta*[a]

	compds[b]	R_t (min)	UV (nm)	$[M-H]^-$, m/z	$MS^2[M - H]^-$, m/z (%)					
					flavones mono-*C*-glycosides					
					−18			−60	Aglc + 71	Aglc + 41
23	Lut-8-*C*-Hex	18.6	256, 268 sh, 350	447					357 (25)	327 (100)
26	Lut-6-*C*-Hex	20.1	256, 268 sh, 350	447	429 (10)			387 (11)	357 (100)	327 (96)
31	Api-8-*C*-Hex	22.3	269, 338	431					341 (17)	311 (100)
34	Api-6-*C*-Hex	26.1	270, 336	431	413 (13)				341 (40)	311 (100)
35	Chrys-8-*C*-Hex	26.8	*c*	461					371 (2)	341 (100)
36	Chrys-6-*C*-Hex	27.3	256 sh, 270, 346	461	443 (4)				371 (40)	341 (100)
					flavones di-*C*-glycosides					
					−18	−60	−90	−120	Aglc + 113	Aglc + 83
9	Lut-6,8-di-*C*-Hex	10.4	258 sh, 272, 346	609	591 (2)		519 (16)	489 (100)	399 (25)	369 (12)
12	Lut-6-*C*-Hex-8-*C*-Pent	13.5	258 sh, 270, 348	579	561 (20)	519 (2)	489 (11)	459 (100)	399 (40)	369 (19)
13	Api-6,8-di-*C*-Hex	13.8	*c*	593	575 (1)		503 (25)	473 (100)	383 (50)	353 (50)
14	Lut-6-*C*-Hex-8-*C*-Pent	14.4	258 sh, 270, 348	579	561 (7)	519 (7)	489 (49)	459 (100)	399 (32)	369 (27)
15	Lut-6-*C*-Pent-8-*C*-Hex	15.1	258 sh, 270, 348	579	561 (7)	519 (6)	489 (100)	459 (3)	399 (72)	369 (48)
17	Api-6-*C*-Pent-8-*C*-Hex	16.4	*c*	563	545 (20)	503 (65)	473 (75)	443 (50)	383 (78)	353 (100)
18	Api-6-*C*-Pent-8-*C*-Hex	16.9	258 sh, 270, 346	563	545 (10)	503 (50)	473 (58)	443 (38)	383 (89)	353 (100)
21	Api-6-*C*-Pent-8-*C*-Hex	17.7	258 sh, 270, 346	563	545 (13)	503 (62)	473 (83)	443 (12)	383 (100)	353 (39)
22	Lut-6-*C*-Pent-8-*C*-Hex	18.2	*c*	579	561 (12)	519 (18)	489 (100)	459 (12)	399 (35)	369 (25)
24	Api-6-*C*-Hex-8-*C*-Pent	19.0	258 sh, 270, 346	563	545 (7)	503 (10)	473 (55)	443 (85)	383 (86)	353 (100)
25	Chrys-6-*C*-Hex-8-*C*-Pent	19.1	*c*	593	575 (1)		503 (15)	473 (100)	413 (59)	383 (74)
29	Diosmt-6-*C*-Hex-8-*C*-Pent	21.5	*c*	593	575 (9)		503 (54)	473 (90)	413 (30)	383 (100)
33	Api-6-*C*-Hex-8-*C*-Pent	24.4	258 sh, 270, 346	563	545 (13)	503 (2)	473 (45)	443 (100)	383 (29)	353 (77)
					flavones mono-*C*-(*O*-glycosyl)glycosides					
					−90	−120	−150	−180	Aglc + 71	Aglc + 41
11	Lut-6-*C*-(6-*O*-Hex)Hex	12.1	256, 270, 348	609					357 (100)	327 (98)
16	Api-6-*C*-(6-*O*-Hex)Hex	16.4	*c*	593					341 (19)	311 (100)
27	Lut-6-*C*-(2-*O*-Pent)Hex[b]	20.4	*c*	579		459 (72)	429 (100)		357 (52)	327 (68)[d]
32	Api-8-*C*-(2-*O*-Pent)Hex	23.8	270, 336	563	473 (12)	443 (14)	413 (100)		341 (7)	311 (1)[d]
					flavones di-*C*-glycoside-*O*-glycosylated					
					−18	−60	−90	−120	−162	−180
8	Api-6-*C*-Pent-8-*C*-Hex-7-*O*-Hex[e]	9.5	*c*	725	707 (4)	665 (17)	635 (14)	605 (33)	563 (100)	
19	Lut-6-*C*-(3-*O*-Hex)Hex-8-*C*-Pent[f]	16.9	*c*	741	723 (9)	681 (21)	651 (26)		579 (38)	561 (100)
28	Api-6-*C*-Pent-8-*C*-(2-*O*-Hex)Hex[g]	21.2	*c*	725		665 (1)	635 (7)	605 (5)	563 (100)	545 (11)
30	Api-6-*C*-(2-*O*-Hex)Hex-8-*C*-Pent[h]	21.7	*c*	725	707 (4)		635 (12)	605 (9)	563 (100)	545 (20)
					flavones *O*-glycosides					
								−146	−164	Agl − H
37	Lut-7-*O*-Rhmn(1→2)Hex	28.2	257, 266 sh, 348	593				447 (60)	429 (8)	285 (100)
38	Chrys-7-*O*-Hex	31.6	*c*	461						299 (100)
39	Chrys-7-*O*-Rhmn(1→6)Hex	32.2	255, 266, 348	607						299

[a]Main observed fragments. Other ions were found, but they have not been included. [b]Lut, luteolin; Api, apigenin; Chrys, chrysoeriol; Diosmt, diosmetin; Hex, hexoside; Pent, pentoside; and Rhmn, rhamnoside. [c]Coelutes with other compounds or are in trace amounts and its spectrum cannot be properly observed. [d]Other observed ions (Aglc + 41-18): (27) m/z 309 (10%) and (32) m/z 293 (45%). [e](**8**) $-MS^3$ (725 → 563): 545 (23, −18), 503 (65, −60), 473 (62, −90), 443 (53, −120), 383 (100, Aglc + 113), 353 (62, Aglc + 83). [f](**19**) $-MS^3$ (741 → 579): 561 (4, −18), 489 (22, −90), 459 (100, −120), 399 (45, Aglc + 113), 369 (21, Aglc + 83). [g](**28**) $-MS^3$ (725 → 563): 545 (19, −18), 503 (54, −60), 473 (59, −90), 443 (50, −120), 383 (86, Aglc + 113), 353 (100, Aglc + 83). [h](**30**) $-MS^3$ (725 → 563): 545 (9, −18), 503 (26, −60), 473 (42, −90), 443 (22, −120), 441 (100, −120 − 2H), 383 (40, Aglc + 113), 353(40, Aglc + 83).

