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Phenolic Profile of *Cydonia oblonga* Miller Leaves

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Cydonia oblonga Miller leaves phenolic compounds were analyzed by reversed-phase HPLC/DAD and HPLC/UV. Qualitative and quantitative analysis of phenolics were carried out in a total of 36 samples of quince leaves from three different geographical origins of Northern (Bragança and Carrazeda de Ansiães) and Central Portugal (Covilhã) and three collection months (June, August, and October of 2006). These leaves presented a common phenolic profile composed by nine compounds: 3-*O*-, 4-*O*- and 5-*O*-caffeoylquinic acids, 3,5-*O*-dicafeoylquinic acid, quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside, kaempferol-3-*O*-glycoside, kaempferol-3-*O*-glucoside, and kaempferol-3-*O*-rutinoside. 5-*O*-caffeoylquinic acid was the major phenolic compound (36.2%), followed by quercetin 3-*O*-rutinoside (21.1%). Quince leaves are characterized by higher relative contents of kaempferol derivatives than fruits (pulp, peels, and seeds), especially in what concerns kaempferol-3-*O*-rutinoside (12.5%). *C. oblonga* leaves total phenolic content was very high, varying from 4.9 to 16.5 g/kg dry matter (mean value of 10.3 g/kg dry matter), indicating that these leaves can be used as a good and cheap source of bioactive constituents. Significant differences were observed in 3-*O*-caffeoylquinic and 3,5-*O*-dicafeoylquinic acids contents, according to geographical provenance and harvesting month, suggesting a possible use of these compounds as geographical origin and/or maturity markers.

KEYWORDS: *Cydonia oblonga* Miller; quince leaves; phenolic compounds

INTRODUCTION

Epidemiological studies have consistently shown that there is a clear significant positive association between intake of fruits and vegetables and reduced rate of heart disease mortalities, common cancers, and other degenerative diseases as well as aging (1). This association is often attributed to the antioxidant substances present in these plant products, mainly to phenolics such as phenolic acids and flavonoids (2).

Phenolic compounds are secondary metabolites that are quite widespread in nature. Despite this almost ubiquity, experimental evidence has demonstrated that some of them can work as useful markers for the botanical and geographical origin of several plant foods (wine, coffee beans, and jams). Nevertheless, within each species, the nature of these compounds can vary from organ, to organ and several other factors can contribute to the variability in the phenolics distribution, such as cultivar and

genetics, geographical origin, maturity, climate, position on the tree, and agricultural practices (3).

In the past decade, several studies about *Cydonia oblonga* Miller fruit and its derivatives have been performed by our research group. First, several analytical methods were developed to determine phenolic compounds, organic acids, and free amino acids in quince fruit and jam (3–6). Among these chemical parameters, phenolic profile determination seemed to be the most useful in the discrimination of the different parts of quince fruit (pulp, peel, and seed) (3, 7–9) and in the evaluation of the genuineness of quince puree (10), jam (3, 4, 9, 11, 12), and jelly (13). The influence of jam processing upon the contents of phenolics, organic acids, and free amino acids in quince fruit was evaluated (14), and the antioxidant activity of the methanolic extracts of quince pulp, peel, seed, and jam was also reported (2). Recently, other authors described antioxidant, antimicrobial (antibacterial and anti-influenza viral), and anti-ulcerative properties of quince fruit phenolics (1, 15–18).

The importance of many plants as natural cheap sources of polyphenols and as nutrition promoting human health is well-established (1). As far as we know, for *C. oblonga* leaves, few chemical studies have been developed. Some α - and β -ionol glycosides have been isolated from these leaves and identified

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Table 1. Quince Leaves Samples Characterization