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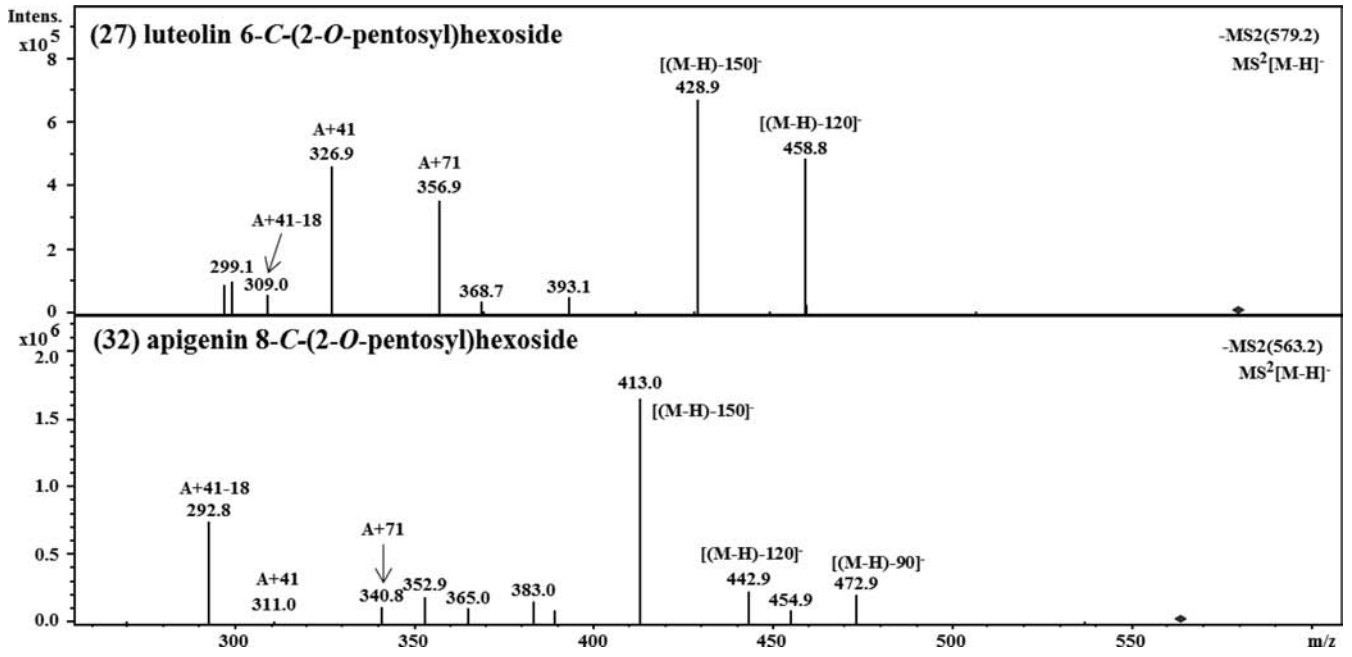


Figure 2. $MS^2[M - H]^-$ analysis of luteolin-6-*C*-(2-*O*-pentosyl)hexoside (**27**) and apigenin-8-*C*-(2-*O*-pentosyl)hexoside (**32**).

the collision gas, with voltage ramping cycles from 0.3 up to 2 V. MS data were acquired in the negative ionization mode. MS^2 was carried out in the automatic mode on the most abundant fragment ions in MS. MS^3 was carried out in the manual mode.

Compounds in Figure 1A were numbered following elution order (compounds **1**−**39**). In Figure 1B, the same criteria were used; therefore, compounds that had been previously labeled kept the same number. Table 1 shows the compounds grouped by the type of structural substitution (mono-*C*-glycosylflavonoids, di-*C*-glycosylflavonoids, and *O*-glycosyl-*C*-glycosylflavonoids) and following their elution order.

HPLC-DAD Quantitative Analyses. *Phenolics Quantification.* Redissolved aqueous extract (ca. 50 mg/mL in water) was analyzed on an analytical HPLC-DAD unit (Gilson) using a Luna C18 column (250 mm × 4.6 mm, 5 μm particle size; Phenomenex). The mobile phase consisted of two solvents: water−acetic acid (1%) (A) and methanol (B), starting with 20% B and using a gradient to obtain 35% B at 30 min, 50% B at 40 min, 90% B at 42 min, and 100% B at 50 min. The flow rate was 1 mL/min. Spectral data from all peaks were collected in the range of 200−400 nm, and chromatograms were recorded at 320 nm for hydroxycinnamic acids and at 340 nm for flavonoids. The data were processed on Unipoint System software.

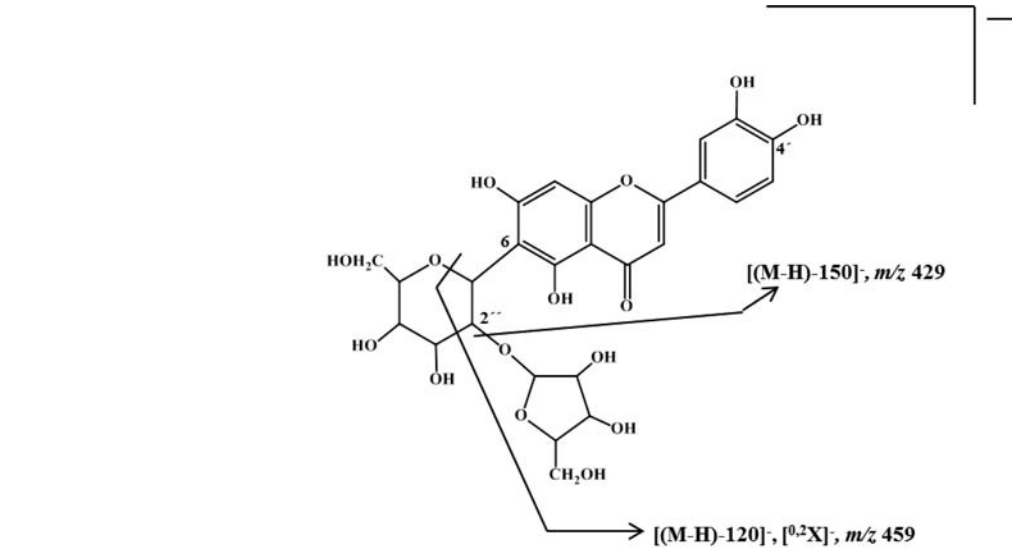
Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external calibration standards. Because there is no commercially available standard, di-*C*-glycosides, mono-C-(*O*-glycosyl)glycosides, and di-*C*-(*O*-glycosyl)-glycosides of luteolin, apigenin, and chrysoeriol were quantified as luteolin-3′,7-di-*O*-glucoside, apigenin-6-C-glucoside-7-*O*-glucoside, and chrysoeriol, respectively. Luteolin-7-*O*-rhamnosyl(1→2)hexoside was also determined as luteolin-3′,7-di-*O*-glucoside and chrysoeriol-7-*O*-rhamnosyl(1→6)hexoside as chrysoeriol. For the same reason, caffeic acid derivatives were quantified as caffeic acid and caffeoylquinic acid derivatives as 5-*O*-caffeoylquinic acid. In the cases of coelution, the UV spectra enabled the identification of the most abundant compound. Thus, apigenin-6-*C*-(6-*O*-hexosyl)hexoside, apigenin-6-*C*-pentoside-8-*C*-hexoside isomers, and luteolin-6-*C*-(3-*O*-hexosyl)hexoside-8-*C*-pentoside (compounds **16**−**19**) were quantified together as apigenin-6-*C*-glucoside-7-*O*-glucoside, as well as the pair diosmetin-6-*C*-hexoside-8-*C*-pentoside plus apigenin-6-*C*-(2-*O*-hexosyl)hexoside-8-C-pentoside (**29** + **30**); the pair luteolin-6-*C*-hexoside plus luteolin-6-*C*-(2-*O*-pentosyl)hexoside (**26** + **27**) was quantified as luteolin-6-*C*-glucoside; compound **41** (dihydrocaffeoylquinic acid derivative) and compound **9** (luteolin 6,8-di-*C*-hexoside) were quantified together as 5-*O*-caffeoylquinic acid. The other compounds were quantified as themselves.