sample identification	geographical origin	collection month
1	Bragança, Pinheiro Manso	June
2	Bragança, Pinheiro Manso	August
3	Bragança, Pinheiro Manso	October
4	Bragança, Quinta	June
5	Bragança, Quinta	August
6	Bragança, Quinta	October
7	Bragança, Tecnologia	June
8	Bragança, Tecnologia	August
9	Bragança, Tecnologia	October
10	Bragança, Vale de Álvaro	June
11	Bragança, Vale de Álvaro	August
12	Bragança, Vale de Álvaro	October
13	Carrazeda de Ansiães, Barrancas	June
14	Carrazeda de Ansiães, Barrancas	August
15	Carrazeda de Ansiães, Barrancas	October
16	Carrazeda de Ansiães, Botelho	June
17	Carrazeda de Ansiães, Botelho	August
18	Carrazeda de Ansiães, Botelho	October
19	Carrazeda de Ansiães, Cortinha	June
20	Carrazeda de Ansiães, Cortinha	August
21	Carrazeda de Ansiães, Cortinha	October
22	Carrazeda de Ansiães, Gorgulão	June
23	Carrazeda de Ansiães, Gorgulão	August
24	Carrazeda de Ansiães, Gorgulão	October
25	Covilhã, Mina	June
26	Covilhã, Mina	August
27	Covilhã, Mina	October
28	Covilhã, Peso	June
29	Covilhã, Peso	August
30	Covilhã, Peso	October
31	Covilhã, Quinta Ortigal	June
32	Covilhã, Quinta Ortigal	August
33	Covilhã, Quinta Ortigal	October
34	Covilhã, Silveira	June
35	Covilhã, Silveira	August
36	Covilhã, Silveira	October

by De Tommasi et al. (19, 20) and Lutz et al. (21). Additionally, the first research group also identified four flavonol glycosides (20).

In Italy, quince leaves have been used in folk medicine for the treatment of various skin diseases, while in Portugal, when submitted to decoction, these leaves have been utilized in traditional medicine because of their sedative, antipyretic, antidiarrheic, and antitussive properties (19).

Interest in edible plants as a source of natural antioxidant prompted us to investigate phenolic constituents of *C. oblonga* leaves. So, in continuation of our investigation on this plant species, the work herein represents a contribution to the composition of quince leaves, concerning their phenolic profile. It was also our purpose to study the possible influence of factors, such as geographical origin and collection month, in the phenolics content.

MATERIALS AND METHODS

Samples. Thirty-six healthy quince leaves samples from Portugal cultivar were collected in four different places in each one of the three geographical origins of northern and central Portugal—Bragança, Carrazeda de Ansiães, and Covilhã—in the beginning of June, August, and October of 2006 (Table 1). Each sample was dried in a stove (Memmert UL6D, Germany) at 30 ± 2 °C for 5 days (in the dark). The mean drying yield was 49.82%.

Standards. 5-*O*-Caffeoylquinic acid and quercetin-3-*O*-rutinoside standards were from Sigma (St. Louis, MO), and quercetin-3-*O*-galactoside, kaempferol-3-*O*-glucoside, and kaempferol-3-*O*-rutinoside standards were from Extrasynthèse (Genay, France). Methanol and formic and hydrochloric acids were obtained from Merck

(Darmstadt, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA).

Solid-Phase Extraction (SPE) Columns. The Chromabond C18 SPE columns (70 mL/10 000 mg) were purchased from Macherey-Nagel (Duren, Germany).

Extraction of Phenolic Compounds. Each dried sample (ca. 0.5 g) was thoroughly mixed with methanol (10 × 25 mL; until negative reaction to 20% NaCl), at 40°C. The methanolic extract was filtered, concentrated to dryness under reduced pressure (40°C), and redissolved in acidic water (pH 2 with HCl; ca. 25 mL). The aqueous solution obtained was passed through an SPE C18 column, previously conditioned with 30 mL of methanol and 70 mL of acidic water (pH 2 with HCl). The phenolic fraction remaining in the column was then eluted with methanol (ca. 50 mL; until negative reaction to 20% NaCl). The methanolic extract was evaporated to dryness under reduced pressure (40°C) and redissolved in methanol (2 mL), and 20 µL were analyzed by HPLC.

HPLC/DAD System for Qualitative Analysis. The extracts were analyzed on an analytical HPLC unit (Gilson), using a C18 Spherisorb ODS2 (25.0 × 0.46 cm; 5 µm particle size) column from Waters (Ireland). The solvent system used was a gradient of water–formic acid (19:1; A) and methanol (B), starting with 5% methanol and installing a gradient to obtain 15% B at 3 min, 25% B at 13 min, 30% B at 25 min, 35% B at 35 min, 45% B at 39 min, 45% B at 42 min, 50% B at 44 min, 55% B at 47 min, 70% B at 50 min, 75 % B at 56 min, and 80% B at 60 min, at a solvent flow rate of 0.9 mL/min (2–4, 8–14). Detection was achieved with a Gilson diode array detector (DAD). Spectral data from all peaks were accumulated in the range 200–400 nm, and chromatograms were recorded at 350 nm. The data were processed on a Unipoint System software (Gilson Medical Electronics, Villiers le Bel, France).

The compounds in each sample were identified by comparing their retention times and UV–vis spectra in the 200–400 nm range with the library of spectra previously compiled by the authors (2–4, 7–14).

HPLC/UV System for Quantitative Analysis. The extracts were analyzed on an analytical HPLC unit (Agilent, 1100 Series), using a C18 Lichrocart 250-4 column (25.0 × 0.40 cm; 5 µm particle size) from Merck (Germany). The solvent system used was the same as that presented in the HPLC/DAD System for Qualitative Analysis section. Detection was achieved with an Agilent (1100 Series) UV detector set at 350 nm.

Phenolics quantification was achieved by the absorbance recorded in the chromatograms relative to external standards. 3- and 4-*O*-caffeoylquinic acids and 3,5-*O*-dicaffeoylquinic acid were quantified as 5-*O*-caffeoylquinic acid. Kaempferol-3-*O*-glycoside was quantified as kaempferol-3-*O*-glucoside. The other compounds were quantified as themselves.

Statistical Analysis. All statistical analyses involving experimental data were performed by using SAS software (9.1 version). The evaluation of statistical significance was determined by ANOVA followed by Tukey LSD test. The level of significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

The leaves of *C. oblonga* Miller presented a chemical profile composed of nine phenolic compounds: 3-*O*-, 4-*O*-, and 5-*O*-caffeoylquinic acids, 3,5-*O*-dicaffeoylquinic acid, quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside, kaempferol-3-*O*-glycoside, kaempferol-3-*O*-glucoside, and kaempferol-3-*O*-rutinoside (Table 2 and Figure 1). Comparing this qualitative profile with that of quince fruit parts (pulp, peels, and seeds), we found that there are some differences. Quince pulps were characterized by the presence of only six of these compounds: 3-*O*-, 4-*O*-, and 5-*O*-caffeoylquinic acids, 3,5-*O*-dicaffeoylquinic acid, quercetin-3-*O*-galactoside, and quercetin-3-*O*-rutinoside (2, 3, 9, 12, 14). Usually, quince peels contained 13 phenolics, the compounds presented in leaves, plus four not totally identified compounds: two quercetin glycosides acylated with *p*-coumaric acid and two kaempferol glycosides acylated with