Method Validation. The "giant white" variety was used, as it was the sample available in higher amounts. The HPLC-DAD method was validated using the reference standards of three phenolic acids (caffeic, 5-*O*-caffeoylquinic, and *p*-coumaric acids), six flavonoid glycosides (apigenin-8-*C*-glucoside, apigenin-6-*C*-glucoside, luteolin-8-*C*-glucoside, luteolin-6-*C*-glucoside, apigenin-6-*C*-glucoside-7-*O*-glucoside, and luteolin-3′,7-*O*-diglucoside), and one free flavonoid (chrysoeriol). Linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy were determined. The calibration curves were obtained by the external standard method, by injecting five concentration levels of standard solutions (8.5−68 μg/mL for luteolin-8-*C*-glucoside, 27.1−217 μg/mL for luteolin-6-*C*-glucoside, 2.34−18.75 μg/mL for apigenin-8-*C*-glucoside, 4.13−33 μg/mL for apigenin-6-*C*-glucoside, 6.38−102 μg/mL for apigenin-6-*C*-glucoside-7-*O*-glucoside, 6.86−110 μg/mL for luteolin-3′,7-*O*-diglucoside, 1.03−66 μg/mL for chrysoeriol, 4.13−132 μg/mL for caffeic acid, 5.63−90 μg/mL for 5-*O*-caffeoylquinic acid, and 7.5−60 μg/mL for *p*-coumaric acid) three times. Chromatographic peak areas were recorded at 320 nm for hydroxycinnamic acids and at 340 nm for flavonoids and were plotted against the known concentrations of the standard solutions. LOD and LOQ were calculated from the residual standard deviation of the regression (σ) line and the slope (S), as follows: LOD = $3.3\sigma/S$; LOQ = $10\sigma/S$.

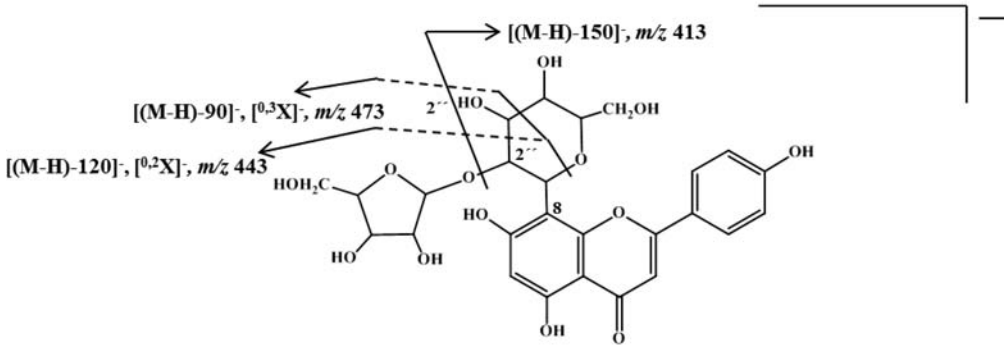
Repeatability (intraday assay) was assessed by triplicate analysis of three different concentrations of standard solutions in the same day and expressed as relative standard deviation (RSD). The intermediate precision (interday assay) was evaluated by analyzing the middle concentration of the curve three times a day, on three different days; the RSD of the peak area was calculated as a measure of interday precision.

Recoveries were determined by adding to the sample low, medium, and high amounts of compounds found in it: caffeic acid (3.00, 8.00, and 13.00 μg/mL), *p*-coumaric acid (3.00, 8.00, and 13.00 μg/mL), apigenin-8-*C*-glucoside (2.00, 7.00, and 12.00 μg/mL), apigenin-6-*C*-glucoside (4.00, 9.00, and 14.00 μg/mL), luteolin-8-*C*-glucoside (2.00, 4.00, and 6.00 μg/mL), and luteolin-6-*C*-glucoside (50.00, 100.00, and 150.00 μg/mL).

Statistical Analysis. Data were analyzed using GraphPad Prism software (version 5.02 for Windows). Two-way analysis of variance



(27) luteolin-6-*C*-(2-*O*-pentosyl)hexoside



(32) apigenin-8-*C*-(2-*O*-pentosyl)hexoside

Figure 3. MS fragmentation scheme of luteolin-6-*C*-(2-*O*-pentosyl)hexoside (**27**) and apigenin 8-*C*-(2-*O*-pentosyl)hexoside (**32**).

(ANOVA), using the Bonferroni's multiple comparison test, was carried out on data obtained from triplicate determinations of each sample. A level of statistical significance set at $p < 0.05$ was used.