Table 2. Phenolic Composition of Quince Leaves Samples^a

sample	phenolic compound (%)									Σ (g/kg)
	3-CQA	4-CQA	5-CQA	3,5-diCQA	Q-3-Gal	Q-3-Rut	K-3-Gly	K-3-Glu	K-3-Rut	
1	9.97 ± 0.01	0.18 ± 0.02	33.60 ± 0.08	6.14 ± 0.06	3.48 ± 0.05	21.43 ± 0.11	8.82 ± 0.16	2.02 ± 0.02	14.36 ± 0.15	10.92
2	5.56 ± 0.13	0.36 ± 0.02	32.15 ± 1.92	1.71 ± 0.03	4.33 ± 0.09	25.63 ± 0.12	10.72 ± 0.34	4.24 ± 0.29	15.31 ± 1.13	8.43
3	5.55 ± 0.07	0.39 ± 0.01	32.22 ± 0.50	2.08 ± 0.05	9.48 ± 0.22	22.27 ± 0.17	9.84 ± 0.03	5.26 ± 0.02	12.91 ± 0.31	8.28
4	10.05 ± 0.10	0.39 ± 0.01	36.95 ± 0.32	3.18 ± 0.03	1.99 ± 0.01	23.31 ± 0.16	8.80 ± 0.01	1.45 ± 0.01	13.87 ± 0.05	9.06
5	7.26 ± 0.05	0.20 ± 0.00	33.95 ± 0.23	3.76 ± 0.08	4.77 ± 0.02	23.32 ± 0.18	8.99 ± 0.26	3.13 ± 0.04	14.63 ± 0.05	11.09
6	4.58 ± 0.15	0.41 ± 0.02	32.38 ± 0.96	1.97 ± 0.01	7.92 ± 0.07	23.24 ± 0.54	10.57 ± 0.04	4.64 ± 0.03	14.31 ± 0.21	10.07
7	7.86 ± 0.05	0.17 ± 0.01	33.44 ± 0.33	3.50 ± 0.01	5.54 ± 0.33	25.91 ± 0.40	8.88 ± 0.47	1.60 ± 0.10	13.09 ± 0.57	11.61
8	7.39 ± 0.06	0.19 ± 0.03	31.78 ± 0.22	3.77 ± 0.12	7.36 ± 0.03	23.92 ± 0.08	8.76 ± 0.01	3.57 ± 0.02	13.26 ± 0.02	9.69
9	4.91 ± 0.01	0.30 ± 0.01	37.29 ± 0.41	2.79 ± 0.02	9.91 ± 0.08	20.72 ± 0.06	8.81 ± 0.20	4.04 ± 0.01	11.22 ± 0.03	11.72
10	14.63 ± 0.13	0.70 ± 0.01	30.76 ± 0.17	3.69 ± 0.10	1.60 ± 0.02	28.39 ± 0.43	8.22 ± 0.10	0.99 ± 0.01	11.01 ± 0.17	6.61
11	6.90 ± 0.21	0.47 ± 0.02	32.73 ± 0.84	3.39 ± 0.13	5.74 ± 0.22	23.76 ± 0.94	8.59 ± 0.36	3.59 ± 0.18	14.82 ± 0.28	9.58
12	4.04 ± 0.19	0.29 ± 0.01	20.78 ± 0.89	4.47 ± 0.14	9.48 ± 0.44	29.50 ± 2.08	10.55 ± 0.58	5.30 ± 0.08	15.60 ± 1.27	9.00
13	17.93 ± 0.12	1.89 ± 0.02	40.32 ± 0.20	5.70 ± 0.30	1.86 ± 0.01	15.95 ± 0.03	5.81 ± 0.06	1.95 ± 0.10	8.59 ± 0.04	10.44
14	14.31 ± 0.50	0.20 ± 0.01	38.80 ± 1.42	3.47 ± 0.34	2.45 ± 0.04	19.78 ± 0.60	7.09 ± 0.05	2.99 ± 0.07	10.91 ± 0.08	8.69
15	8.42 ± 0.02	0.35 ± 0.01	41.02 ± 0.12	3.70 ± 0.04	4.65 ± 0.17	17.85 ± 0.08	7.77 ± 0.24	5.00 ± 0.16	11.23 ± 0.24	8.99
16	10.45 ± 0.22	0.46 ± 0.01	39.20 ± 2.06	5.99 ± 0.01	4.65 ± 0.14	20.67 ± 1.13	7.01 ± 0.14	1.96 ± 0.08	9.61 ± 0.29	16.51
17	8.64 ± 0.16	0.21 ± 0.01	30.74 ± 0.64	5.51 ± 0.13	6.86 ± 0.19	25.52 ± 0.31	8.28 ± 0.12	2.91 ± 0.02	11.33 ± 0.14	13.31
18	7.18 ± 0.06	0.27 ± 0.01	31.44 ± 0.06	4.09 ± 0.20	8.33 ± 0.22	23.70 ± 0.03	6.95 ± 0.29	3.77 ± 0.08	14.27 ± 0.39	10.22
19	17.39 ± 0.15	1.07 ± 0.01	40.14 ± 0.20	8.42 ± 0.06	2.33 ± 0.01	13.18 ± 0.09	6.72 ± 0.03	1.74 ± 0.03	9.02 ± 0.01	14.07
20	13.95 ± 0.20	0.23 ± 0.02	37.02 ± 0.50	4.92 ± 0.08	2.70 ± 0.07	18.75 ± 0.25	7.95 ± 0.05	2.80 ± 0.15	11.69 ± 0.04	12.30
21	8.36 ± 0.05	0.36 ± 0.02	38.95 ± 0.34	3.21 ± 0.09	6.20 ± 0.13	16.37 ± 0.20	9.05 ± 0.20	5.47 ± 0.07	12.03 ± 0.35	12.49
22	15.10 ± 0.02	3.01 ± 0.02	39.76 ± 0.64	5.76 ± 0.29	1.88 ± 0.06	15.33 ± 0.16	7.27 ± 0.14	1.63 ± 0.05	10.25 ± 0.09	14.77
23	10.18 ± 0.01	0.62 ± 0.02	40.25 ± 0.14	3.95 ± 0.01	4.25 ± 0.17	19.07 ± 0.08	7.32 ± 0.02	3.58 ± 0.03	10.78 ± 0.30	11.42
24	7.31 ± 0.12	0.43 ± 0.01	39.18 ± 0.52	3.91 ± 0.04	7.37 ± 0.11	18.46 ± 0.10	5.39 ± 0.02	6.48 ± 0.04	11.47 ± 0.12	10.77
25	14.13 ± 0.13	0.31 ± 0.01	36.95 ± 0.25	4.33 ± 0.08	3.47 ± 0.04	20.61 ± 0.04	8.03 ± 0.01	1.14 ± 0.01	11.02 ± 0.02	9.04
26	7.55 ± 0.07	0.30 ± 0.01	39.93 ± 0.32	2.69 ± 0.01	6.92 ± 0.05	20.97 ± 0.02	8.06 ± 0.02	3.04 ± 0.01	10.54 ± 0.04	10.33
27	6.17 ± 0.11	0.49 ± 0.04	41.85 ± 0.78	2.07 ± 0.01	7.82 ± 0.18	18.50 ± 0.49	7.93 ± 0.03	4.10 ± 0.10	11.08 ± 0.03	5.20
28	9.46 ± 0.05	0.17 ± 0.01	39.22 ± 0.18	4.27 ± 0.03	4.48 ± 0.01	19.10 ± 0.01	8.36 ± 0.01	1.98 ± 0.08	12.97 ± 0.03	11.64
29	5.32 ± 0.03	0.36 ± 0.01	33.69 ± 0.21	2.11 ± 0.08	8.52 ± 0.24	20.91 ± 0.20	10.35 ± 0.29	5.10 ± 0.23	13.63 ± 0.33	12.27
30	3.05 ± 0.08	0.32 ± 0.01	29.71 ± 0.38	1.08 ± 0.12	13.67 ± 0.03	23.76 ± 0.11	7.34 ± 0.05	6.65 ± 0.06	14.42 ± 0.25	9.49
31	11.92 ± 0.23	0.55 ± 0.02	40.91 ± 0.92	4.84 ± 0.06	1.77 ± 0.07	20.36 ± 0.23	6.37 ± 0.04	nd	13.28 ± 0.08	10.98
32	7.72 ± 0.06	0.19 ± 0.01	43.32 ± 0.14	2.64 ± 0.35	3.61 ± 0.22	19.89 ± 0.32	7.80 ± 0.14	2.99 ± 0.14	11.85 ± 0.74	7.76
33	4.98 ± 0.02	0.26 ± 0.01	44.72 ± 0.25	1.28 ± 0.14	6.13 ± 0.01	18.17 ± 0.16	8.76 ± 0.02	4.12 ± 0.04	11.57 ± 0.18	4.94
34	10.54 ± 0.02	0.15 ± 0.02	37.45 ± 0.15	3.63 ± 0.01	3.59 ± 0.03	20.22 ± 0.09	8.65 ± 0.12	1.62 ± 0.07	14.16 ± 0.17	11.71
35	7.56 ± 0.02	0.43 ± 0.04	31.25 ± 0.01	1.18 ± 0.01	4.41 ± 0.04	23.49 ± 0.29	10.86 ± 0.05	4.01 ± 0.07	16.82 ± 0.10	8.08
36	5.15 ± 0.05	0.29 ± 0.03	40.46 ± 0.02	1.54 ± 0.03	10.50 ± 0.12	18.88 ± 0.49	6.35 ± 0.21	4.88 ± 0.07	11.86 ± 0.37	7.94
mean	8.93	0.47	36.23	3.63	5.56	21.14	8.25	3.42	12.47	10.26
maximum	17.93	3.01	44.72	8.42	13.67	29.50	10.86	6.65	16.82	16.51
minimum	3.05	0.15	20.78	1.08	1.60	13.18	5.39	nd	8.59	4.94
SD	3.82	0.54	4.87	1.62	2.93	3.54	1.37	1.53	1.94	2.44