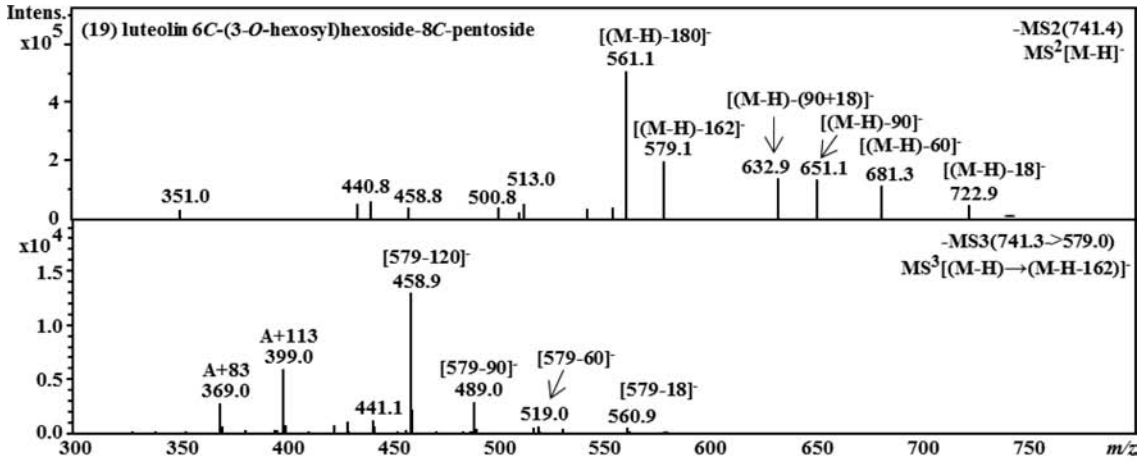
RESULTS AND DISCUSSION

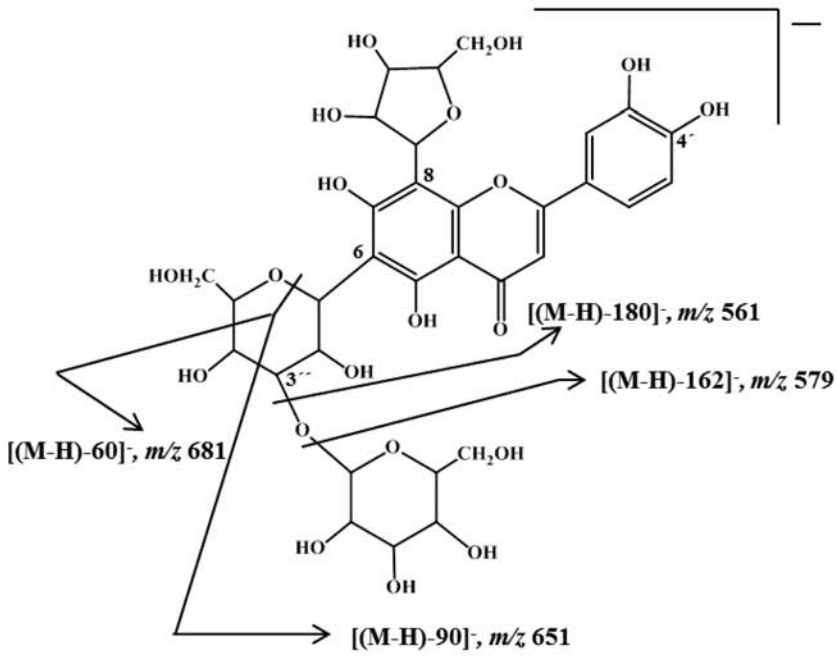
Characterization of Phenolic Compounds. The HPLC-DAD-ESI/MS^n study of the aqueous extract of the leaves from the two varieties of *C. esculenta* ("giant white" and "red") showed the presence of a high number of hydroxycinnamic acid derivatives (**1**−**7**, **10**, **20**, **40**, and **41**) and glycosylflavonoids (**8**, **9**, **11**−**19**, and **21**−**39**), their chromatographic profile being quite similar, differing only in the relative abundance and the presence or absence of some of the acyl derivatives (Figure 1A,B). The anthocyanins described in previous works[13,15] were not detected in the analyzed samples.

Hydroxycinnamic Acid Derivatives. A series of isomers with UV spectra characteristics of caffeoyl derivatives (UV: 298 sh, 326 nm) and deprotonated molecular ion at m/z 371 was detected (compounds **1**−**6**, R_t: 4.4, 5.2, 5.4, 6.4, 6.7, and 7.1 min). Their MS^2 fragmentations showed ions with m/z 353 (10%, −18), 209 (100%, −162), and 191 (20%, −162−18), indicating that these compounds could be caffeoylglucaric acid isomers. Compound **1** was not detected in the "red" variety. D-Glucaric acid is found in fruits, vegetables, and mammals. It is available as a dietary supplement in the form of calcium D-glucarate and has been studied for therapeutic purposes, including cholesterol reduction and cancer chemotherapy.[16]

Compound **7** was characterized as glucosylsinapic acid {R_t: 7.8 min; UV: 292 sh, 330 nm; MS: 385 $[M - H]^-$, MS^2 (385): 223 (100%, −162)}. Compounds **10** and **20** were characterized as caffeic and *p*-coumaric acids, respectively (**10**, R_t: 11.0 min; UV: 298 sh, 325 nm; MS: 179 $[M - H]^-$, MS^2 (179): 134 (100%, −45); **20**, R_t: 17.2 min; UV: 296 sh, 310 nm; MS: 119 $[(M - H) - 44]^-$). Compounds **40** and **41**, which were found only in "red" variety (Figure 1), are dihydrocaffeoylquinic acid isomers, showing the same UV and mass spectrum {**40**, R_t: 9.1 min; **41**, R_t: 10.3 min; UV: 298 sh, 318 nm; MS: 355 $[M - H]^-$, MS^2 (355): 191 (100%, −164)}.

Flavonoids. The two studied varieties present the same flavonoids chromatographic profile, differing only in their





(19) luteolin-6-*C*-(3-*O*-hexosyl)hexoside-8-*C*-pentoside, $[M-H]^-$, *m/z* 723

Figure 4. $MS^2[M - H]^-$ and $-MS^3[(M - H) \rightarrow (M - H - 162)]^-$ analysis and −MS fragmentation scheme of luteolin-6-*C*-(3-*O*-hexosyl)hexoside-8-*C*-pentoside (**19**).

abundance. Nineteen *C*-glycosylflavones (compounds **9**, **12**−**15**, **17**, **18**, **21**−**26**, **29**, **31**, and **33**−**36**), eight *O*-glycosyl-*C*-glycosylflavones (compounds **8**, **11**, **16**, **19**, **27**, **28**, **30**, and **32**), and three *O*-glycosylflavones (compounds **37**−**39**) were detected (Figure 1), all of them presenting UV spectrum characteristic of flavonoids[17] (Table 1).

The MS of compounds **23**, **26**, **31**, and **34**−**36** showed typical fragmentations of mono-*C*-hexosylflavones with losses of 90 and 120 amu matching with the ions (Aglc + 71) and (Aglc + 41) that characterize the aglycones of mono-*C*-glycosylflavones:[18] **23**/**26** luteolin (Aglc + 71/41, *m/z* 357, 327), **31**/**34** apigenin (*m/z* 341, 311), **35**/**36** trihydroxy-methoxy-flavone (*m/z* 371, 341) probably chrysoeriol (Table 1). The order of elution, as well as the observation of water loss (18 amu) in compounds **26**, **34**, and **36**, indicate that the *C*-glycosylation is at C-6, while for compounds **23**, **31**, and **35**, it is at C-8.[18] The detection of orientin (luteolin-8-*C*-glucoside), isoorientin (luteolin-6-*C*-glucoside), vitexin (apigenin-8-*C*-glucoside), and isovitexin (apigenin-6-*C*-glucoside) in *C. esculenta* reported by Iwashina et al.[14] and Leong et al.[4] confirms the identity of compound **23** as orientin, **26** as isoorientin, **31** as vitexin, **34** as isovitexin, and, tentatively, **35** as scoparin (chrysoeriol-8-*C*-glucoside) and **36** as isoscoparin (chrysoeriol-6-*C*-glucoside).

Compounds **9** and **13** are 6,8-di-*C*-hexosyl derivatives of luteolin and apigenin, respectively, as demonstrated by the presence of the ions (Aglc + 113) and (Aglc + 83) (Table 1), which characterize the aglycones of di-*C*-glycosylflavones.[18] As Iwashina et al.[14] have also detected apigenin-6,8-di-*C*-glucoside,

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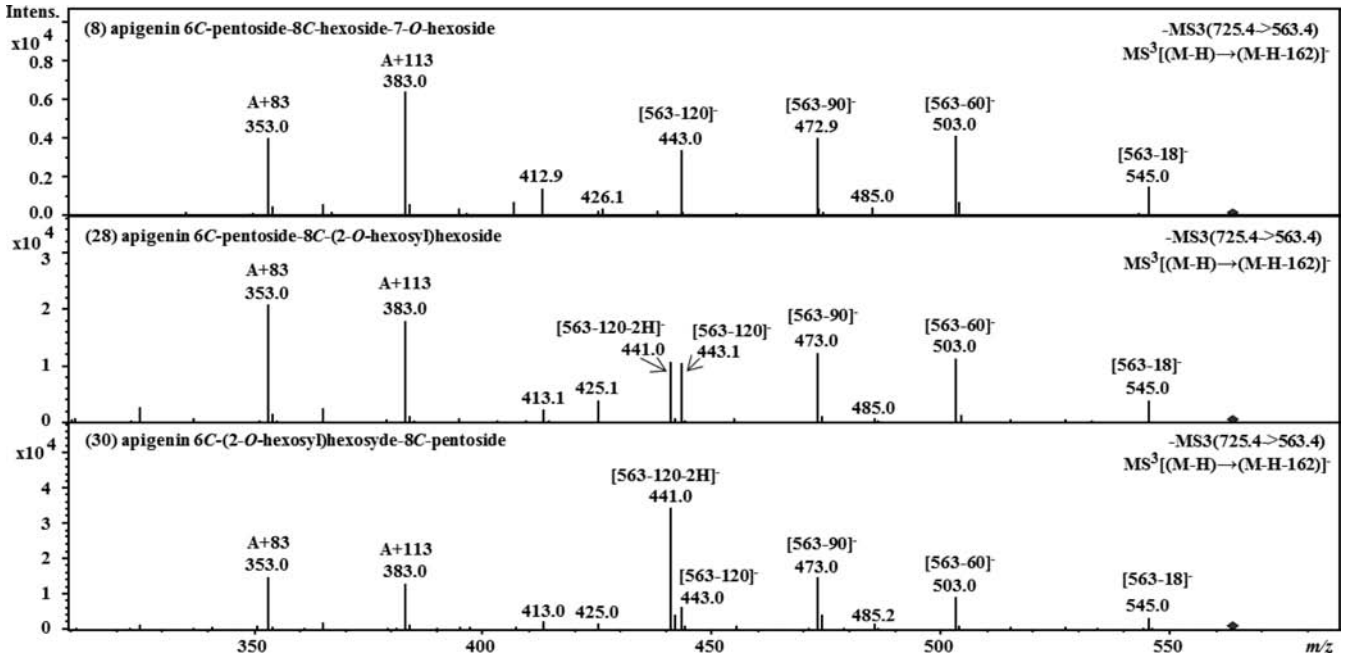

Figure 5. $MS^3[(M - H) \rightarrow (M - H - 162)]^-$ analysis of apigenin-6-*C*-pentoside-8-*C*-hexoside-7-*O*-hexoside (**8**), apigenin-6-*C*-pentoside-8-*C*-(2-*O*-hexosyl)hexoside (**28**), and apigenin-6-*C*-(2-*O*-hexosyl)hexoside-8-*C*-pentoside (**30**).

Table 2. Regression Equations, LOD, and LOQ for Phenolic Compounds HPLC-DAD Analysis

			μg/mL	
compd	regression equations	r^2	LOD	LOQ
caffeic acid	$2.18 \times 10^9 x - 1.93 \times 10^6$	0.99	0.05	0.16
5-*O*-caffeoylquinic acid	$1.42 \times 10^9 x - 6.38 \times 10^5$	0.99	0.25	0.77
p-coumaric acid	$2.20 \times 10^9 x + 6.65 \times 10^5$	0.99	0.98	3.27
apigenin-8-*C*-glucoside	$6.34 \times 10^8 x - 2.44 \times 10^4$	0.99	0.23	0.71
apigenin-6-*C*-glucoside	$1.04 \times 10^9 x + 5.63 \times 10^4$	0.99	0.30	0.91
luteolin-8-*C*-glucoside	$8.08 \times 10^8 x + 1.29 \times 10^6$	0.98	0.04	0.13
luteolin-6-*C*-glucoside	$1.05 \times 10^9 x + 2.50 \times 10^6$	0.99	1.11	3.36
apigenin-6-*C*-glucoside-7-*O*-glucoside	$7.70 \times 10^8 x + 2.95 \times 10^5$	0.99	0.01	0.03
luteolin-3′,7-di-*O*-glucoside	$6.63 \times 10^8 x + 5.38 \times 10^5$	0.99	0.11	0.33
chrysoeriol	$1.53 \times 10^9 x + 2.88 \times 10^5$	0.99	0.04	0.11

compounds **9** and **13** can correspond to luteolin-6,8-di-*C*-glucoside (lucenin-2) and apigenin-6,8-di-*C*-glucoside (vicenin-2), respectively.

Compounds **12**, **14**, **15**, and **22** are isomers, presenting a deprotonated molecular ion at *m/z* 579 and ions that characterize their aglycone as luteolin (Aglc + 113/83, *m/z* 399/369), being, therefore, luteolin-*C*-hexoside-*C*-pentoside isomers. Their MS^2 fragmentation exhibited the ions $[(M - H) - 18]^-$, $[(M - H) - 60]^-$, $[(M - H) - 90]^-$ and $[(M - H) - 120]^-$, the latter being very abundant (base peak) in **12** and **14** and of low abundance in **15** and **22** (Table 1). Therefore, taking into account that the sugar at C-6 is the one which undergoes preferential fragmentation,[18] compounds **12** and **14** may be

Table 3. Repeatability and Intermediate Precision for Phenolic Compounds HPLC-DAD Analysis

	repeatability		intermediate precision	
compd	concn (μg/mL)	RSD (%)	concn (μg/mL)	RSD (%)
	4.13	0.39		
caffeic acid	16.50	0.68	16.50	0.71
	45.00	2.30		
	5.63	1.68		
5-*O*-caffeoylquinic acid	45.00	2.98	45.00	3.11
	90.00	3.31		
	7.50	3.83		
p-coumaric acid	15.00	3.67	15.00	3.69
	30.00	3.64		
	2.30	3.04		
apigenin-8-*C*-glucoside	4.70	2.29	4.70	2.35
	9.40	0.56		
	4.10	3.58		
apigenin-6-*O*-glucoside	8.30	4.12	8.30	3.75
	16.50	3.42		
	8.50	0.21		
luteolin-8-*C*-glucoside	17.00	0.99	17.00	1.23
	50.00	0.87		
	2.70	1.76		
luteolin-6-*C*-glucoside	5.40	1.74	5.40	1.32
	11.00	4.17		
	6.40	0.05		
apigenin-6-*C*-glucoside-7-*O*-glucoside	25.50	0.97	25.50	0.95
	51.00	1.69		
	6.88	0.56		
luteolin-3′,7-di-*O*-glucoside	27.50	2.49	27.50	2.03
	55.00	2.60		
	1.03	1.14		
chrysoeriol	8.25	4.31	8.25	3.97
	33.00	2.21		