^a Values as percentages are expressed as mean ± standard deviation of three assays for each sample. Abbreviations: nd, not detected; SD, standard deviation; Σ, sum of the determined phenolics; 3-CQA, 3-*O*-caffeoylquinic acid; 4-CQA, 4-*O*-caffeoylquinic acid; 5-CQA, 5-*O*-caffeoylquinic acid; 3,5-diCQA, 3,5-*O*-dicaffeoylquinic acid; Q-3-Gal, quercetin-3-*O*-galactoside; Q-3-Rut, quercetin-3-*O*-rutinoside; K-3-Gly, kaempferol-3-*O*-glycoside; K-3-Glu, kaempferol-3-*O*-glucoside; K-3-Rut, kaempferol-3-*O*-rutinoside.

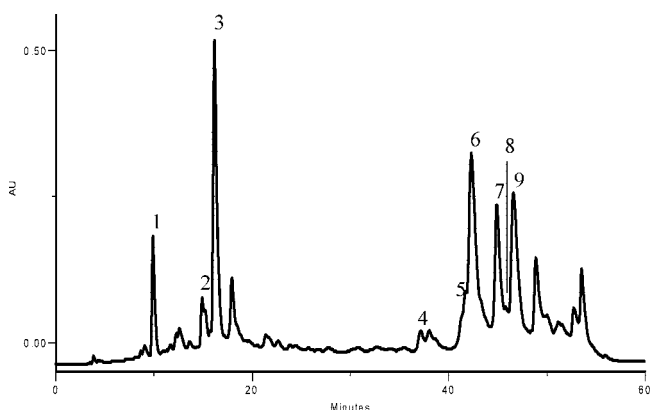


Figure 1. HPLC phenolic profile of quince leaves. Detection at 350 nm. Peaks: (1) 3-*O*-caffeoylquinic acid; (2) 4-*O*-caffeoylquinic acid; (3) 5-*O*-caffeoylquinic acid; (4) 3,5-*O*-dicaffeoylquinic acid; (5) quercetin-3-*O*-galactoside; (6) quercetin-3-*O*-rutinoside; (7) kaempferol-3-*O*-glycoside; (8) kaempferol-3-*O*-glucoside; (9) kaempferol-3-*O*-rutinoside.

p-coumaric acid (2, 3, 9, 12, 14). Quince seeds presented 11 phenolics, the four hydroxycinnamic acid derivatives presented

in leaves and seven *C*-glycosyl flavones: lucenin-2, vicenin-2, stellarin-2, isoschaftoside, schaftoside, 6-*C*-pentosyl-8-*C*-glucosyl chrysoeriol, and 6-*C*-glucosyl-8-*C*-pentosyl chrysoeriol (2, 7, 8).

C. oblonga leaves total phenolics content varied from 4.9 to 16.5 g/kg dry matter. Even considering the drying yields of quince leaves samples (about 50%), the total content (between 2.5 and 8.3 g/kg fresh matter; mean value of 5.1 g/kg fresh matter) was much higher than that found for pulps (varying from 11.7 to 518.6 mg/kg fresh matter), peels (between 278.8 and 1962.4 mg/kg fresh matter), and seeds (varying from 107.4 to 116.4 mg/kg fresh matter) (8, 9).

The most abundant compound in quince leaves was 5-*O*-caffeoylquinic acid (36.2%), followed by quercetin-3-*O*-rutinoside (21.1%; **Table 2**). Generally, in pulps and seeds, the most abundant phenolic compound was also 5-*O*-caffeoylquinic acid, representing approximately 59% and 21% of the total phenolics content, respectively, while the major compound in peels was quercetin-3-*O*-rutinoside (ca. 38%) (8, 9).

As quince peels, leaves of *C. oblonga* revealed the presence of kaempferol glycosides. Nevertheless, quince leaves presented the highest relative contents of kaempferol derivatives, especially

Table 3. Phenolic Composition of Quince Leaves, According to Geographical Origin^a

geographical origin	phenolic compound (%)									Σ (g/kg)
	3-CQA	4-CQA	5-CQA	3,5-diCQA	Q-3-Gal	Q-3-Rut	K-3-Gly	K-3-Glu	K-3-Rut	
Bragança	7.39 ± 2.99 b	0.34 ± 0.15 a	32.34 ± 4.13 b	3.37 ± 1.21 b	5.97 ± 2.88 a	24.28 ± 2.64 a	9.30 ± 0.88 a	3.32 ± 1.49 a	13.70 ± 1.47 a	9.67 ± 1.52 b
Carrizada de Ansiães	11.60 ± 3.92 a	0.76 ± 0.86 a	38.07 ± 3.42 a	4.89 ± 1.48 a	4.46 ± 2.29 a	18.72 ± 3.47 b	7.22 ± 1.00 a	3.36 ± 1.58 a	10.93 ± 1.50 b	12.00 ± 2.37 a
Covilhã	7.80 ± 3.22 b	0.32 ± 0.12 a	38.29 ± 4.68 a	2.64 ± 1.33 b	6.24 ± 3.43 a	20.41 ± 1.77 ab	8.24 ± 1.35 a	3.60 ± 1.65 a	12.77 ± 1.81 a	9.12 ± 2.43 b