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Table 4. Recovery of Phenolic Compounds

compd	recovery		
	concn (μg/mL)	mean (%)	RSD (%)
caffeic acid	3.00	72.40	0.92
	8.00	133.90	4.49
	13.00	68.74	3.06
p-coumaric acid	3.00	87.78	3.12
	8.00	79.14	4.49
	13.00	70.33	3.70
apigenin-8-*C*-glucoside	2.00	116.50	3.27
	7.00	136.30	6.99
	12.00	85.44	4.54
apigenin-6-*O*-glucoside	4.00	122.10	13.80
	9.00	123.10	15.30
	14.00	88.59	3.63
luteolin-8-*C*-glucoside	2.00	97.34	7.43
	4.00	132.10	5.94
	6.00	71.72	3.66
luteolin-6-*C*-glucoside	50.00	119.10	3.77
	100.00	118.60	2.94
	150.00	80.70	1.51

characterized as luteolin-6-*C*-hexoside-8-*C*-pentosides and **15** and **22** as luteolin-6-*C*-pentoside-8-*C*-hexosides.

In a similar way, compounds **17**, **18**, **21**, **24**, and **33** are apigenin-*C*-hexoside-*C*-pentoside derivatives (Aglc + 113/83, *m*/*z* 383/353). The high abundance of the ions $[(M - H) - 60]^-$ and/or $[(M - H) - 90]^-$ in **17**, **18**, and **21** indicates the substitution with a pentose at position 6, while in **24** and **33**, the ion $[(M - H) - 120]^-$ is very abundant and characteristic of an hexose at the 6-position (Table 1). Thus, compounds **17**, **18**, and **21** can be labeled as apigenin-6-*C*-pentoside-8-*C*-hexosides and **24** and **33** as apigenin-6-*C*-hexoside-8-*C*-pentosides. Leong et al.[4] had previously described the occurrence of schaftoside (apigenin-6-*C*-glucoside-8-*C*-arabinoside) and isoschaftoside (apigenin-6-*C*-arabinoside-8-*C*-glucoside) in *C. esculenta*.

Compounds **25** and **29** are trihydroxy-methoxyflavone-6-*C*-hexoside-8-*C*-pentosides ($[M - H]^-$, *m*/*z* 593; Aglc + 113/83, *m*/*z* 413/383 and abundant $[(M - H) - 120]^-$, Table 1), probably chrysoeriol (5,7,4′-trihydroxy-3′-methoxyflavone) and diosmetin (5,7,3′-trihydroxy-4′-methoxyflavone) derivatives. Chrysoeriol derivatives elute before the corresponding diosmetin isomers;[19] on the other hand, the presence of an ion at *m*/*z* 563 ($[(MH) - 30]^-$) in compound **29** with an abundance of 15 versus 2% in compound **25** (data not shown in Table 1) confirms the presence of a 4′-methoxy group in compound **29** and characterizes these compounds as chrysoeriol-6-*C*-hexoside-8-*C*-pentoside (**25**) and diosmetin-6-*C*-hexoside-8-*C*-pentoside (**29**).

The MS^2 fragmentation of compounds **11**, **16**, **27**, and **32** presents the ions Aglc + 71/41, characterizing them as flavonoids mono-*C*-glycosides. Because their $[M - H]^-$ are characteristic of flavonoid diglycosides (Table 1), these compounds are *C*-glycosylflavonoid-*O*-glycosylated.

Compounds **11** and **16** are luteolin and apigenin-*C*-(*O*-hexosyl)hexosides, respectively, and their MS^2 spectra show no fragment between the deprotonated molecular ion and the Aglc + 71, indicating an *O*-glycosylation at position 6 of the sugar of the *C*-glycosylation, since the interglycosidic linkage (1→6) is difficult to break, and only the internal cleavage of the sugar of the *C*-glycosylation by positions 0,2 to yield the ions $[^{0,2}X]^-$ ($[(M - H) - (120 + 162)]^-$, Aglc + 41) and $[^{0,3}X]^-$ ($[(M - H) - (90 + 162)]^-$, Aglc + 71) is observed.[20] Therefore, these compounds will be 6″-*O*-hexosyl derivatives of **23**/**26** and **31**/**34**. The high abundance of **26** and **34** indicates that they could be tentatively labeled as luteolin-6-*C*-(6-*O*-hexosyl)hexoside (**11**) and apigenin-6-*C*-(6-*O*-hexosyl)hexoside (**16**).

Regarding compounds **27** and **32**, in addition to the ions characteristic of mono-*C*-glycosyl-flavones, a very abundant ion was also observed $[(M - H) - (132 + 18)]^-$, which indicates the presence of a pentose linked to a nonphenolic hydroxyl group, thus belonging to the sugar involved in the *C*-glycosylation (Table 1). The low abundance of the ions arising from the fragmentation of the sugar moiety of *C*-glycosylation in compound **32** suggests that it is located at the 8-position, while in **27** it is located at C-6 (Table 1 and Figure 2). The loss of 120 amu from the deprotonated molecular ion indicates that positions 3−6 of the sugar moiety at *C*-glycosylation are free; therefore, the pentose is attached to the hydroxyl group at position 2 of the hexose (Figure 3). Accordingly, these compounds could be labeled as luteolin-6-*C*-(2-*O*-pentosyl)-hexoside (**27**) and apigenin-8-*C*-(2-*O*-pentosyl)hexoside (**32**). Iwashina et al.[14] detected in *C. esculenta* two *O*-glycosylated derivatives of orientin and isovitexin and vitexin-X″-glucoside that do not match with those characterized in this work.