^a In the same column, means with different letters are significantly different ($p \leq 0.05$); 3-CQA, 3-*O*-caffeoylquinic acid; 4-CQA, 4-*O*-caffeoylquinic acid; 5-CQA, 5-*O*-caffeoylquinic acid; 3,5-diCQA, 3,5-*O*-dicaffeoylquinic acid; Q-3-Gal, quercetin-3-*O*-galactoside; Q-3-Rut, quercetin-3-*O*-rutinoside; K-3-Gly, kaempferol-3-*O*-glycoside; K-3-Glu, kaempferol-3-*O*-glucoside; K-3-Rut, kaempferol-3-*O*-rutinoside.

Table 4. Phenolic Composition of Quince Leaves, According to Collection Month^a

	phenolic compound (%)									
month	3-CQA	4-CQA	5-CQA	3,5-diCQA	Q-3-Gal	Q-3-Rut	K-3-Gly	K-3-Glu	K-3-Rut	Σ (g/kg)
Bragança										
June	10.63 ± 2.85 a	0.36 ± 0.25 a	33.69 ± 2.54 a	4.13 ± 1.36 a	3.15 ± 1.79 b	24.76 ± 3.04 a	8.68 ± 0.31 a	1.52 ± 0.43 c	13.08 ± 1.48 a	9.55 ± 2.24 a
August	6.78 ± 0.84 b	0.31 ± 0.13 a	32.65 ± 0.95 a	3.16 ± 0.98 a	5.55 ± 1.34 b	24.16 ± 1.01 a	9.27 ± 0.98 a	3.63 ± 0.46 b	14.51 ± 0.88 a	9.70 ± 1.09 a
October	4.77 ± 0.63 b	0.35 ± 0.06 a	30.67 ± 7.00 a	2.83 ± 1.15 a	9.20 ± 0.88 a	23.93 ± 3.85 a	9.94 ± 0.83 a	4.81 ± 0.60 a	13.51 ± 1.88 a	9.77 ± 1.50 a
Carrazada de Ansiães										
June	15.22 ± 3.41 a	1.61 ± 1.10 a	39.86 ± 0.50 a	6.47 ± 1.31 a	2.68 ± 1.33 b	16.28 ± 3.16 a	6.70 ± 0.64 a	1.82 ± 0.16 b	9.37 ± 0.72 b	13.95 ± 2.55 a
August	11.77 ± 2.80 a,b	0.32 ± 0.20 b	36.70 ± 4.19 a	4.46 ± 0.92 b	4.07 ± 2.03 a,b	20.78 ± 3.19 a	7.66 ± 0.55 a	3.07 ± 0.35 b	11.18 ± 0.41 a,b	11.43 ± 1.98 a
October	7.82 ± 0.66 b	0.35 ± 0.07 b	37.65 ± 4.24 a	3.73 ± 0.38 b	6.64 ± 1.59 a	19.10 ± 3.19 a	7.29 ± 1.53 a	5.18 ± 1.12 a	12.25 ± 1.39 a	10.62 ± 1.45 a
Covilhã										
June	11.51 ± 2.01 a	0.30 ± 0.18 a	38.63 ± 1.80 a	4.27 ± 0.50 a	3.33 ± 1.13 b	20.07 ± 0.67 a	7.85 ± 1.02 a	1.58 ± 0.42 b	12.86 ± 1.32 a	10.84 ± 1.24 a
August	7.04 ± 1.15 b	0.32 ± 0.10 a	37.05 ± 5.55 a	2.16 ± 0.70 b	5.87 ± 2.26 a,b	21.32 ± 1.53 a	9.27 ± 1.56 a	3.79 ± 0.99 a	13.21 ± 2.72 a	9.61 ± 2.11 a,b
October	4.84 ± 1.30 b	0.34 ± 0.10 a	39.19 ± 6.56 a	1.49 ± 0.43 b	9.53 ± 3.29 a	19.83 ± 2.64 a	7.60 ± 1.01 a	4.94 ± 1.20 a	12.23 ± 1.49 a	6.89 ± 2.20 b
All Sites Combined										
June	12.45 ± 3.29 a	0.75 ± 0.87 a	37.39 ± 3.23 a	4.95 ± 1.51 a	3.05 ± 1.34 c	20.37 ± 4.30 a	7.75 ± 1.07 a	1.51 ± 0.58 c	11.77 ± 2.09 a	11.45 ± 2.70 a
August	8.53 ± 2.90 b	0.31 ± 0.14 a	35.47 ± 4.22 a	3.26 ± 1.27 b	5.16 ± 1.92 b	22.08 ± 2.47 a	8.73 ± 1.28 a	3.50 ± 0.68 b	12.96 ± 2.08 a	10.25 ± 1.84 a,b
October	5.81 ± 1.70 b	0.35 ± 0.07 a	35.83 ± 6.71 a	2.68 ± 1.17 b	8.46 ± 2.38 a	20.95 ± 3.70 a	8.98 ± 1.62 a	4.98 ± 0.93 a	12.66 ± 1.58 a	9.09 ± 2.30 b

^a In the same column, means with different letter are significantly different ($p \leq 0.05$); 3-CQA, 3-*O*-caffeoylquinic acid; 4-CQA, 4-*O*-caffeoylquinic acid; 5-CQA, 5-*O*-caffeoylquinic acid; 3,5-diCQA, 3,5-*O*-dicaffeoylquinic acid; Q-3-Gal, quercetin-3-*O*-galactoside; Q-3-Rut, quercetin-3-*O*-rutinoside; K-3-Gly, kaempferol-3-*O*-glycoside; K-3-Glu, kaempferol-3-*O*-glucoside; K-3-Rut, kaempferol-3-*O*-rutinoside.

of kaempferol-3-*O*-rutinoside, which represented 12.5% of the total phenolics content (against ca. 4% in peels and an absence in pulps and seeds). The high flavonoidic content of quince peels and leaves is not surprising because these types of compounds act as UV filters, protecting some fragile cell structures, such as chloroplasts, from UV radiation. Absorption of UV light is a general feature of phenolic compounds (2, 9, 22, 23). These filters consist mainly of flavonols and are located in the skins of fruits and leaves (9, 22). In addition, because of their antioxidant properties, polyphenols can serve as protection against photooxidation caused by UV light (2, 9, 22, 23).

Significant differences ($p \leq 0.05$) were found in the phenolic profiles of quince leave samples from different geographical origins, in terms of 3-*O*- and 5-*O*-caffeoylquinic acids, 3,5-*O*-dicaffeoylquinic acid, quercetin-3-*O*-rutinoside, kaempferol-3-*O*-rutinoside, and the total phenolic content (Table 3). Generally, considering the content of these compounds, samples from Carrizada de Ansiães were significantly different from those from Bragança and Covilhã.

Significant differences ($p \leq 0.05$) were also found among samples harvested in the three different months, considering each geographical origin or all of them at the same time (Table 4), especially concerning 3-*O*-caffeoylquinic and 3,5-*O*-dicaffeoylquinic acids, quercetin-3-*O*-galactoside, and kaempferol-3-*O*-glucoside contents. While the amounts of these caffeoylquinic acids decrease with harvesting time, quercetin-3-*O*-galactoside and kaempferol-3-*O*-glucoside contents increase, which can be related with the high temperature verified during the summertime that implies physiological adaptations of plants. Samples collected in June were characterized by higher total phenolics content.

Nowadays, quince fruit is recognized as a good, cheap, and important source of health promoting compounds, because of its antioxidant (1–3, 9, 15, 16, 18), antimicrobial (1, 15), and antiulcerative (16) properties. Several studies suggest that phenolic compounds are mainly responsible for these activities and consequently the possible health benefits associated (1–3, 15–18). So, in conclusion, this study suggests that leaves from *C. oblonga* can be used as a better and cheaper source of bioactive compounds and may have relevance in the prevention of diseases in which free radicals are implicated. Considering the high phenolics content of quince leaves, in future studies, we intend to evaluate their antioxidant and antimicrobial potential.

Additionally, significant differences were found in 3-*O*-caffeoylquinic and 3,5-*O*-dicaffeoylquinic acid contents, according to geographical origin and collection season, which indicates a possible use of these compounds as geographical provenance and/or maturity markers.

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