Four flavonoids triglycosides were also detected in this work (compounds **19**, $[M - H]^-$ *m*/*z* 741 and **8**, **28**, and **30**, *m*/*z* 725), and their MS^3 spectra enabled the identification of the ions that characterize the aglycones of di-*C*-glycosyl derivatives (Aglc + 113/83, **19**: *m*/*z* 399/366, luteolin; **8**, **28**, and **30**: *m*/*z* 383/353, apigenin) (data shown at the footnote of Table 1), thus being di-*C*-glycosylflavonoids-*O*-glycosylated. In addition to some ions from the fragmentation of the *C*-glycosylation sugars, the MS^2 spectra of **19**, **28**, and **30** showed the ions $[(M - H) - 162]^-$ and $[(M - H) - 180]^-$ in high abundance, indicating the *O*-glycosylation with hexose of a nonphenolic hydroxyl, that is, interglycosidic linkage on *C*-glycosylation sugars. On the other hand, for compound **8**, the ion $[(M - H) - 180]^-$ was not noticed, pointing to the *O*-glycosylation of a phenolic hydroxyl group.[20]

The MS^3 spectra of the ion produced by the loss of the *O*-hexosyl radical ($MS^3[(M - H) \rightarrow (M - H - 162)]^-$) of compound **19** (data shown at the footnote of Table 1 and Figure 4, MS^3 analysis) is characteristic of luteolin-6-*C*-hexoside-8-*C*-pentoside, with a base peak produced by loss of 120 amu ($[(M - H - 162) - 120]^-$). However, in the MS^2 spectra of **19** (Table 1 and Figure 4, MS^2 analysis), the ion $[(M - H) - 120]^-$ was not observed, indicating that the *O*-glycosylation occurs in a hydroxyl of the 6-*C*-hexose distinct from the 2″-position. The presence of the ions $[(M - H) - 90]^-$ and $[(M - H) - 60]^-$ confirms the position of the *O*-glycosylation in 3″ (Figure 4). Hence, compound **19** was characterized as luteolin-6-*C*-(3-*O*-hexosyl)hexoside-8-*C*-pentoside.

As already done for compound **19**, for the elucidation of compounds **8**, **28**, and **30**, *O*-glycosylated derivatives of apigenin-*C*-hexoside-*C*-pentoside, it is appropriate to invoke the MS^3 $[(M - H) \rightarrow (M - H - 162)]^-$ (data shown at the footnote of Table 1 and Figure 5, MS^3 analysis), that is, the fragmentation of the ion produced by the loss of the *O*-hexosyl radical. The MS^3 of **8** and **28**, showing abundant $[(M - H) - 60]^-$ ions, is typical of 6-*C*-pentoside-8-*C*-hexoside, while that of compound **30**, with an abundant $[(M - H) - 120 - 2H]^-$ ion, corresponds to 6-*C*-hexoside-8-*C*-pentoside. Thus, compound **8**, which, as mentioned above, presents one *O*-glycosylation on the phenolic hydroxyl

Table 5. Phenolic Composition of Varieties of *C. esculentum* Leaves (mg/kg Dry Basis)[a]

	compd[b]	concn "Giant white" var.	concn "Red" var.
	phenolic acids		
1	Caf acid derivative	119.89 ± 4.49 a	NI b
2	Caf acid derivative	70.70 ± 1.94 a	61.96 ± 1.64 a
3	Caf acid derivative	158.06 ± 6.99 a	136.38 ± 6.79 a
4	Caf acid derivative	219.10 ± 9.79 a	171.67 ± 6.99 a
5	Caf acid derivative	155.41 ± 10.3 a	223.49 ± 21.14 b
6	Caf acid derivative	212.87 ± 15.9 a	152.22 ± 11.63 b
7	Sin Hex	NQ a	NQ a
10	Caf acid	129.49 ± 2.25 a	58.19 ± 2.23 b
20	*p*-Coum acid	139.17 ± 4.04 a	362.75 ± 36.02 b
40	Dihydrocaf quinic acid derivative	NI	421.44 ± 12.72
41	Dihydrocaf quinic acid derivative	NI	310.40 ± 12.82
	flavones mono-*C*-glycosides		
23	Lut-8-*C*-Hex	833.37 ± 53.39 a	620.59 ± 16.75 b
26	Lut-6-*C*-Hex	1836.56 ± 100.77 a	1412.78 ± 76.61 b
31	Api-8-*C*-Hex	160.78 ± 9.62 a	143.23 ± 9.13 a
34	Api-6-*C*-Hex	299.97 ± 15.14 a	289.84 ± 12.86 a
35	Chrys-8-*C*-Hex	33.11 ± 0.33 a	NQ a
36	Chrys-6-*C*-Hex	100.80 ± 5.35 a	95.99 ± 7.77 a
	flavones di-*C*-glycosides		
9	Lut-6,8-di-*C*-Hex	NQ	quantified with **41**
12	Lut-6-*C*-Hex-8-*C*-Pent	583.16 ± 4.39 a	662.06 ± 25.96 b
13	Api-6,8-di-*C*-Hex	NQ a	65.18 ± 4.60 b
14	Lut-6-*C*-Hex-8-*C*-Pent	996.87 ± 56.18 a	1087.87 ± 38.93 b
15	Lut-6-*C*-Pent-8-*C*-Hex	276.96 ± 15.44 a	283.96 ± 12.77 a
17	Api-6-*C*-Pent-8-*C*-Hex	43.04 ± 2.04 a	29.79 ± 0.31 a
18	Api-6-*C*-Pent-8-*C*-Hex	quantified with **17**	quantified with **17**
21	Api-6-*C*-Pent-8-*C*-Hex	393.92 ± 27.29 a	446.78 ± 34.46 a
	flavones di-*C*-glycosides		
22	Lut-6-*C*-Pent-8-*C*-Hex	750.51 ± 49.44 a	734.94 ± 37.41 a
24	Api-6-*C*-Hex-8-*C*-Pent	328.15 ± 16.66 a	299.65 ± 8.15 a
25	Chrys-6-*C*-Hex-8-*C*-Pent	NQ a	NQ a
29	Diosmt-6-*C*-Hex-8-*C*-Pent	118.10 ± 7.58 a	142.89 ± 2.86 a
33	Api-6-*C*-Hex-8-*C*-Pent	232.42 ± 13.69 a	187.41 ± 15.77 a
	flavones mono-*C*-(*O*-glycosyl)glycosides		
11	Lut-6-*C*-(6-*O*-Hex) Hex	119.79 ± 3.51 a	177.29 ± 6.38 b
16	Api-6-*C*-(6-*O*-Hex) Hex	quantified with **17**	quantified with **17**
27	Lut-6-*C*-(2-*O*-Pent) Hex	quantified with **26**	quantified with **26**
32	Api-8-*C*-(2-*O*-Pent) Hex	151.94 ± 5.52 a	164.97 ± 15.92 a
	flavones di-*C*-glycoside-*O*-glycosylated		
8	Api-6-*C*-Pent-8-*C*-Hex-7-*O*-Hex	NQ a	NQ a
19	Lut-6-*C*-(3-*O*-Hex) Hex-8-*C*-Pent	quantified with **17**	quantified with **17**
28	Api-6-*C*-Pent-8-*C*-(2-*O*-Hex)Hex	149.49 ± 4.56 a	152.61 ± 4.14 a
30	Api-6-*C*-(2-*O*-Hex) Hex-8-*C*-Pent	quantified with **29**	quantified with **29**
	flavones *O*-glycosides		
37	Lut-7-*O*-Rhmn(1→2) Hex	60.04 ± 4.27 a	96.74 ± 6.52 a
38	Chrys-7-*O*-Hex	NQ a	NQ a
39	Chrys-7-*O*-Rhmn (1→6)Hex	80.28 ± 2.11 a	107.77 ± 5.31 a
Σ		8770.573	9100.89

[a]Results are expressed as means ± standard deviations of three determinations;NI, not identified; NQ, not quantified; in the same row, different lowercase letters mean significant differences ($p < 0.05$). [b]Caf, caffeoyl; Sin, sinapoyl; *p*-Coum, *p*-coumaric; Dihydrocaf, dihydrocaffeoyl; Lut, luteolin; Api, apigenin; Chrys, chrysoeriol; Diosmt, diosmetin; Hex, hexoside; Pent, pentoside; and Rhmn, rhamnoside.

(absence of $[(M - H) - 180]^-$) can be tentatively characterized as apigenin-6-*C*-pentoside-8-*C*-hexoside-7-*O*-hexoside. Compounds **28** and **30** are *O*-glycosyl derivatives over a nondetermined sugar from the *C*-glycosylation, probably on the hydroxyl at position 2 of the hexose, and could be tentatively labeled as apigenin-6-*C*-pentoside-8-*C*-(2-*O*-hexosyl)hexoside (**28**) and apigenin-6-*C*-(2-*O*-hexosyl)hexoside-8-*C*-pentoside (**30**).

Three flavonoids *O*-glycosides eluted at the end of the chromatogram (Figure 1). According to their UV and MS spectra, they were characterized as luteolin-7-*O*-rhamnosyl-(1→2)hexoside (**37**), chrysoeriol-7-*O*-hexoside (**38**), and chrysoeriol-7-*O*-rhamnosyl(1→6)hexoside (**39**) (Table 1).

HPLC-DAD Phenolic Compounds Quantification. *Method Validation.* For the validation of the analytical method, linear regression analysis was performed by using external calibration curves. The regression equations and coefficients of correlation are shown in Table 2. A good linearity was found for all of the analytes peak areas at the tested concentrations ($r^2 \geq 0.98$). The LOD and LOQ values (Table 2) were experimentally verified by the standards solutions. The results indicate that the HPLC-DAD method is sufficiently sensitive and has a good linearity for quantification of phenolic compounds present in low concentrations in *C. esculenta* leaves extracts.

The low intra- and interday variations of peak areas (RSD ≤ 4.31%) indicate a high precision of the chromatographic system (Table 3). The accuracy of the analytical procedure was evaluated by recovery tests, proving to be generally good (Table 4). Thus, the HPLC-DAD method is suitable for the determination of phenolic compounds in *C. esculenta* leaves.

Phenolic Compounds in C. esculenta Leaves. The HPLC-DAD method allowed the quantification of 41 phenolic compounds in a single run. The phenolics content in the aqueous extracts of the two varieties was similar, ca. 9 g/kg (dry basis) (Table 5), although quantitative differences were observed for individual compounds. The phenolic profile of "giant white" and "red" varieties contains, respectively, ca. 14 and 21% of hydroxycinnamic acids, 37 and 28% of flavones-mono-*C*-glycosides, 42 and 43% of flavones-di-*C*-glycosides, 3 and 4% of flavones-mono-*C*-(*O*-glycosyl)glycosides, and both of them ca. 2% of flavones-di-*C*-(*O*-glycosyl)glycosides and 2% of flavones-*O*-glycosides (Figure 6). As mentioned above, some flavones were already described in this species.[4,14] On the other hand, Lako and colleagues reported the presence of flavonols in the leaves of *C. esculenta*.[21] The phenolic fraction of the tuber of *C. esculenta* from Vanuatu was also described to be rich in flavonols like hyperoside and isorhamnetin-3-*O*-glucoside, along with flavanols, such as catechin and epicatechin.[22] Thus, one may speculate the existence of infraspecific chemical variability in this species. Apigenin derivatives, luteolin derivatives, and

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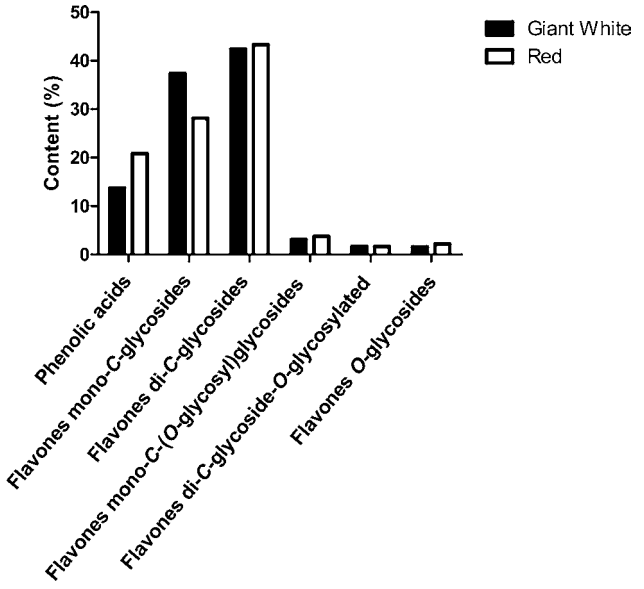

Figure 6. Contents of the different classes of phenolic compounds found in each *C. esculenta* variety.

chrysoeriol derivatives represent ca. 20, 64, and 3% of the determined flavonoids in "giant white" variety, respectively, while in the "red" one, they correspond to ca. 20, 57, and 2%, respectively. Considering phenolic acids, "red" taro extract is richer in hydroxycinnamic acid derivatives than "giant white" one (ca. 12 and 17% for "giant white" taro and "red" taro, respectively). As far as we are aware, this is the first report on the occurrence of hydroxycinnamic acid derivatives in the leaves of *C. esculenta*. Champagne et al.[22] found only traces of caffeoylquinic acid derivatives in the tuber. Luteolin-6-*C*-hexoside (**26**) was the main compound in both varieties, representing ca. 21 and 16% of the determined phenolics in "giant white" and "red" varieties, respectively. Apigenin-6-*C*-glucoside (**34**) was reported to be the main compound in the leaves of a different *C. esculenta* variety (var. *aquatilis*).[4] For some compounds, the differences observed between the two varieties in Figure 1 are not reflected in the quantification data shown in Table 5, once the extractive conditions were distinct. The concentration of compounds **1**, **6**, **10**, **23**, and **26** was significantly higher in "giant white" variety, while the "red" one showed considerably higher contents of compounds **5**, **11**−**14**, and **20**. Hence, it may be inferred that there is an infraspecific quantitative chemical variability, since the samples from the two *C. esculenta* varieties share the same geographical origin.

In conclusion, HPLC-DAD-ESI-MS^n proved to be a useful technique for the characterization of the phenolic composition of two *C. esculenta* varieties. As far as we are aware, among the 41 identified metabolites, 34 are being reported for the first time, thus improving the knowledge on the species. Furthermore, the application of a precise, accurate, and reproducible HPLC-DAD method to the quantification of the identified phenolics allowed finding significant differences between the two varieties. Thus, the validated HPLC-DAD method will not only facilitate the quality control of *C. esculenta* varieties but could also improve the phylogenetic systematics investigation of the distribution of flavonoids in the species. In addition, the compounds identified herein are important for the nutritional value of *C. esculenta*.

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Notes

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